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SUMMARY

GENETICS AND BREEDING

NUTRITION

REPRODUCTION, PHYSIOLOGY, ANATOMY

TECHNOLOGIES OF ANIMAL HUSBANDRY

TECHNOLOGIES OF THE AGRO FOOD PRODUCTS PROCESSING

WILD LIFE MANAGEMENT, FISHERY AND AQUACULTURE

GENETICS AND BREEDING

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RESEARCH REGARDING SOME SPECIFIC FESTURES OF MOLDOVAN KARAKUL LAMBS HIDE

Ion BUZU

Institute of Zoology of Academy of Sciences of Moldova,
MD-2028, Chişinău, str. Academiei, 1, Republic of Moldova, Phone: +373.22.73.98.09,
Fax: + 373.22.73.98.09, Email: izoolasm@yahoo.com

Coresponding author email: ionbuzua@gmail.com

Abstract

The purpose of this research was to evaluate the particularities of Moldovan Karakul lambs hide features and identify factors influencing its quality. The research was conducted on Karakul sheeps in sovhoz "Kotovschi" from Căinari district and Experimental Station of the National Institute of Animal Husbandry and Veterinary Medicine, from Anenii Noi district, Republic of Moldova. The lambs hide thickness was determined according to the purpose of research, through the palpation and measurement methods using cutimeter (foldimeter) on the lambs evaluation, made on the next day after being born, and measuring under the microscope using eyepiece micrometer on histological samples of hide, taken for evaluation purpose. The density and hide reserves were determined by the palpation method at the evaluation. The research results have shown that the qualities of Karakul lambs hide is related to a number of factors both internal and external. The Moldovan Karakul lambs have thicker hide compared with Asian Karakul lambs, average hide thickness measured with cutimeter on the evaluation constituting 2.0 to 2.6 mm. Features of lamb hide qualities are correlative link between them and a string of other characters, such as curls size, curls type, lamb constitution, class of evaluation, age of ewes-mothers at birth, etc. Hide thickness of Moldovan Karakul lambs is in a positive phenotypic correlation with the size of their curls and ewes- mothers at birth, and in negative correlation with hide density, reserve hide, fertility, type of curls and general class of its own. The hide of Moldovan Karakul lambs after evaluation is suitable dense (83.8% of individuals) and very dense (12.8% of individuals), being in positive phenotypic correlation with the type of curls and in negative correlation with hide thickness and lamb constitution. Karakul lamb's hide reserve after evaluation is, in the majority, free (from 46.1 to 72.0% of individuals) and folds (at 12.9 - 26.9% of lambs), being in positive phenotypic correlation with the type of curls, hide density and class of its own, and in negative correlation with the thickness of the hide and its own constitution.

Key words*: Karakul lambs, features, hide, thickness, density, reserve.*

INTRODUCTION

Karakul lambs hide, unlike other animals, represent a particulary interest, given thatdermal tissue papilla is covered with a shiny, elastic and silky curls exposed as the original, forming a noble fur. The following properties are valued live lamb important: thickness, density, reserve (area) and flexibility (elasticity).

The skin thickness is important because this determines the character of hide weight, respectively coat made of them, and her durability. Is considered valuable skins have a thinner thickness and the worthless - thicker. Skins thin - normal are required because these clothes are made of lighter than the thicker skins. One should remember that skins too thin is not always appreciated because of weak resistance of the dermis, for which can be easily broken in industrial processing and exploitation.

Like other animals, Karakul lamb hide histological structure consists of three layers: the *epidermis* - the outer layer of skin, the *dermis* - the main layer (middle), and *hypodermis* - bottom layer, the connection between the hide and the body itself animal. Among these, interest in the first place, the epidermis and the dermis, the latter being formed in the substrate *papillary* and *reticular*. The epidermis is the germination substrate (of Malpighi) which generates follicle sheath formation and keratinized epidermal substrates. In the dermis, papillary substrate is about 70% of its total thickness. It is composed of connective tissue with collagen and elastin fibers. In this layer are

plunged hair follicles, sebaceous and sweat glands, blood capillaries, arrectori muscles and nerve fibers. The substrate reticular dermis is made up of strips of elastic collagen fibers thick printed with a developed network of blood vessels and nerve threads by means of which the receiving power of the upper substrates of the hide and hair root sheath. Given that Karakul lamb skin sacrificed 1-5 days after birth, has a cargo of fur own features, such as thickness, density, reserve (area) and flexibility (elasticity) are of particular importance. They are subject to multiple factors such as the orientation of the collagen fibers and the connection character (which determines to a large extent, the density of the dermis), the correlation thickness dermal substrate, the histological structure. Each of these traits hide deserves a separate study and a proper assessment as part of skin qualities as a whole, causing its value.

Among the many general functions of the hide, draws attention of researchers, primarily producing the hair fiber. Between the structure, characteristics of its skin and follicle sheath there is a very close functional link because the fibers are derived hide.

Therefore, knowledge of the structure, functions and features of utmost importance hide to identify the possibility of influencing the process of formation and growth of hide and follicle coating to improve their qualities (Рахматов, 1978; Баратов et al., 1975).

In this context, the aim of this research was to evaluate the particularities of Karakul lambs hide features Moldovan and identify factors influencing quality.

MATERIALS AND METHODS

The research was conducted on Karakul sheep in farms: Sovhoz "Kotovschi" s. Cârnățănii Noi, Căinnari adistrict and Experimental Station of the National Institute of Animal Husbandry and Veterinary Medicine, Maximovca, Anenii Noi district.

The hide's qualitative assessment of lambs was performed by evaluation marks the second day after birth, according to the Instructions of evaluation of Karakul sheep breeding principles in the Republic of Moldova (Buzu et al., 1996), with supplemented and amended modifications (Buzu, 2012). The evaluation marks lambs were investigated following hide properties: *thickness, density*, *reserve* and *flexibility*.

Lambs *hide thickness* was determined according to the purpose of research by one of the following methods: *farm method* (field testing) or *laboratory method*.

The *method of farm* field was applied to the evaluation marks and lambs was carried out by one of the ways: first - the determination of the thickness of the hide tactilo-visual (palpation), and the expression level of hide thickness words and scores (from 1 to 10 points), and the second - measured by using foldimeter (callipers) and expression of hide thickness in millimeters.

In determining hide thickness by means of probing fingers grab a fold of hide on lamb rump stretched up. In this case researcher simultaneously assesses several properties such as thickness, book and flexibility. The hide thickness at probing was expressed in words: *thin*, *medium, thickened,* and *thick*. *Thin* hide - normal is fine, dense and elastic. This leather is assessed by 8-10 points and enrolls in the register of evaluation symbol "thin". *Medium thick*hide on palpation is moderately fine, dense with the feeling of being resistant and elastic. The register of evaluation falls under the symbol "med". *Thickened* hide on palpation is slightly thicker, less dense, with feeling low elasticity. Thickened hide on evaluation marks is assessed only by 3-4 points. This hide is part of the registry with the "tknd". *Thick*hide on palpation, is considerably bulky and coarse sponge. Thick hide, the evaluation marks, is assessed by 1-2 and entered in the register of evaluation with "thick".

In determining hide thickness using cutimeter, the thickness of skin folds was measured (ie, double thickness hide) with accuracy of 0.5 mm. By dividing two fold size was determined thickness of the hide itself. This method is more objective than the probing method, but the value increased skin thickness is more than its absolute thickness, being conditioned by the subcutaneous layer coating roots and taken into the fold.

Laboratory method (most accurate) was applied to measure the absolute thickness of

skin on hide samples taken from histological evaluation marks lamb. The hide thickness, including the histological part of each layer was determined with a microscope equipped МБИ-1 eyepiece micrometer gradually with the increase of 280 times. For this, hide samples taken from the evaluation marks lamb rump were frozen and cut using microtome, and prepared by special methods (Диомидова, 1960) and fixed on microscope slides. Histological preparations were prepared as the raw hide, and the hide samples preserved formaldehină solution with a concentration of 10%.

Hide *density* was estimated by the method of probing the evaluation marks lambs. To do this, grab a fold of hide with your fingers (double) of the thigh, squeezing it between your fingers and feel so determining the degree of relative density of the hide. According to the instructions of evaluation Karakul lambs were observed following degrees of hide density: *very dense, fit* dense, *low* and *loose*. *Very* dense hide, to touch, produce very strong feeling of dermal tissue (contrary soft). The clamping fingers are not deep into the fold of hide that opposes a relatively stiff resistance against their action. The evaluation marks, very dense hide is assessed by 8-10 points and entered in the register of evaluation with the rating "v.dens". Fit dense hide according to palpation, produce the sensation of hard tissue, but less hard than the hide very dense. The clamping fingers deepened assessor little slack, but not too much. Hide resistance to palpation is also sentient. The evaluation marks, such lambs skin are appreciated by 5-7 points and entered the adjective "fit". Low density hide sensation on palpation produce dermal tissue less hard (to soft). When clamping, researchers fingers deep into the skini. The hide resistance to palpation is poor. At evaluation marks, such hide is appreciated only by 3-4 points and entered in the register of evaluation with the rating "low". Th hide with soft density (sponge) on palpation, produce the sensation of a sponge and has a soft dermal tissue. When clamping fingers researcher fingers deepen in tyhe hide tissue. The hide resistance to palpation is very poor. The evaluation marks, loose skin (sponge) is appreciated only by 1-2 and entered in the register of evaluation with the rating "loos".

Karakul lambs *reserve* hide was assessed by examination and palpation of the evaluation marks of folds of skin in different body regions usually, on the rump. The reserve is understood, by property of the hide to form (after detachment from the chassis) some additional area of hides usually larger than the body of the lamb alive. This additional area is formed due to hides suppleness (elasticity, stretching property) and folds (creases) natural hide that can be lamb. The notion of slack should be distinguished two kinds: natural fold (folds) and fold artificial hide formed by adding assessor fingers. To assess suppleness, hide fold should be stretched with fingers over the lamb. The search for more stretching, the hide is more supple, therefore, and the hide is greater reserve.

According to the instructions of evaluation in force following degrees were outstanding character development reserve Karakul lambs hide: reserve *folds*, *free, tight* and *insufficient*. Reserve Karakul lambs hide folds is observed visually free (without probe) and is characterized by the presence of natural folds in the neck, ribs, rump and tail. On palpation and stretch, bend naturally extends far beyond the body and after cessation extent, retains some time enlarged. The hide is very elastic, thin to medium thickness and produces the feeling that her body poorly stitched. Reserve hide folds is estimated at 8-10 points and evaluation marks in the register of evaluation with the rating "fold". Free reserve is characterized by placing free hide on the body surface, but without folds (folds). On palpation, the hide on the rump gets down slightly, forming box with fingers and extends moderately over the body. Hide elasticity is moderate. Reserve free hide evaluation marks is estimated at 5-7 points and entered in the register of evaluation with the rating "fre".

Tight reserve is characterized by confusion, indeed, stretched hide on the body. On palpation, the hide of the thigh hardly gets down to form less crease and extends over the body. The elasticity of the hide is reduced. Reserve hide stretched evaluation marks is estimated at only 3-4 points and entered in the register with the qualification "tig".

Insufficient reserve is characterized by very large settlement body lamb hide. On palpation, the hide on the rump gets down very hard, so it is very difficult to form a fold fingers. The hide does not stretch at all over the body and creates a feeling that her tightly against the body. Hide elasticity is sufficiently low (almost absent). The evaluation marks, this reserve is considered the lowest, with only 1-2 points and entered in the register of the mark "ins".

The data obtained were statistically experience using computer software "STATISTICA - 6" and rated their certainty, according to statical variational biometric methods (Плохинский, 1969).

RESULTS AND DISCUSSIONS

Research results have shown that the qualities Karakul lambs hide is linked to a number of factors both internal and external. Hide quality are related (connection) rather complicated with other phenotypic characteristics of the parcel roots, the curls and the development of the whole body and body constitution (Buzu, 2001, 2012).

Hide thickness is hereditary determined and influenced by many factors, such as during intrauterine development of fetal, lambs sex, maternal age at birth, pregnant sheep nutrition etc. Heritability coefficient is not higher hide thickness h^2 = 0,201 (Закиров, 1987). Therefore, the thickness of the hide is very much influenced by external factors, in particular the environment, such as the second feeding sheep in gestation period.

In the Republic of Moldova, more favorable in terms of fodder base compared with the conditions of Central Asia, there is a tendency of Karakul lambs hide in thickening. Under the same conditions for growth and maintenance, according to information from other regions rams young is a thickening of the rams there, compared with hide ewes (Table 1).

Thus, according to Кошевой, 1975, newborn lambs population share with thin hide ewes was 16.3% higher than of the rams ($P < 0.001$), while the share of individuals with thickened hide and thick of rams was 9.1% higher than of the ewes ($P < 0.001$). In our research, the essential differences between hide thickness of ewes and rams were not recorded, but obviously it is noted that the Republic of Moldova lambs hide thicker than those investigated by Кошевой M.A. in Uzbekistan.

Table 1.The thickness of Karakul lambs hide depending on the sex

Lambs sex	Head	including hide thickness					
		Thin		Medium		Bold thick	
		head	%	head	%	head	%
Our researches (Republic of Moldova)							
Rams	334	70	21.0	159	47.6	105	31.4
Ewes	292	59	20.2	145	49.7	88	30.1
After Кошевой M.A. (Uzbekistan)							
Rams	890	501	56.3	188	21.1	201	22.6
Ewes	992	720	72.6	138	13.9	134	13.5

In domestic conditions, the weight of lambs with thin hide is less compared to those in Uzbekistan, with 35.3% rams and 52.4% to lamb ewes ($P < 0.001$). The share of medium thick lamb hide is higher by 26.5% of the rams ($P < 0.01$) and 35.8% of the ewes ($P < 0.001$), while the share of individuals with thickened hide tends to to be higher in both groups of lambs local sex. These differences could be explained not only by various madiu and feeding conditions but also by the biology (genetics) the types of Karakul sheep Asian and Karakul sheep Moldovian bred in these regions.

The hide thickness is related to the type of buclaj of lambs (Table 2).

Table 2. Type of the curls of Karakul lambs depending of hide thickness

Hide thickness	n	Including curls type, %			
		jacket	coastal	flat	kaukazian
Thin	133	31.6	30.8	23.3	14.3
Medium	299	38.5	29.1	15.4	17.0
Thickened	176	30.7	24.4	12.5	32.3
Thick	18	11.1	38.9	5.6	44.4

Research has shown that Karakul lambs in Republic of Moldova, most of them have medium skin thickness (47.8%) and slightly thickened (28.1%).

Type jacket lambs were mostly middle-thickness skin (38.5%) and thin (31.6%). Lambs to the type of curls costal have a thicker hide. Among these are 38.9% of individuals with thick hide, 24.4% - thickened hide and 29.1% - medium hide. Lambs with

thick hide in this beatch belong to the thick rib apron. The lambs with flat type curls have thinner hide, from the first two beatchs. In this beatch predominates individuals with thin hide (23.3%), while in this beatch are lambs and 15.4% and 12.5% medium hide of lambs with thickened hide. The lambs with type of curls kaukazian compared with the first three batches were much thicker hide. In this batch predominates both thick-skinned individuals (44.4%) and with the thickened (32.3%).

Mentioned that the lambs with thin hide and medium generates valuable curls types. Thus, the batch of lambs with thin hide was recorded the highest share valuable summary of curls types lambs (jacket, coastal and flat) and the lowest percentage of individuals with unwanted curls type (kaukazian). However, in batches of lambs with thickened hide and thick is worth the largest share of individuals with unwanted curls type (kaukazian).

Karakul lambs hide thickness is relatively strong relationship with lamb class (Table 3).

Table 3. Hide thickenss of Moldovan Karakul lambs depending on class

Class	n	Including hide thickness, %			
		thin	medium	thickened	thick
Elite	160	20.1	51.1	27.5	1.3
Class I	315	20.0	51.0	28.0	1.0
Class II	144	22.5	40.1	30.6	6.8
Bad	7	14.3	14.3	57.1	14.3

Due to the fact that the class is an index reflected in the final synthetic hide qualities as a whole, resulting from the coating and buclaj roots which are produced in the hide, then it is natural that these qualities are related to the hide, and vice versa, the hide is related to class lamb.

The lambs of higher class (elite and class I) were hide predominantly middle (51.1-51.0%) and thin (20.0 to 20.1%), and slightly thickened (28.0 to 27.5%). Individuals with thick hide, among these batch, were very rare (1.0 to 1.3%). However, lambs from lower classes (class II and brac) have hide predominantly thickened (30.6 -57.1%) and medium (14.3 to 40.1%), and less thin (14.3 - 22.5%). Among these lambs, often meet individuals with thick hide (6.8 to 14.3%). Therefore, the superior qualities of skins, such as lambs and elite class, is associated with thick hide and thin middle and lower ones, such as lambs and class II brac, thicker hide or thick.

A relationship was found between the rather obvious hide thickness and size curls Karakul lambs (Table 4).

Table 4. Hide thickness depending on curls size of Moldovan Karakul lambs

Curls size	n	Including hide thickness, %			
		thin	medium	thickened	thick
Large	156	12.7	38.0	43.0	6.3
Medium	372	20.5	53.5	24.4	1.6
Small	98	35.9	43.4	19.8	0.9

The research shows that lambs curls possess high usually thickened hide (43.0%) and medium (38.0%).

Those with medium curls and hide were predominantly middle (53.5%) and thickened (24.4%). This relationship is reciprocal, so lambs of thickened hide usually have larger loops and thin cell lambs were smaller and shorter loops. Lambs with small loops have a thinner hide thickness (35.9%) and medium (43.4%). Therefore, as the loop is smaller, and the hide is thinner and, conversely, the loop is higher, and the skin is thicker. Also we found that both hides too thin hide and the hide too thick, not required by manufacturers cunt because too thin hide has insufficient resistance to traction and exploitation clothing and hide generates a weight too heavy too great Karakul lambs hide clothing.

The measuring done with cutimeter, the average thickness of the hide Moldovian Karakul lambs varies on average, depending on the type of curls, class, and size of the curls from 2.00 to 2.88 mm (Table 5).

Obvious differences were established between sole lambs hide thickness and the twins hide. The twin lambs hide is definitely thinner than sole contemporaries, like by type of curls and curls size. The elite class with twin lambs with curls middle and curlstype jacket, had thinner hide to 0.24 mm sole congeners or 9.3% ($P < 0.01$), twin lambs with curls class type Jackets and middle curls had thinner hide to 0.31 mm the sole congeners or 12.1% ($P < 0.001$), and twin lambs curls class with large loop type jacket and had hide thinner peers to sole 0.45 mm and 16.4% ($P < 0.001$). Such significant differences were seen in batchs of

lambs buclaj type of cost, payment, kaukazian and mestizos Karakul x Ostfriz of F1 generation.

Table 5. Hide thickness (meassured with cutimeter) of black Moldovan Karakul lambs, depending on the curls type, class and size loop (mm)

Curls type, class, curls size	Solo lambs			Twin lambs		
	n	$x \pm s_x$	v %	n	$x \pm s_x$	v %
Jacket						
Elite, sm	8	2.25±0.13	17.2	-	-	-
Elite, med	108	2.57±0.03	12.7	15	2.33±0.09	14.9
Elite, high	9	2.85±0.15	15.2	-	-	-
Class I,sm	32	2.28±0.05	12.0	12	2.08±0.12	19.3
Class I, md	210	2.57±0.02	12.1	63	2.26±0.04	15.0
Class I, hg	103	2.75±0.04	15.8	14	2.30±0.07	11.8
Coastal						
Elite	53	2.74±0.05	12.1	2	2.50±0.00	0.00
Class I	59	2.73±0.04	10.8	4	2.12±0.24	22.5
Class II	9	2.63±0.14	16.0	-	-	-
Flat						
Elite	24	2.53±0.08	15.8	3	2.17±0.16	12.9
Class I	46	2.61±0.05	14.1	9	2.22±0.09	11.8
Class II	16	2.53±0.07	11.9	9	2.19±0.13	17.8
Kaukazian						
ClassII,sm	5	2.10±0.06	6.7	2	2.00±0.00	0.00
ClassII,md	55	2.56±0.05	13.5	16	2.20±0.09	17.7
ClassII, hg	30	2.88±0.07	12.8	6	2.83±0.11	9.1
Kark-Ostf, crossed F_1	32	2.66±0.05	11.1	4	2.00±0.20	25.0

The data demonstrate that the thickness of the hide, measured by cutimeter also is closely correlated with the size of the curls. The thin hide is found in lambs with small curls. The thick hide is observed in lambs with large curls.

The lambs middle curls has a medium thick hide. For example, type lambs jacket with small loop elite class had hide thickness of 2.25 mm, the middle curls- 2,57 mm, and those with large curls - 2.85 mm. In these batchs, small curls lambs were thinner hide to middle loop congeners, 0.32 mm, or 12.5% ($P < 0.001$) and to congeners with large loop - 0.60 mm, or 21.1% ($P < 0.001$). In the lambs with curls class type jacket, individuals with small curls had thinner hide to middle curls congeners, 0.29 mm, or 13.3% ($P < 0.001$) and to curls congeners high - 0.47 mm, or 17.1% ($P < 0.001$). Among batchs of different type of curls lambs, lambs tended curls a cost thicker hide over other types. The thin hide of solo lambs was recorded in individuals with type kaukazian small curls (2.10 ± 0.06 mm). The thick hide curls was recorded in lambs than kaukazian type (2.88 ± 0.07 mm). The same picture is manifested and twin lambs. The coefficient of variation (Cv) of hide thickness measured by cutimeter, up to a maximum of 25% and 12 – 15% on the average.

Our research performed on histological preparations of raw hide samples collected from the evaluation marks lamb rump, have shown that the total thickness hide Karakul lambs depends on the age of mothers at birth (Table 6).

Table 6. Hide thickness (measured using histological preparates) of Moldovan Karakul lambs depending of mother age, $x \pm s_x$ (mkm)

The age of ewes-mother when calvin	n	Total hide thickness	The thicknes of the layer		
			epidermis	papillary	reticulary
13-14 months	15	1910±39	15,9±0,7	1145±32	717±36
2,0-2,2 years	30	2100±43	17,2±0,4	1223±23	830±25
>3years adults	10	2828±104	36,0±1,2	1921±86	854±24

The thin hide was registered in lambs obtained from sheep seeded at an early age (8 - 9 months) and calved at age 13-14 months. The total thickness of the hide in these lambs was 1910 ± 39 mkm. The thick hide was observed in lambs obtained from adult ewes (over 3 years) was 2828 ± 104 and mkm.

Primiparous sheep, which were seeded at 18-20 months calved medium thick-skinned descendants - 2100 ± 43 mkm. Lambs born to ewes calved mothers at an early age (13-14 months) had thin hide mkm 190 or 9.1% ($P < 0.001$) compared to peers born from primiparous sheep calved at age 23-25 months, with 918 mkm or 32.5 % ($P < 0.001$) compared to peers born from adult ewes older than 3 years.

Epidermal layer of the hide is the thinnest, representing only 0.8-1.2% of the total thickness, and has great importance to the Karakul skins. The thick layer of hide is important *papillary layer*, which is 1145-1921 mkm, or 58-68% of the total thickness of the hide. In this layer fibers are plunged their hair follicles, sebaceous and sweat glands, blood vessels and nerve fibers. *Papillary layer*, specific histological structure is responsible for organizing the groups and the rows of hair fibers, the fibers structure, therefore, the

quality of the curls coating and roots as a whole. *Reticulary layer* is the second layer upon thickness and inferior to the papillary layer via hypodermic link to the animal. The thickness at Karakul lambs is about 717 - 854 mkm, or 30 - 39% of the total thickness of the hide. The structure and arrangement of cells reticular dermis hide resistance depends on the processing and exploitation. The thickness of the hide and its layers ranges at different regions having different values of hide. If the region rump hide thickness is 100%, then the back is 117.4%, the withers - 104.1%, and the sides- 92.9% (Закиров, 1987). This is explained by the difference in terms of the formation of substrates hair in these areas.

Density hide Karakul lambs is determined inherited and conditioned by many factors such as hide thickness, type of curls, type constitution etc. After Иванов, 1964, thin hide, dense and elastic produce the most valuable curls, although fine and durable; thin and loose hide (sponge) generates rare fibers, thin, long and scattered curls; thick curls and loose fibers causes thick, rare and curls overgrown, loose, sandy and normal elasticity, forming curls annular, peas, corkscrew.

Our research showed that the density of Karakul lambs hide is directly related to its thickness (Table 7).

Table 7. Relation between hide thickness and density of Moldovan Karakul lambs

Hide thickness	n	Hide density, %			
		Very dense	Fit	Low	Loose
Thin	129	67.2	32.1	0.7	-
Medium	304	12.8	83.8	3.4	-
Thickened	172	5.9	40.9	51.1	2.1
Thick	21	-	7.7	53.8	38.5

The Moldovan Karakul lambs, thin hide is usually in 67.2% of cases, very dense and in 32.1% of cases - according dense. The hide is medium thick, mostly in 83.8% of cases, moderately dense.

The hide with a thickened density, the majority (51.1%) and reduced in 40.9% of cases. The thick hide is associated in most cases with low density (53.8%) and loose (sponge) - 38.5%. Once the hide is thickening of the degree to thickened hide decreases very dense weight lambs from 67.2% to 5.9%, or 61.3% ($P < 0.001$) and also increase the share of the lambs the low density of the hide, from 0.7% for those with thin hide, to 51.1% for individuals with thickened skin, and up to 53.8% in those with thick hide, or 50.4 and, respectively, 53.1% ($P < 0.001$). In this case, simultaneously increase the density lambs loose weight to 2.1% in individuals with thickened skin and to 38.5% in those with thick hide.

So, this relationship demonstrates that with lambs hide thickening, and its density decreases and, conversely, hide thinning increases its density. Thus, selecting lambs with thin hide - normal middle coach contributes indirectly to improving its density. Selection after these two qualities, is quite effective, because the correlation between them is quite high. Skins with dense dermis (right) and very dense are required by buyers because they are more resistant to industrial processing and exploitation of their clothing and, conversely, skins with loose dermis are vulnerable to exploitation and processing and therefore less demand on market outlets.

Karakul lamb hide density is related to type curls of the skin (Table 8).

Table 8. Hide density of Moldovan Karakul lambs depending of curls type

Curls type	n	Including hide density, %			
		Very dense	Fit	Low	Loose
Jacket	213	19.6	65.9	14.5	-
Coastal	178	28.3	55.6	14.4	1.7
Flat	101	20.8	62.4	16.8	-
Kaukazian	127	15.0	57.5	25.2	2.3
Bad	7	-	14.3	71.4	14.3

We have established that the densest lambs hide possesses curls type jacket, coastal and flat.

Among the thousand of these groups are 19.6 to 28.3% of individuals with very thick hide and from 55.6 to 65.9% - according to the dense hide. The loose hide was observed in lambs kaukazian and bad curls type. Share lambs with low density and loose hide in these batchs constitute 71.4 and, respectively, 14.3% and 25.2 bad lambs, respectively, 2.3% kaukazian type lambs. With the increasing amount curls type from the poor lambs (bad)

and worthless (kaukazian) to the most valuable (jacket), essentially increase the share of individuals with hide summary dense and dense right from 14.3% to 85.5% or 71.2% (P < 0.001), and decreased at the same time a summary of the individual weight of low density and the loose hide from 85.7% to 14.5%, of the same 71.2% (P < 0.001). Therefore, how curls type of lambs is more valuable, the hide is less dense and, conversely, how curls type of lambs is less valuable, the lower the density of their hide and loose. The positive correlation between these features allow the coach to improve hide density while performing selection by type curls lambs only.

Lambs curls density is addictive and their constitution (Table 9).

Table 9. The density of Moldovan Karakul lambs hide depending on their constitution

Constitution	n	Hide density, %							
		V. dense		Fit		Low		Loose	
		num	%	num	%	num	%	num	%
V.fine (bad)	2	1	50.0	1	50.0	-	-	-	-
Fine	167	124	74.2	35	21.0	8	4.8	-	-
Robust	806	464	57.6	204	25.3	138	17.1	-	-
Coarsely	7	-	-	1	14.3	2	28.6	4	57.1

We found that the densest lambs hide possesses a fine constitution. Lambs in this batch, the overwhelming majority of them have a very thick hide and dense right. Among them, the weight of lambs with very thick hide is 74.2%, while that of the right dense hide - 21.0%.

The lambs with a robust constitutionwere also very thick hide (57.6%) and under dense (25.3%). However, among this batch lambs is a minor proportion of individuals with low density of the hide, which is 17.1%. After the share of individuals with hide dense, fine constitution lambs surpassed the constitution robust 16.6% (P <0.001). In the batch of lambs with coarsely constitution, most individuals have loose hide density (57.1%) and low (28.6%). With the robustness constitution lambs from very fine to coarse drops summary weight of individuals with very thick hide and dense right from 100 % to 14.3%, or 85.7% (P < 0.001) and simultaneously increase the share of individuals with low density brief and loose hide from 4.8% in lambs with fine constitution, to 85.7% in lambs with coarsely constitution, or by 80.9% (P < 0.001) .

The reserve is a hereditary hide and conditioned by a number of factors, such as hide thickness, type of curls, hide density, constitution lamb, class etc. Reserve hide is correlative with multiple links caracters and features a string of lamb and hide. Reserve hide is closely related to its thickness (Table 10).

Table 10. Relation between hide's reserve and thickness of Moldovan Karakul lambs

Hide thicknes	n	Hide reserve, %			
		Folds	Free	Tight	Insuffi-cient
Thin	129	26.9	61.2	11.9	-
Middle	304	18.2	72.0	9.8	-
Thickned	172	12.9	55.4	31.2	0.5
Thick	21	-	46.1	46.2	7.7

Our research shows that most of Moldovan Karakul lambs hide isfree reserves (46.1 to 72.0%). The largest reserves of hide was recorded in lambs with thin hide and those with medium thick hide. The lambs examined, the highest share of lambs was registred reserve folds in batch lambs with thin hide. With thinning hide thickened lambs from grade to the thin folds increase the share of individuals with reserves from 12.9% to 26.9%, or 2.1- fold (P < 0.001), and vice versa, with thickening of the hide from the thin to the thick drops summary weight of lambs with free reserve and folds from 88.1% to 46.1%, or 42% (P < 0.001), and increasing the share of individuals with reserve summary large and insufficient hide from 11.9% to 53.9%, and the same 42% (P < 0.001). Therefore, as the hide is thinner, the reserve is higher and, conversely, the hide is thicker and thicker hide is less reserve. Lambs with hide folds and free reserves and flexibility have improved as a result skins surface is higher.

The reserve of hide is in direct relation with the type of curls of the lambs (Table 11).

In our research we found that most of Moldovian Karakul lambs hide free reserves (58.4 to 63.3%). The type of lambs curls of jacket and spare coastal tend hide higher than those with type kaukazian curls flat.

Table 11. Relation between Moldovan Karakul lambs hide reserve and curls types

Curls type	n	Hide reserve %			
		Folds	Free	Tight	Insuffi-cient
Jacket	213	25.2	63.3	11.2	-
Coastal	178	27.8	61.1	11.1	-
Flat	101	19.8	58.4	21.8	-
Kaukazian	127	12.6	61.4	25.2	0.8
Bad	7	-	57.1	14.3	28.6

The jacket and coastal type lambs were 25.2 and, respectively, 27.8% of lambs hide folds reserves against only 19.8 and, respectively, 12.6% in lambs with flat curls type kaukazian. The weakest reserve found in lambs hide type curls bad. Among these lambs were 28.6% with insufficient reserves and 14.3% with tight reserves. For comparison we report that according to data of Кошевой, 1975, most Asian Karakul lambs were also reserve free of hide approximately equal weight to all curls. On this basis, the author concludes that the Karakul lambs of any type curls, provided free hide is characteristic of this race.

The hide reserve is closely linked with its density (Table 12).

Table 12. Relation between Moldovan Karakul lamb hide reserves and density

Hide density	n	Hide reserve, %			
		Folds	Free	Tight	Insuffi-cient
V. dense	145	38.6	48.3	13.1	-
Fit	362	27.1	62.2	10.7	-
Low	116	17.3	43.1	37.9	1.7
Loosed	3	-	-	33.3	66.7

We found that lambs with very dense hide and dense fit are mostly reserve folds (27,1 - 38.6%) and free (48.3 to 62.2%). Among those batchs lambs are few individuals with hide tight reserve (10.7 to 13.1%) and those with insufficient reserves, absolutely lacking. In the batch of lambs with low hide density decreases and the weight of lambs with folds and free reserves, and also increases the weight of lambs with tight reserves (37.9%) and insufficient (1.7%), almost three times. The lambs with loosed hide density has usually a tight reserve (33.3%) and insufficient (66.7%). With the increasing density of the gradation lamb hide reduced to the very dense, essentially increases the weight of the hide of individuals with the proviso folds from 17.3% to 38.6%, or 2.2 - fold ($P < 0.001$) and, at the same time, decreases the weight of the summary of the individual with the reserve tight and insufficient hide from 39.6% to 13.1%, or 3.0 - fold ($P < 0.001$). Therefore, as lamb hide is thicker, the subject reserve thereof is greater, and conversely, the lamb hide has a density low or loose, so it is reserve less subject.

The hide reserve is related Karakul lambs and their constitution (Table 13).

Table 13. The hide reserve depanding on constitution of Modlovan Karakul lambs

Consti-tution	n	Hide reserve, %							
		Folds		Free		Tight		Insuffi-cient	
		num	%	num	%	num	%	num	%
Coarsely	4	-	-	2	50.0	1	25.0	1	25.0
Robust	809	238	29.4	446	55.1	125	15.5	-	-
Fine	163	61	37.4	90	55.2	12	7.4	-	-
V. fine	3	-	-	2	66.7	1	33.3	-	-

We found that most robust constitution lambs were provided free hide (55.1%), folds (29.4%) and less tight (15.5%). Most reserves have lambs hide with fine constitution. Among these are 37.4% of individuals with hide folds reserves, 55.2% - with free reserves and 7.4% of individuals with hide tight reserve.

As the number of lambs with coarsely and very fine constitution is very small, their hide reserve certain conclusions can not be made, but can only find that there is some tendency for lower reserve their hide to lambs with robust constitution and fine. This trend, in fact, is considered by coach to evaluation marks lambs.

The hides reserve is closely related to the general class of lamb (Table 14).

The data obtained in research is clearly observed that the lambs higher class (and elite class) reserves the hide is higher, and vice versa, the lambs hide of the lower classes reserve is limited.

Table 14. Hide reserve depending on the classes of Moldovan Karakul lambs

Lambs classe	n	Hide reserve, %			
		Folds	Free	Tight	Insufficient
Elite	160	40.6	52.5	6.9	-
Class I	315	24.8	60.0	15.2	-
Class II	144	12.9	57.8	28.6	0.7
Bad	7	-	42.8	42.9	14.3

Most reserves have lambs hide elite class, of which 40.6% have hide folds reserves, 52.5% were provided free and only 6.9 % have tight reserves hide. The smallest reserve lambs hide possesses bad. Most of them have tight reserves (42.9%) and insufficient (14.3%). With the increase in ranking lambs, lambs grow essentially share the book folds to 12.9% in batch II class lambs, up from 24.8% in the batch of class I and up to 40.6% in batch class lambs elite or 1.9 and, respectively, 3.1 times ($P < 0.001$). Along with this, decreases summary weight of lambs with low density and loose hide from lambs bad 57.2%, up from 15.2% in batch I and class lambs 6.9% in group lambs elite class, or 3.8, respectively, 8.3 times ($P <0.001$). Therefore, the higher the ranking is higher lambs, the hide is the largest book, and conversely, the ranking is lower lambs, and the reserve of their hide is less. Selecting permanent evaluation marks lambs reserve free hide folds can improve herd this important character.

Generalizing the results of research on the assessment of Karakul lambs hide features, in particular those of Moldovan type, we find that these and the conclusions drawn are in general consistent with the data of multiple researchers in the field (Adametz, 1927; Brădăţean et al., 2001; Kechawartz, 1958; Nicov, 1936; Taftă et al., 1997; Авсаджанов, 1968; Баратов et al., 1975; Диомидова, 1954; Дъячков, 1950; Иванов, 1964; Ильев, 1969; Кошевой, 1975; Рахматов, 1978; Юдин, 1964;et al.).

However, the above-mentioned peculiarities of Karakul lambs hide Moldovan qualities and their correlations with other features and characters of skin will help to guide and efficient selection for genetic improvement of populations of sheep hide after qualities.

CONCLUSIONS

The Moldovan Karakul lambs have a thicker hide compared with Asian Karakul lambs, the average skin thickness measured with cutimeter having the marks constituting 2.0 to 2.6 mm.

The features of lambs hide are correlative relation between each other and with a number of other characters, such as curls size, the type of curls, constitution of lamb, class of evaluation, age ewes-mothers at farrowing, etc.

The Moldovan Karaul lambs hide thickness have a positive phenotypic correlation with the size of their curls and age ewes-mothers at farrowing, and in negative correlation with hide density, hide reserve, twins, type of curls and general class of its own.

Moldovan Karakul lambs hide at the evaluation marks is suitable for dense (83.8% of individuals) and dense (12.8% of individuals), being in a positive phenotypic correlation with the type of curls and in negative correlation with thickness lamb hide and constitution.

The reserve of Karakul lambs hide at the evaluation, in the vast majority of free (from 46.1 to 72.0% of individuals) and folds (at 12.9 - 26.9% of lambs), being positive phenotypic correlation curls type, hide density and class of its own, and in negative correlation with the thickness of the hide and its own constitution.

REFERENCES

Adametz L., 1927. Uber die Herkunft der Karakulschafe Bocharas und die Entstehung der Lockenbildung am Lammvliese dieser Rasse. Zeitschrift fur Tierzuchtung und Zuchtungsbiologie. Band VIII, 1, Wien.

Brădăţan Gh., Chiorescu I., 2001. Influenţa furajării hiperproteice a oilor gestante Karakul de Botoşani asupra însuşirilor calitative ale pielii mielului nou născut. Simpozion Ştiinţific Jubiliar „50 ani de învăţământ superior zootehnic la Iaşi 1951 – 2001", Universitatea de Ştiinţe Agricole şi Medicină Veterinară „Ion Ionescu de la Brad", Iaşi, 192.

Buzu I., Zelinschi N., Evtodienco Silvia, 1996. Instrucţiuni de bonitare a ovinelor Karakul cu principii de ameliorare în Republica Moldova (în două limbi: Md şi Ru). Departamentul Edituri, Poligrafie şi Comerţul cu Cărţi al Tipografiei Centrale. Chişinău, 72.

Buzu I., 2001. Corelaţia lungimii corporale a mielului Karakul la naştere cu unele însuşiri de pielicică. Universitatea de Ştiinţe Agricole şi Medicină Veterinară din Iaşi. Facultatea de Zootehnie. Simpozion ştiinţific jubiliar „50 ani de învăţământ superior zootehnic la Iaşi", Iaşi, 172-173.

Buzu I., 2012. Tip de ovine Karakul Moldovenesc: teoria şi practica creării şi perfecţionării. Academia de Ştiinţe a Moldovei. ISBN 978-9975-4369-9-1, Tipogr. „Elena V.I.", Chişinău, 514.

Kechawartz M.N., 1958. La formasion et levolution de la boucle chez le foetus et lagneau Karakul. Annales de le Institut national de la Recherche Agronomique. Annales de Zootechnice. Paris, 7 (1), 25-68.

Nicov Th., 1936. Die Karakulzucht in Rumânien. z. Halle, 213.

Taftă V., Vintilă I., Zamfirescu Stela, 1997. Producţia, ameliorarea şi reproducţia ovinelor. Bucureşti, „Ceres", 525.

Авсаджанов Г.С., 1968. Развитие кожи и шерстного покрова новорожденных ягнят в зависимости от уровня кормления маток в суягный период.Биология кожи и волосяного покрова животных.Тезисы докладов МОИП, Москва.

Баратов Ю.А., Хидоятов Х., Рахматов Н., 1975. Особенности строения кожи ягнят жакетного смушкового типа в зависимости от размера завитка. Каракулеводство. Сборник трудов ВНИИК, вып. IV, Ташкент, 75-81.

Диомидова Н.А., 1954. Эмбриональное развитие кожи и шерсти у овец.«Известия АН СССР.Серия билогическая», №6.

Диомидова Н.А., Панфилова Е.П., Суслина Е.С., 1960. Методика исследования волосяных фолликулов у овец.Институт морфологии животных им.Северцова Академии Наук СССР.Москва, 38.

Дъячков И.Н. и др., 1950. Вопросы влияния различного кормления овец на развитие плода и на формирование каракульского завитка.Труды ВНИИК, вып.IV.

Закиров М., Каримов К., 1987. Смушковедение. Изд.«Мехнат», Ташкент, 191.

Иванов М.Ф., 1964. Овцеводство. Полное собрание сочинений, том 3. Москва, изд.«Колос», 15 – 26.

Иванов М.Ф., 1964. Сортировка черных каракульских смушков и ее научные основы.Полное собрание сочинений, том 3.Москва, «Колос», 376 – 398.

Ильев Ф.В., 1975. Крештереа оилор ын Молдова. Ед.«Картеа Молдовенеаскэ», Кишинэу, 1969, 88.

Кошевой М. А. Селекция и условия разведения каракульских овец.Ташкент, изд.«Фан», 247.

Плохинский Н.А., 1969. Руководство по биометрии для зоотехников.Москва, «Колос», 255.

Рахматов Н., 1978. Связь структуры кожи каракульских баранчиков с качеством смушка. Каракулеводство. Сборник трудов ВНИИК, вып. 9, Ташкент, 69-74.

Юдин В.М., 1964.К вопросу о конституциональных типах каракульских овец.В книге «Полное собрание сочинений» под редакцией Иванова М.Ф., т. 3, изд.«Колос», Москва, 526 – 529.

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GENEALOGICAL STRUCTURE OF MOLDOVAN KARAKUL TYPE SHEEP

Ion BUZU

Institute of Zoology of Academy of Sciences of Moldova,
MD-2028, Chişinău, str. Academiei, 1, Republic of Moldova, Phone: +373.22.73.98.09,
Fax: + 373.22.73.98.09, Email: izoolasm@yahoo.com

Corresponding author email: ionbuzua@gmail.com

Abstract

The purpose of this research was to study the genealogical structure and characterization of elite lines of basic herd of sheep Moldovan Karakul type. The research has been done on Karakul sheep from Experimental Section of the National Institute of Animal Husbandry and Veterinary Medicine, Maximovca, from Anenii Noi district. The qualitative assessment of lamb's skins was performed using evaluation methods in the second day after birth, according to the available Instructions on evaluation of Karakul sheep. Elite genealogical lines were created through stringent selection of rams that where founders and followers of the line. The main objectives common to all genealogical lines were: typical exterior of the Karakul race, high body mass at all stages of assessment, according to criteria for the type requested*, high milk production of rams mothers line significantly higher than the average herd. The specific objectives were: black line - excellent qualities of the skin, black colour and intense pigmentation; greyish line - medium or dark shade light greyish colour, with an excellent uniformity of staining required or appropriate; gray colour line - excellent gray colour and suitable expressed staining required. As a result of research and selection work has improved the genetics of herd type of Moldovan Karakul sheep, which resulted in forming a genealogical structure of three elite lines, differentiated by colour: black line Corpolent 7094, greyish line Delicat 2049 and gray line Buhar 7001. Each of these lines have common characteristics of selected characters, mainly, on the development of significant weight of the rams at birth 5.5 to 6.8 kg, at 6 months 40-47 kg and at 18 months 70-83 kg, an increased milk production of mothers founders and followers within 80-180 kg per lactation, the weight of lambs was increased in descendants elite and class I (80.8 to 86.8%) and also some specific characteristics of curls, particularly related to colour and colouring follicle sheath, such as blue marble - at the greyish colour, and gold, bronze and diamonds - at the gray colour. The proportion af the animals of each elite lines is quite significant genealogical distributed in the structure of the herd (20.5 to 37.4 %), which provides over a relatively long period, an increase effect in independent pure breeding- this way reducing the high risk of inbreeding.*

Key words*: genealogical lines, Karakul rams, sheep types.*

INTRODUCTION

In husbandry breeding at creation of new races, types intraracial, a special importance shall be given to the construction of the genealogical structure of the population, in particular at the stage of genetic typing and consolidation of the main characters and many of its qualities by means of increase in pure race-based lines and families (Борисенко, 1967). The structure problems of Karakul race, breed type, genealogical, flock (population) were described in the works of a string of researchers both classics of traditional from geographical regions with tradition in breeding this race (Гигинейшвили, 1976; Дъячков, 1980; Иванов, 1964; Кошевой, 1975; Юдин, 1943), as well as in other regions, relatively new (Adametz, 1927; Pecuta, 1938; Ле Ру Дж, 1975; Нел Дж, 1975), which was extended this world wide unique race.

For example, Иванов М.Ф., 1964, noted that increasing the sheep in the genealogical lines is important in establishing qualitative traits in progenity and subsequent application of crossbreeding to avoid negative impact of inbreeding. Researcher Дьячков И. Н., 1980, in describing the principles and methods of creating lines and new populations of sheep Karakul told that for new bloodlines, and those with common origin is characteristic a common provenience of elite interlinear, but last ones differ from first through a uniformity (typification) of morpho-productive characters and a constant transmission of hereditary descent. To create a new type of sheep Karakul

of Surhandaria, Гигинейшвили Н.С., 1976, started the selection with the creation of two genealogical lines of the elite on the basis of two remarkable Rams Halbai-4733 and Al-baş-22. Subsequently, selecting stew, grandchildren and great-grandchildren were created a string of other elite lines under this type.

In the breeding farm "Ulus" (Uzbekistan), Кошевой М.А., 1975, has consolidated a genetically population of black sheep with population over 20,0 thousand list, creating 6 genealogical lines of elite with different specific curls particularities.

According to information of Ле Ру Дж. П., 1975, as a result of the work of perennial selection and testing the Rams after their descent into the herd of sheep Karakul of the Experimental Station Neidam, Namibia (South Africa-West) was built upon the structure of the genealogical line of elite 4, which differed by some features of modelling, fibers drawing of the type and form of the curls, with the average body weight of lambs at birth 4.05 - 4.28 kg. Referring to the same flock, Дж Нел. А., 1975, mentions, that the sheep population of Neidam has a predominantly genetic impact (52%) of the genealogical structure of the race (African Karakul intraracial type) as a whole, because of the pressure for improvements carried out by providing breeding rams from elite genealogical lines obtained with inbreeding moderated after Rait (3.51-3.59%.

In the former Bessarabia, early research on the structure of a flock of sheep Karakul were undertaken (under the auspices of prof. A. Cardaş, director of the laboratory of Animal Husbandry of the Faculty of Agricultural Sciences in Chisinau) by agricultural-engineer Pecuta N., 1938, which has conducted an analysis of hereditary cattery „Oniţcani-Synadino", founded in 1883 by the importing a pure-bred of Karakul animals from the Emirate of Bukhara in 445 females and 22 males, at which, in the years 1928-1936, were imported from the farm 3 rams from Zootechnical Institute in Halle (Germany) and 1 ram from Farm of prof. Adametz, Vienna, Austria. In the structure genealogical of the flock, the author of the differentiated 4 lines of rams ("groups of families" according to the phrase of agronom Pecuta N.) the transmission characteristically with a "faithful" of one or more characters of their offspring. For example: the rams from line A to transmit well through heredity character extension of curls; B-line specific to shine; C-line for shape and type of curls; D-line for the resistance and elasticity of loops; line E-the grade of closing of the curls. The author concludes that "The structure of the herd or flock of sheep in recent years provide a visible progress in improving the productive qualities of animals. From this flock of Karakul sheep were exported in southern France, Bulgaria, Algeria and Portugal, which were acclimatized very well".

In Republic of Moldova, research and selection work on the construction of genealogical structure of Karakul sheep populations were performed Ильев Ф.И., 1957; Iliev Theodor V., 1992; Богданович Н.И., 1957; Вогданович Н.И. et al., 1983; Бузу И.А. et al., 1989. Further research and multiannual work of selection and genetic improvement of Karakul sheep (Buzu I., 1997, 2012) have been successfully completed, creating a new type of Moldavian Karakul sheep, approved by the State Commission of the Ministry Agriculture and Food Industry (Order no. 238 of 29.12.2007) and recognized by the State Agency for Intellectual Property like an invention (selection achievement) in animals (patent MD 3825 G2 2009.02.28).

In this context, the aim of this work was analyze of the structure genealogical and characterization of elite lines of basic herd of sheep type Moldovan Karakul.

MATERIALS AND METHODS

The researches have been conducted on Karakul sheep in the household of the Experimental Section of the National Institute of Animal Husbandry and Veterinary Medicine, Maximovca, Anenii Noi district. The assessing of skin qualities of the lambs was carried out by evaluation the day after the birth, according to the Instructions of evaluation with principles of sheep Karakul breeding in the Republic of Moldova (Buzu I. et al., 1996), with subsequent amendments and additions (Buzu I., 2012). Elite genealogical lines were created by exigent selection of rams both founders and followers of the line. To do this, from the annual generations of ram lambs,

destined at evaluation for breeding, were selected the most valuable individuals after its phenotypic characters and after the parents, being included in batch of the young rams in testing after descent qualities through biometric method of score (Buzu I., 2014). The creation of the genealogical line of elite was taken in consideration of the purpose and the targets so common to all lines, as well as specific to each line individually.

The main objectives were:

- to create black line: exterior the typical for the Karakul race, higher body weight at all stages of assessment, according to the criteria of the type requested, excellent qualities skin, black and intense pigmentation, mother's milk production significantly greater than the average of the flock;
- to create greyish line: exterior the typical for the Karakul race, higher body weight at all stages of assessment, according to the criteria of the type requested, excellent or appropriate qualities skin, greyish colour medium or dark shade, the staining required (blue, marble, pearl, silver, gray) with excellent uniformity or suitable, mother's milk production significantly greater than the average flock;
-to create gray the colour line: exterior the typical for the Karakul race, higher body weight at all stages of assessment, according to the criteria of the type requested, excellent qualities skin, excellent colour and suitable expression, staining required (gold, silver, diamond, bronze), the mother's milk production significantly greater than the average flock with certainty at least one threshold of the theory of probability forecasts without error ($P < 0.05$);

The data obtained were statistically processed experiences using computer software "STATISTICS - 6" and appreciated their certainty, according to the statistics, recent various methods, after methods of Плохинский H. A., 1969.

RESULTS AND DISCUSSIONS

At the early stages of creation of a new type of sheep have been using a number of different enhancers rams genealogical lines of Asian origin from various breeding farms, as they were: „Karnab", „Kenimeh" „Gagarin", „Nurata" and others. Following, in the flock were breeding rams own use including with half breed origin, which correspond to the standard-purpose type requested.

Since 1997, of the many genealogical lines and their ramifications, exiting in herds, have been identified three lines of perspective that works have been carried out by the creation and consolidation of the new lines of their own breeding sheep. As the founders, were elected 3-copulent breeding rams, top-ranking elite, with curls type requested: jacket, costal and flat and increased milk production of mothers. Productive characters and hints of the outside of the line of the high and meet the demands were made of breeders.

For foundation lines were selected by breeders rams exterior features well manifested and typical Karakul race and also so as founders and the successors of lines were preferred hornless rams (polled) or with small horns, rudimentary.

The black line Corpolent 7094. Founder of the ram line was selected without horns, with the identification 7094, with black pigmented intensive elite class, with costal type curls. Body weight of this ram, 2.5 years of age was 100 kg (Figure1).

Figure 1. Ram founder of the black line Corpolent 7094

During the use of this ram within 4 years were obtained 146 lambs, from which 131 heads the elite class I (Table 1), being regarded as having the qualities of descent, breeder of grade I and grade II. In the period 1999-2000, as followers of this line, were selected sons of founder, the rams with identification 9206 (Figure 2) and 0230 (Figure 3).

Table 1. Characteristics progeny obtained of black line Corpolent7094

Ser. No.	Regist number	Year of birth	Class, type of curls	Name of kinship	Year of test	N	Incl. elite+class I		Value of breed
							heads	%	
1	7094	1995	El. costal	Founder	1997	146	131	89.7	*II gr. br* ***
2	9206	1999	El. jacket	Son	2001	29	25	85.2	*Ord. br* **
3	0230	2000	El. jacket	Son	2002	28	27	96.4	*Rel. br**
4	**1226**	2001	El. flat	Son	2005	219	181	82.6	*Rel. br**
5	**1129**	2001	El. jacket	Nephew t	2005	131	116	88.5	*I gr. br* ****
Total per line				1998 - 2005		553	480	86.8±1.4	t_d=6.15
Total perflock				1998 - 2005		2809	2160	76.9±0.8	P<0.001

Remark: Rel. br – breeder relatively; Ord. br** - ordinary breeder; II gr. br*** second degree breeder; I gr. br**** - first degree breeder.*

Figure 2. Son nr. 9206 follower of the Figure 3. Son nr. 0230 follower of the black line 7094 black line 7094

Table 2. Feature progeny obtained from greyish line Delicat 2049

Ser. No	Regist number	Year of birth	Class, type of curls, coloration	Name of kinship	Year of test	N	Incl. el.+class I		Value
							heads	%	
1	2049	1995	El. gr. bluish	Founder.	1997	263	208	79.1	*II gr. br*
2	9085	1999	El. gr. marble	Son	2004	140	105	75.0	*Ord. br*
3	**0286**	2000	El. gr. bluish	Son	2005	187	157	84.0	*Ord. br*
4	**1237**	2001	El. gr. bluish	Son	2005	185	161	87.0	*Ord. br*
5	**2127**	2002	El.cost. marble	Nephew	2005	130	116	89.2	*Ord. br*
Total per line				1998-2005		905	747	82.5±1.3	t_d=3.66
Total per flock				1998-2005		2809	2160	76.9±0.8	P<0.001

Figure 4. Ram founder of greyish line Delicat 2049

a) b) c)

Figure 5. Moldovan Karakul lambs from greyish line Delicat 2049 a) Lamb 9085 greyish-marble; b) Lamb 0286 greyish-bluish; c) lambskin greyish-bluish to the right.

Table 3. Feature of progeny obtained from grey line Buhar 7001

Ser. No	Register number	Year of birth	Class, type of curls, coloration	Name of kinship	Year of test	N	Including elite+class I		Value
							head	%	
1	7001	1997	Elite flat grey	Founder	1999	196	156	79.6	*II gr. br*
2	0285	2000	Elita flat diamont	Son	2001	44	36	81.8	*I gr. br*
3	0130	2000	Elite jacket grey	Son	2002	44	38	86.4	*I gr. br*
4	0267	2000	Elite flat grey	Son	2003	74	56	75.7	Neutral
5	1054	2001	Elite costal grey	Son	2003	54	44	81.5	*Ord. br.*
6	**2501**	2002	Elite flat grey	Nephew	2005	41	36	87.8	Neutral
7	**2500**	2002	Elite flat grey	Nephew	2005	47	38	80.8	*Ord. br*
Total per line				1999-2005		500	404	80.8±1.8	t_d=2.62
Total per flock				1999-2005		2421	1826	75.4±0.9	P<0.01

Figure 8. Moldovan Karakul lambs from genealogical line grey Buhar 7001

Table 4. Milk production of mothers and daughters of rams from Buhar 7001 line

Ser. No	Regist. number of ram	Year of birth	Name of kinship	Milk production		
				mother, kg	daughters of ram	
					N	M ±m, kg
1	7001	1997	Founder	180	28	79.4 ± 3.7
2	0285	2000	Son	125	15	78.3 ± 4.2
3	0130	2000	Son	80	12	74.5 ± 2.8
4	0267	2000	Son	81	11	80.4 ± 5.1
5	1054	2001	Son	84	-	-
Total per line				110.0±24	66	78.4 ± 4.6 (t_d = 2.2; P<0.05)
Total per flock					267	67.6 ± 2.0

They, as the founder, were the elite class, with curls type requested. The curls were very long and wonderfully shaped. The fibers had a great silky and luster. After the exterior, these rams were black-intense, hornless, well developed, with characters well cast for new Karakul type breed. The mother of the first ram, sheep nr.1257, had an increased productivity of milk, for 116 kg, during the whole lactation. The result of the testing after these lads progeny qualities were appreciated as soil improvers. The share of elite and class I lambs in their descent was 85.2 – 96.4%.

In 2001, from the progeny, as followers, 2 rams were selected, among which-the son, with the identification 1226, and the grandson of the founder, with the identification 1129. On new type approval Moldovian Karakul sheep, these rams (no.1226 and no.1129) were alive and active in the flock. Both rams had the qualities of excellent lambskin and black colour-intense. On the surface of their lambskin were large and very long curls, which formed a drawing of concentric parallel, the type of fibers was silky excellent and high-gloss.

After pace of development body, both rams were precocious. For example, son nr. 1226 at the age of 6 months have body mass 46 kg, at 1.5 years - 80 kg, and adulthood (2.5 years) body weight has reached the level of 103 kg. From this ram have been obtained 219 lambs,

including 181 heads, or 82.6%, descendants elite and I class. From the founder's grandson, with the registration 1129, were obtained from 131 lambs, of which 88.5% - higher ranking (elite and class I).

Black line on average, since 1998, the male line, followers was obtained a total of 553, including descendants 480 of descendants elite and class I. Average rate of lambs seek, high ranking (first class and elite), the black line is 86.8 ± 1.4 %, which significantly exceeds the average flock with 9.9 % ($P < 0.001$).

Selection works with this line continued to the date of approval of the new type of sheep. Then, in the flock were select 3 grandchildren followers line-class elite young rams, nr. 5366 and nr. 5337, which have been using the service since the age of 18 months, and grandson with the registration 7248, elite class, with curls flat-type, was included in the test after their descent from the age of 6 months.

In the structure of the herd or flock, the sheep share the full-bodied 7094 constitutes 34.7%.

The greyish line Delicat 2049. Based on this line of the ram founder registration 2049, greyish colour, blue coloration (*Fig*ure 4). The rams founder was of elite class and has a close jacket type curls. The body conformation was smooth and specifies where the name appeared Delicat. Characters exterior of the ram were typical Karakul for the race. It was rather corpulent and hornless, have robust constitution. Body weight, at age of 2.5 years, was 100 kg. Since 1997, was tested after the quality of descent, and recognized by multiple times as a breeder (*Table* 2).

From the founder of the line have been obtained a total of 263 lambs, including 208 thousand elite and first class.

The qualities of lambskin of the progeny were excellent (*Figure 5*), coloration marble and bluish. On average, the period of use at this breeding ram lambs share of elite and first class called type was 79.1%. According to the results obtained in the test, after the qualities of descent, was praised as a valuable and awarded in the category of breeder second grade.

In 1999, the progeny of the founder's story has been selected son-follower line with registration 9085 (*Figure 5, point a)*). He, like his father, was greyish in colour and possesses the requested colour-marble with excellent uniformity, silkiness and fibre-excellent gloss. After ranking elite class, was the size of large curls with costal curls type. Black and white fibres were very short and virtually equal, 7, and respectively 8 mm. Silkiness and gloss were excellent fibers. After the body was development of early type. At the age of 6 months had 43 kg body weight at the age of 1.5 years has reached 83 kg weight.

The body weight into adulthood was 107 kg. This ram had received a total of 140 people, including 105 descendants elite and class I. Based on the results of testing after the qualities of descent, was praised as a breeder.

In 2000, the founder of the progeny was selected over a valet-line, ram works with the registration 0286 (*Figure 5, point b)*). He was at birth a great body and development weighed 5.5 kg. In the autumn, at the age of 6 months had 44 kg, at the age of 1.5 years reached the weight of 73 kg and 100 kg into adulthood. This ram is greyish in colour and coloration bluish. The coloration has an excellent uniformity. On the surface of the skin were scattered tubular long and bob curls, forming an excellent drawing of parallel-concentric type. From this ram during use in breeding, have obtained 187 descendants, including 157 lambs elite and class I. Share of higher ranking lambs was at this ram 84.0%. After the test results, he was awarded the category of breeder.

In 2002, from the progeny selected for breeding as the followers of a grandson of the founder, ram nr.2127 elite class, with costal-type curls, new coloration-marble.

After the exterior parameters, comply with new type. He is a development hornless body good. On the surface of the skin, at evaluation, have been recorded in coastal and long tubular curls, which formed an excellent model of parallel-type scale. The fibers were excellent and luster silkiness. Personal development was pretty good. At the age of 6 months has reached 39 kg body weight, 1.5 years have 68 kg and adulthood had 88 kg. After testing this ram quality progeny, he was assigned to the category of breeder. Of the 130 descendants obtained, 116 were heads of elite and class I. The share of high ranking lambs was 89.2 %, which exceeded the average of the flock with 12.3 % ($P < 0.001$).

At the date of approval of the type new Moldovan Karakul, in the flock were alive and is used in breeding rams with registration 0286, 1237 and 2127. As a prospective followers, two rams were selected, grandchildren, line numbers 5205 and 5988 projects,
both from the elite class and the type curls of jacket, bluish coloration, with excellent luster and silkiness. After they meet the requirements of exterior standard-aim, hornless, well developed and represents a potentially valuable genetic. According to the genealogical structure of sheep flock, Delicat line 2049 occupies a share of **31.9%.**

The grey line Buhar 7001. This line was founded on the basis of ram with the registration 7001, born in 1997. The founder was grey colour and goldish coloration, elite class and has a flat-type curls. After the development of the outer body, body weight complied with the standard-purpose. Hornless, was robust and peculiarities characteristic of the Karakul race type. After conformation and development was full bodied. At the age of 3.5 years reached 94 kg body weight. At the same time, with special colour and requested, in this line, have selected ewes with increased milk production. Thus, the mother of the founder of the line had increased milk productivity of 180 kg per lactation. In the result of the matings over many years, from this ram has been obtained 196 descendants, including 156 heads or 79,6% were elite and first class (*Table 3*).
Improvement category, obtained as a result of testing after their descent has been breeder second grade.
In the period 2000-2005, in this line, as followers, were selected for breeding 4 rams-sons grey colour, with registration numbers 0285, 0130, 0267, 1054 and 2 grandchildren rams - 2500 and 2501 (*Figure 6 and 7*).
For example, ram 0285, at birth, had body mass 6 kg, and at the age of 6 months has reached mass of 40 kg. From him was obtained 44 offspring, of which 36 heads, or 81.8 % were elite and first class. Ram with nr. 0130 elite class have curls type gray coloured jacket and goldish coloration. From this ram, yielded a total of 44 offspring, of which 38 or 86.4 % were head and elite class I. Followers of the line had traits similar to the line the founder. They had good body development and meet the requirements of type. After breeding value, this ram was awarded the degree breeder category I.

Figure 6. Ram nr.2500, follower of grey line Buhar 7001

Figure 7. Ram nr.2501, follower of grey line Buhar 7001

The ram with the registration number 0267, in mature age, at weighing in autumn 2005, reached 99 kg body weight. From followers obtained (67 heads) from this ram, the majority, 56 cap, or 75.7%, where superior class, elite and first class.
After the qualities of descendant, this ram was recognized as the breeder of class I. In 2002, in this line, as were selected from followers growing grandchildren of the grey colour founder, with the registration number 2500 and 2501, elite class, both with flat curls type. From this rams where obtained 88 descendants, from which, 74 heads, or 84.1 % where elite and class I, to the category as breeders. Most of the lambs obtained from this lambs superior, curls very special, the follicle was shine and extremely silky. The predominant follicle sheath surface curls shaped long waves, which formed an excellent modelling concentric or parallel-scale (*Figure 8*).
The grandson of the founder, ram nr. 2500 in the breeding had the qualities of the lamb skin. After the breed value of their descendants, they classified period of use, have obtained a total of 47 descendants, of which 38 heads or 80,8 %, or elite and class I. On the time of approval

new Moldovian Karakul sheep type, both rams, no. 2500 and 2501, were alive and were used in the flock. From the progeny of ram 2500 since 2005 was selected and used in the flock for testing young ram nr. 5393 elite, with the jacket curls type, with big and long curls, with silky and excellent gloss. At birth the ram had body weight of 6.8 kg! And at the age of 6 months has reached 45 kg! At the age of 1.5 years, the ram had body weight of 70 kg. The progeny of this line, the new type-approval year of sheep, ram was selected with the registration No 7975 costal elite, which has reached the age of 6 months body weight 47 kg.

Subsequently, this ram will be tested after the qualities of descent. In the total on line Buhar 7001 were obtained 500 descendants, of which 404 heads or 80.8 ± 1.8% were elite and class I. After the share of elite and class I individuals, these descendants are significantly above average on the flock with 5.4% ($P < 0.01$).

It is worth mentioning that the ewes of this line have increased slightly more in skills milk production. For confirmation, we present below (Table 4) data about productivity of milk mothers line Buhar 7001 rams and their daughters.

From the table, we see that mothers of all rams Buhar 7001 line have increased milk yields and is on average 110.0 ± 24 kg per lactation. Followers of line, daughters also have increased milk productivity equal to 78.4 kg, which exceed the average throughout the whole flock with 16.0% ($P < 0.05$). The higher production of milk had no daughters of rams 0267, which constituted in average per lactation 80.4 ± 5.1 kg milk.

According to the data obtained, it can be concluded that the sheep in line potentially possess genetic 7001 Buhar valuable, so after the original color of the sheath of the follicle and the quality of skins, and after bodily development skills (meat production) and milk production. In the structure of the flock, the sheep grey color line Buhar 7001 share total is **20.5%.**

Most obviously, the weight of the animal genealogical lines mentioned above in the genealogy researched population structure can be viewed in the chart (Figure 9).

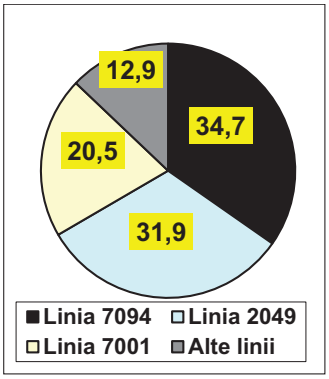

Figure 9. Share of animals in the population bloodlines

In the diagram we see that the largest share in the genealogical structure of populations occupying ancestral animals of the black line 7094 (34.7%) - and typical colour for the race traditional Karakul from all geographical regions, followed by the greyish line 2049 (31.9%) and those from the grey 7001 (20.5%). The rest of the population they occupy the other genealogical lines, showing no particular object selection.

There are multiple outcomes of external characteristics of rams founders followers of genealogical lines and elite, mention that, in addition to personal development and typical forms for most of the race, the rams were hornless (without horns), and only some individuals have had rudimentary horns.

CONCLUSIONS

Genealogy of the type structure of sheep Moldovan Karakul consists of three elite lines differentiated by colour: black line Corpolent 7094, greyish line Delicat 2049 and the grey line Buhar 7001. Each of these lines have common characteristics of selected characters, in particular, on the development of large body of rams from birth 5.5-6.8 kg, 6.0 months 40 – 47 kg, to 18 months 70-83 kg, increased milk production of mothers and followers within the limits of 80 -180 kg, the weight of the descent of lambs elite and class I (80.8 - 86.8%) and, at the same time, some features of curls, and in particular, the colour and the coloration of the sheath follicles, such as: bluish, marble - the

greyish colour, and goldish, bronze, and diamond - at the grey colour.

The share of each elite line is significant enough in the genealogical structure of the flock (20.5 - 37.4%), which provides, for a long period, a relatively independent increase in breeding pure bred without applying high degrees of inbreeding.

REFERENCES

Adametz L., 1927. Uber die Herkunft der Karakulschafe Bocharas und die Entstehung der Lockenbildung am Lammvliese dieser Rasse. Zeitschrift fur Tierzuchtung und Zuchtungsbiologie. Band VIII, 1, Wien.

Buzu I., Zelinschi N., Evtodienco Silvia, 1996. Instrucţiuni de bonitare a ovinelor Karakul cu principii de ameliorare în Republica Moldova (în două limbi: Md şi Ru). Departamentul Edituri, Poligrafie şi Comerţul cu Cărţi al Tipografiei Centrale. Chişinău, 72p.

Buzu I., 1997. Particularităţile metodelor de ameliorare, aplicate la crearea tipului de ovine Karakul Moldovenesc. Asigurarea ştiinţifică a sectorului zootehnic şi medicinii veterinare (Materialele conferinţei jubiliare a INZMV din 04 octombrie a. 1996). Chişinău, p. 64-65.

Buzu I., 2012. Tip de ovine Karakul Moldovenesc: teoria şi practica creării şi perfecţionării. Academia de Ştiinţe a Moldovei. ISBN 978-9975-4369-9-1, Tipogr. „Elena V.I.", Chişinău, 514 p.

Buzu I., 2014. Genotypic assessment of Karakul rams by fur skin qualities of progeny. Univrsity of Agronomic Sciences and Veterinary Medicine of Bucharest. Scientific papers. Series D. Animal Science. „CERES" Publ. House. Vol. LVII, Bucharest, p. 15-24.

Iliev T.V., 1992. Ameliorarea animalelor. Editura „Universitas", Chişinău, 220 p.

Pecuta N., 1938. Analiza ereditară a crescătoriei de oi rasa Karakul „Oniţcani-Synadino". Extras din lucrările primului Congres al crescătorilor de oi Karakul, Karakul x Ţurcană şi brumării, ţinut în Chişinău la 12-13 fabruarie 1938. Monitorul Oficial şi Imprimeriile Statului, Chişinău, p. 3-7.

Ursu Elena, Bosînciuc S., Ursu S., 1997. Cercetări privind structura genetică şi însuşirile morfo-productive ale ovinelor de culoare sur din rasa Karakul de Botoşani. Asigurarea ştiinţifică a sectorului zootehnic şi medicinii veterinare. (Materialele conferinţei internaţionale jubiliare a INZMV din 04 octombrie 1996). Chişinău, p. 78.

Богданович Н. И., 1957. Выведение молдавского каракуля в колхозах Згурицкогорайона. Труды Кишиневского с-х института, т. XIV, с. 109-133.

Богданович Н. И., Бузу И. А., Зелинский Н. А., 1983. Высокопродуктивное стадокаракульских овец. Информационный листок №54. Сельское хозяйство. Кишинев4 с.

Борисенко Е.Я., 1967. Разведение сельскохозяйственных животных. Изд. «Колос», Москва, 463 с.

Бузу И. А., Зелинский Н. А., Пинтяк М. А.и др., 1989. Селекционно-племенная работа с каракульскими овцами в племсовхозе им. Котовского Кэинарского р-на Молдавской ССР. Кишинев, «Молдагроинформ-реклама», 49 с.

Гигинейшвили Н. С., 1976. Племенная работа в цветном каракулеводстве. Москва, «Колос», 190 с.

Дъячков И. Н., 1980. Племенное дело в каракульском овцеводстве. Изд. «Фан», Ташкент, 163 с.

Иванов М. Ф., 1964. Овцеводство. Полное собрание сочинений, том 3. Москва, изд. «Колос», с. 15 – 26.

Ильев Ф. В.,1957. Методы скрещивания, применяемые при выведении молдавского Каракуля, и получунные результаты. Труды Кишиневского с.-х. Института им. М. В. Фрунзе, том XIV, Кишинев, с. 25-108.

Кошевой М. А., 1975. Селекция и условия разведения каракульских овец. Ташкент, изд. «Фан», 247 с.

Ле Ру Дж. П., 1975. Генеалогические линии баранов на Опытной станции Нейдам. В сб. Каракулеводство за рубежом. Изд. «Колос», Москва, с. 142-152.

Нел Дж. А., 1975 Исследования по разведению каракульских овец в Юго-Западной Африке. В сб. Каракулеводство за рубежом. Изд. «Колос», Москва, с. 75-104.

Плохинский Н. А.,1969. Руководство по биометрии для зоотехников. Москва, «Колос», 255 с.

Юдин В. М., 1943. Опыт племенной работы с черными каракульскими овцами в племхозе «Кара-Кум» Узбекской ССР. Изд. ВНИИК, Самарканд, 167 с.

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TESTING OF THE BEE QUEENS BY THE QUALITIES OF DESCENDANTS

Valentina CEBOTARI, Ion BUZU, Olga POSTOLACHI, Olesea GLIGA, Valeriu TODERICI

Institute of Zoology of Academy of Science of Moldova (ASM)
1, Academiei Street, MD 2028, Chisinau, Republic of Moldova,
Phone: +373.22.73.98.09, Fax: + 373.22.73.98.09,
Email: izoolasm@yahoo.com

Coresponding author email: valentinaceb@yahoo.com

Abstract

The aim of the research was the identification genotypic methods for estimating the value of bee queen improving, by their testing after qualities of descendant. The researches was conducted on the Apis mellifera Carpathian bee families, have been grown at the experimental apiary of the Institute of Zoology of the Academy of Sciences. The apiary is located at stationary in a clearing at the edge of the forest. The main melliferous sources in this area are white acacia, linden and wild flora, inclusive yellow melilot. In these experiments were genotypic tested five bee queens after qualities of descendant. For this, have been formed 5 similar batches of bee families (each10 families in every batch), in which, at one time was changed old queens and implanted queens young - daughters of one of the queens - mothers supposed testing. The value of improvement queens-mothers was assessed by comparison averages method morph- productive characters of daughters bee families with the average for the entire apiary, calculating the absolute and relative difference of the averages for each character (feature) in part. At the daughter's families were studied following morphs – productive characters: queen prolificacy, family power, resistance to disease, brood viability and honey production. The research results have shown that according of testing 5 queens after qualities descendants, through the method of comparing with average apiary, have been identified 2 queens which improve honey production. After other morph- productive characters studied, the tested queens have a neutral improvement value. At least in the studied population, it was not identified universal - ameliorative queen of all morph - productive characters. Research result have shown, that phenotypic performance of previously selected queens after their qualities and their families-parents, does not guarantee the improvement value without testing them after qualities of descendant.

Key words*: testing, genotypic, queens, qualities, descendants.*

INTRODUCTION

In beekeeping, queens role in the genetic improvement of bee populations is enormous. This is due to their reproductive biological peculiarities, expressed by extraordinarily high prolificacy and relatively short duration of successive generations (Ruttner,

1980; Siceanu, 2012; Totzek, 2013; Билаш, 1991; Гайдар, 2003; Контев, 1989; Кривцов, 1987). The queen carryng of the entire diploid set of chromosomes, transmitted by heredity in descendents its genetic qualities and morpho- productive abilities. At the apiaries of material genitor multiplication, from a queen of breeding family, in a beekeeping season can be taken thousands of eggs or larvae a day for directed growth of daughter queens with relatively predicted breeding value. Therefore, genotypic qualities of queens from maternal breeding families depends of value phenotypic daughter – queens, both those selected for renewal of queen effective to own apiary and creating new elite bloodlines, and those widely disseminated in third apiaries for implantation in bee families and genetic improvement of populations. Thus, the breeding queens have a significant impact on the development levels of bee families morph - productive characters from the entire population of the apiary, line, ecotype, race as a whole. Therefore, selecting the most valuable breeding queens and their intensive use for sampling and multiplication of genitor beekeeping material has a particularly high importance.

In zootechnics, in general, it is known, that with phenotypic selection method of reproduction after their performance and of ascendants, safer is method of their genotypic selection after qualities of descendants.

In beekeeping, the methods of genotypic selection of reproducers have their own specific after descendants' qualities. Their appreciation after descendants' qualities is carried out, through the maternal line.

According to the research by Билаш (Билаш, 1991), it must to perform testing remarkable queens after qualities of descendants, from each of them must be assessed each 30 families - daughters. The daughters - queens' performance can be compared both with their contemporaries and average indicators apiary. The main morph-productive character, after which is estimated performance of families - daughters is honey production.

The researcher Totzek, 2013, in instructions drawn up by them, for testing queens descendants after qualities in Pskov Region (Russia), recommends formation of similar lots of families - daughters by at least 8 families from each queen being tested.

After communications of Кривцов, 1987, in Austria, the system of selection and reproduction in apiculture is conducted by the Austrian Beekeepers Union. Under its guidance, annual are involved in testing of bee queens after qualities of descendants about 1500 - 2000 families - daughters. The remarkable queens with breeding mother families are transmitted for testing at the particular apiaries, which assesses their genotypic qualities after manifestation morpho –

productive characters of 5-15 families - daughters.

According to the information of Вайс, 1982 (cited by Кривцов, 1987), in the Germany bee queens are evaluated at the three test points, after qualities of descendants. In each of these points are tested simultaneously, 10 remarkable queens-mother at the effective of 10 families -daughters from each queen – mother.

In Romania, according to Dragan, 1984, (quoted by Кривцов, 1987), annual, at the district testing apiaries is identify queens - recordist after honey production, profilacity, gentleness, behavior on the combs, tendency swarming etc. This activity is conducted by Research Institute for Apiculture. The queens highlighted by the characters complex are transmitted at the elite apiaries for their using in the improvement of new lines and crossbreeds.

US beekeeping program selection according to business firms' Dadant & Sons "and" Baton Rouge "(cited by Кривцов, 1987) provides production and conservation of inbred queens lines for their use in cross interline producing. The remarkable breeding queens from cross are distributed for testing by the beekeepers participating at the Complex Program of Selection and Amelioration in Beekeeping. The most valuable queens becomes sources for obtaining queens daughters and drones for the reproduction of the subsequent generation. This material of breeding is returned to companies to achieve commercial cross - breeds.This cooperation is based on close contacts of company with selector - beekeepers.

Generalizing these limited data, we conclude that the growth problems of reproductive - queen, selection and assessment of their value of breeding is a current problem, studied and solved in different countries at different levels.

In the Republic of Moldova, in the former Soviet system, driven growth of the apiaries of breeding queens fell apart at the end of the twentieth century. Currently, there are only two breeding apiaries where grow the queens on the basis of selection. The rest, to all other apiaries, the queens are grow by reproduction methods and clandestine, in the rarest cases, based on the phenotypic selection, but in most cases without any selection. At present, there are only two apiaries of breeding where grow queens based on the selection.

In this context, the aim of this paper was to highlight and mediate of genotypic methods for estimating the value of improving of bee queen by their testing after qualities of descendants.

MATERIALS AND METHODS

The research was conducted on the *Apis mellifera carpathian* bee families, increased at the experimental apiary of the Zoological Institute of the Academy of Sciences of Moldova. The apiary

is located at the stationary in a clearing of the forest, near its edge. The main melliferous sources in this area are white acacia, linden and wild flora, including yellow melilot.

In special experiments were conducted research for genotypic testing of 5th bee queens after qualities of descendants. For each queen being tested, were formed similar batches of bee families (10 families in each batch), where, in early June, the same day (02 June 2014), in each family of bees, was changed the old queen and implanted by a young queen, the daughter of one of the 5 queens of families breeding batch, donor of genitor material. The next day, too, were exchanged with young queens and other queens from families when left outside of the apiary experimental batch. Next, the bee families of queens-daughters from all experimental batches were maintained under the same conditions as entire apiary.

The amount of improvement queens-mothers was assessed by method of comparison the averages morph-productive characters of bee families - daughters with the average for the entire apiary (Iliev, 1992), calculating the absolute and relative difference of the averages for each character (qualities) in part. At the daughters families were studied following morph – productive characters: queen prolificacy, family power, resistance to disease, brood viability and honey production. Determining the level of morph-productive characters development by bee families was carried, according to the methodology developed by us (Cebotari, 2010) for Norma Livestock, regarding breeding of bee families, the growth and certification of genitor beekeeping material, approved by Government Decision no. 306 of 28.04.2011 (OJ no. 78-81 of 13.05.2011, art. 366) (Livestock standard, 2011).

The data obtained in experience were statistically processed using computer software "STATISTICA - 6" and evaluated their certainty, according to variation biometric statistics, by methods of Плохинский, 1969.

RESULTS AND DISCUSSIONS

The research results showed that after the value of morph - productive characters –descendants - families (families-daughter), most queens, included in the test were attributed at the *Neutral* category of improvement. However, the daughter's families of some queens were ascertained and some peculiarities of morph - productive characters development (Table 1).

We want to mention, that the level of development of morph - productive characters at the families - daughter was on the whole, quite high, which corresponds to the requirements, submitted to bee populations from breeding apiaries.

Thus, **prolificacy queen**-daughters of mothers tested, varied within 1693 - 1796 eggs / 24 hours. The

highest prolificacy (1796 ± 47 eggs) was registered at the queens daughters of Queen 49R.

The lowest prolificacy showed queens daughters of Queen 22R, is why, it has been attributed to the category of *Reductive-relative* of this important character. The qualifier "relatively" was related, because the negative deference of the average level of development of this character in queen's daughter, compared with the average of the apiary did not have a significant threshold of certainty, according to the probability theory of forecasts without error after Student (Плохинский, 1969).

After value of prolificacy improvement, the other queens-mothers supposed tested, and therefore the daughter queens, had at first glance, slightly higher level, compared to the average of the apiary, were assigned to the *Neutral* category, because the significant differences at this character, has not been established.

Table 1. The test results of bee queen after qualities of descendants (daughters)

Nr. d/o batch	Nr. registration queen	Families-daughters, N	Average of character, M ± m	Difference from the average of apiary, d	t_d	Category of ameliorative
Qeen prolificacy, eegs/24 hours						
1	19R	10	1794 ± 40	+60	1.40	Neutral
2	34R	10	1776 ± 44	+42	0.90	Neutral
3	49R	10	1796 ± 47	+62	1.26	Neutral
4	21R	10	1783 ± 38	+46	1.13	Neutral
5	22R	10	1693 ± 30	-41	1.22	Reductive-relative
Average of apiary		80	1734 ± 15	x	x	x
Family power, kg						
1	19R	10	3.21 ± 0.04	+0.04	0.89	Neutral
2	34R	10	3.20 ± 0.03	+0.03	0.75	Neutral
3	49R	10	3.17 ± 0.04	0.00	0.00	Neutral
4	21R	10	3.18 ± 0.04	+0.01	0.25	Neutral
5	22R	10	3.12 ± 0.06	-0.05	0.83	Reductive-relative
Average of apiary		80	3.17 ± 0.02	x	x	x
Diseas resistance, %						
1	19R	10	91.8 ± 0.5	+0.2	0.37	Neutral
2	34R	10	92.0 ± 0.4	+0.4	0.89	Neutral
3	49R	10	91.8 ± 0.4	+0.2	0.44	Neutral
4	21R	10	92.0 ± 0.3	+0.4	1.11	Neutral
5	22R	10	92.1 ± 0.2	+0.5	1.79	Ameliorative-relative
Average of apiary		80	91.6 ± 0.2	x	x	x
Broods viability, %						

1	19R	10	93.2 ± 0.5	+0.9	1.70	Ameliorative-relative
2	34R	10	92.9 ± 0.5	+0.6	1.13	Neutral
3	49R	10	92.8 ± 0.5	+0.5	0.92	Neutral
4	21R	10	92.6 ± 0.5	+0.3	0.57	Neutral
5	22R	10	92.4 ± 0.5	+0.1	0.19	Neutral
Average of apiary		80	92.3 ± 0.2	x	x	x
Honey production, kg						
1	19R	10	53.97 ± 2.70	+3.79	1.35	Neutral
2	34R	10	55.58 ± 2.47	+5.40	2.09	Ameliorative-ordinary
3	49R	10	56.02 ± 2.57	+5.84	2.18	Ameliorative-ordinary
4	21R	10	54.10 ± 2.42	+3.92	1.55	Neutral
5	22R	10	52.19 ± 1.80	+2.01	1.03	Neutral
Average of apiary		80	50.18 ± 0.76	x	x	x

Given the fact, that the queen prolificacy decisively determine the amount of copped brood, it has a decisive impact on the development of family power. Through these connections, the development of power daughters - families of tested queens had a similar picture at the prolificacy.

After improvement of the power value families, most queens included in the test were assigned to the N*eutra*l category, because significant differences, at this character, compared to the average of apiary, have not been established. However, the highest power level was recorded at the families – daughter of the queen 19R and constituted 3.21 ± 0.04 kg. The lowest power was recorded in families - daughter of the queen 22R and constituted 3.12 ± 0.06 kg. After the development level of the families - daughter power, this queen was attributed to the *Reductive - relative* category, because gives up, and therefore insignificant, the level of development of this character on average on the apiary.

Resistance to diseases of bee families both from the experimental batches, as well the entire apiary, was at a fairly high level, and ranged within average from 91.6 to 92.1%.The highest level of disease resistance was recorded in families - daughters of the queen no. 22R. Resistance to diseases families daughter of the queen has had a clear tendency to be higher, compared to the average on the apiary, 0.5 or 0.5% absolute units (td=1.79; P<0.1). After improvement value of this character, queen no. 22R was assigned to the category improvement-relative of disease resistance character. Families - daughters of the other queen tested showed a level of development close to the average level of the apiary, so with some improving trends. After the improvement value of this

character, the other 4 bee queen tested after qualities of descendants were assigned to the *Neutrals* category, since the differences between the level of development of this character at the families – daughters and entire of the apiary were not significant. More obvious, the level of development morph-productive characters of families - daughters, compared with the average of the apiary, can be viewed in the histogram (Figure 1).

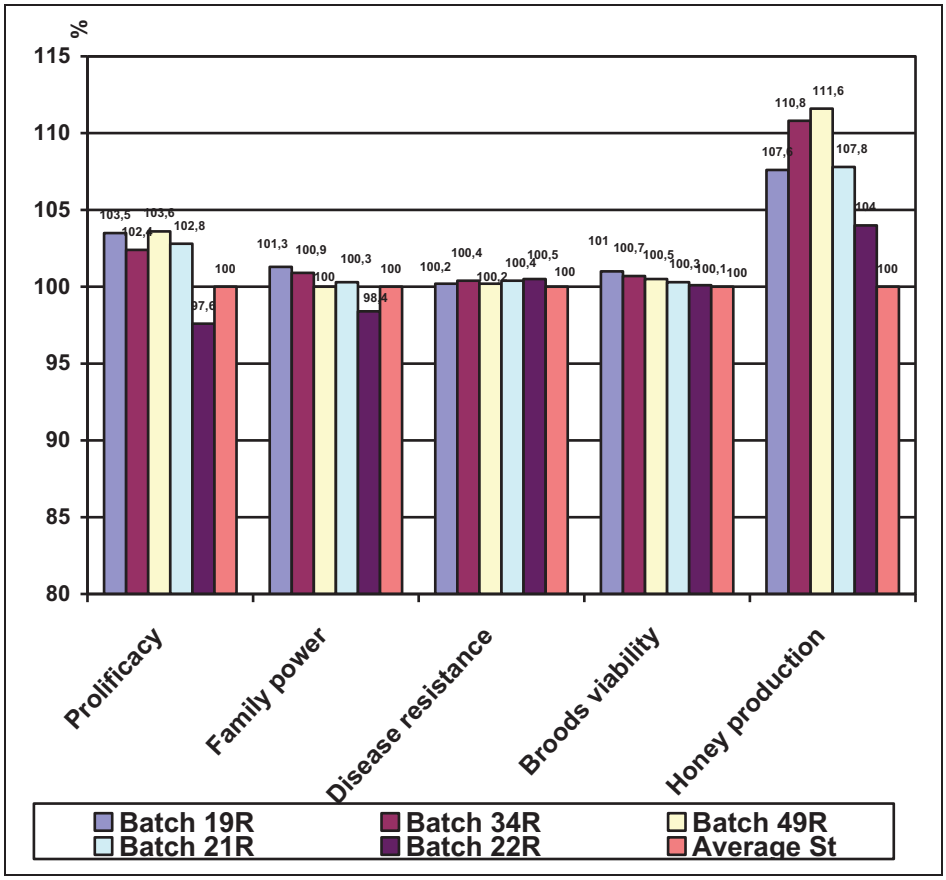

Figure 1. The level of morph – productive characters development of families - daughters, compared with the average on the apiary.

From the histogram can be seen, that all morph - productive characters studied, families - daughters have exceeded tended of apiary level, but clearly these trends were recorded at the prolificacy and honey production. Thus, at the prolificacy, families - daughter of tested queens, except families - daughters of the queen 22R, were on the average level of the apiary. It was observed, that between queens prolificacy and honey production there is a positive correlation. Such correlation registered and anonymous author "Al-Bee", 2013, from web (Al-Bee,

2013). Other researchers, Bar-Cohen et. al., 1978, showed positive correlations between quantity of capped brood and honey production capacity (rxy = 0.27 ± 0.08). **Broods viability** of families - daughter from all experimental batches, as well around the entire apiary, also, was at a rather high level, and ranged on average from 92.3 to 93.2%. The most brood viability was recorded in families - daughters of the queen 19R. Broods viability from these families, has had a clear overcome tendency to the apiary average level of 0.9 or 1.0% absolute units (td = 1.70; P<0.1). After improvement value of this character, queen no. 19R was assigned to the category Improvement - relative. The other tested queen, were assigned to the category of *Neutrals* after qualities of descendant, because the level brood viability of their families - daughters, although have a slight tendency to increase, did not differ significantly compared to average on the apiary.

Honey production, is one of the most important selection characters of bee families, was quite high both in families -daughters of tested queens, and the average for the entire apiary, framing within the averages limits of 50.18 and 56.02 kg. We should mention, that due to progressive targeted selection of bee families, by this character, throughout a long period, has been obtained the increasing effect of its level. More than that, the bee queens were selected mainly by the production of honey, became, in some cases, ameliorative after this character. The highest honey production was recorded at the families - daughters of the queen no. 49R and was 56.02 ± 2.57 kg. After the production of honey, families - daughters of the queen significantly exceeded the average level on the apiary 5.84 kg or 11.6% (td = 2.18; P<0.05), which corresponds to thirst threshold of certainty, according to the probability theory of forecasts contest without error after Student. After ameliorative value of this important morph – productive character, the queen no. 49R has been assigned to the category *Ameliorative - ordinary*. The second level of honey production has been recorded in families - daughters of the queen no. 34R, in average 55.58 ± 2.47 kg. After the production of honey, families - daughters of the queen significantly exceeded the average level on the apiary with 5.40 kg or 10.8% (td = 2.09; P<0.05). After ameliorative value of the families - daughters of this morph – productive character, the queen no. 34R has been assigned, also, to the Ameliorative – *ordinary* category. We should mention that after the improvement value of the other morph – productive characters investigated, both these ameliorative queens were assigned to the category *Neutrals*. Therefore, we can conclude, that selecting these queens for breeding and multiplication of genitor material, we have a significant increase of honey production in bee family's population and, at least,

will not affect their other morph - productive characters.

After improvement value of honey production level families - daughters, the other three queens taken in testing were assigned only to the *Neutral* category. This situation demonstrates, that, in this condition while the queens intended for testing, after qualities of descendants were previously selected by the development of their phenotypic characters and parents families, their genotypic value was no to all true. In our research, from 5 selected queens as performance after their phenotypic qualities and families-parents, just 2 queens, certified their ameliorative value after honey production (40%). In addition, we found that, at least, in our research we have not been identified the queens universal - ameliorative of all morph - productive selected characters.

CONCLUSIONS

In genotypic testing result of 5 bee queens by qualities of descendant, through the method of comparing with the average on the apiary, have been identified two queen-*Ameliorative - ordinary* of honey production.

The phenotypic performance of pre-selected queens after their morph – productive qualities and of families - parent do not guarantee improvement genotypic value without them testing after descendant qualities.

At least, in the studied population, have been not identified universal - ameliorative queens of all morph - productive characters.

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REFERENCES

Al-Bee, 2013. Испытание маток по потомству. // Племенное дело и разведение пчел, с. 37–39. http://www.al-bee.com.

Bar-Cohen R., Alpern G., Bar-Anan R. , 1978. Progeny testing and selecting italian queens for brood area and honey production. Apidologie, 9(2), 95-100.

Cebotari Valentina, Buzu I., 2010. Zootechnical norms regarding the honeybee colonies evaluation, breeding and certification of genetic material in beekeeping.// Contemporary Science Association. Proceedings of the 1st International Animal Health Science Conference: The Beekeeping Conference. Addleton Academic Publishers, New york, (Bucureşti), Library of Congress Control Number, 26-30.

Iliev T.V., 1992. Ameliorarea animalelor. Editura „Universitas”, Chişinău.

Normă zootehnică privind bonitarea familiilor de albine, creşterea şi certificarea materialului genitor apicol, aprobată prin Hotărârea Guvernului nr. 306 din 28.04.2011 (M.O. nr. 78-81 din 13.05.2011, art. 366).

Ruttner Fridrih, 1980. Creşterea mătcilor, baze biologice şi indicaţii metodice. Editura „APIMONDIA”, Bucureşti.

Siceanu Adrian, 2012. Ameliorarea şi înmulţirea albinelor. În: Apicultura – Manualul cursantului. Ediţia I, ICDA, Editura LVS CREPUSCUL", Ploieşti, 148-175.

Totzek H.I., 2013. Оценка качества семьи и ее производительность. http://www.pchelovod.info.

Билаш Н.И., Кривцов Н.И., 1991. Селекция пчел. Изд. „Агропромиздат», Москва.

Гайдар В.А., Левченко И.А., 2003. Сравнительная оценка карпатских и краинских пчел. Журнал «Пчеловодство», Москва, № 4, 37-39.

Контев В.С., Харченко Г.И., 1989. Технология разведения и содержания сильных пчелиных семей. Изд. «Росагропромиздат», Москва, 94.

Кривцов Н.И., 1987. Зарубежный опыт чистопородной селекции. Ж. «Пчеловодство», № 11, 32 - 34.

Плохинский Н.А., 1969. Руководство по биометрии для зоотехников. Изд. «Колос», Москва, 256.

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THE HAEMOGLOBIN, TRANSFERRIN, CERULOPLASMIN AND GLUTATHIONE POLYMORPHISM OF NATIVE GOAT BREEDS OF TURKEY, I- ANGORA AND HAIR

Ayse Ozge DEMIR[1], Nihat MERT[2]

[1]University of Yuzuncu Yil, Faculty of Agriculture, Department of Animal Science, 65080, Van, Turkey, Phone: +90 538 654 61 61, Email: aodemir@yyu.edu.tr
[2]University of Yuzuncu Yil, Faculty of Veterinary Medicine, Department of Biochemistry, 65080, Van, Turkey, Phone: +90 532 376 13 18, Email: mertnihat@hotmail.com

Corresponding author email: aodemir@yyu.edu.tr

Abstract

This study has been carried out in order to determine the polymorphic traits of various biochemical parameters in goat breeds which are native to Turkey. For this purpose, Angora and Hair goat breeds were chosen as live materials. Two different herds for each breed were selected from Ankara and Antalya, respectively. Blood samples were taken from a total of 120 goats aged between 2 and 4 which was made up of 60 Angora goats and 60 Hair goats. All which derived equally from 4 lots of herds. Analyses were performed for the polymorphic determination of the haemoglobin (Hb), transferrin (Tf), ceruloplasmin (Cp) and glutathione (GSH). Hb types were determined by starch gel electrophoresis and Tf types were detected by SDS-PAGE electrophoresis. Furthermore, Cp and GSH analyses were performed by spectrophotometrically. Following the analysis, Hb types were found as 3 genotypes (AA, AB, BB) controlled by 2 allel genes (Hb^A, Hb^B). Tf types were found as 6 genotypes (AA, AB, AC, BB, BC, CC) controlled by 3 allele genes (Tf^A, Tf^B, Tf^C). Findings for Hb were in line with the Hardy-Weinberg Equilibrium (HWE) in Angora goats while the Hair goat was not found to be in line. Moreover, Tf was found in line with the HWE for 2 separate goat breeds. The levels of Cp and GSH of two breeds were significantly different from other ($P<0.0001$). The findings are recorded as a source of reference for prospective polymorphism studies.

Key words: *Electrophoresis, genetic resource, goat, spectrophotometer.*

INTRODUCTION

Living things are adapted to a particular region, maintained presence here and widely grown. This condition is referred as genetic diversity. Moreover, native gene pool is consisted in species belonging to the races. Protection of the shrinking and verge of extinction domestic breeds are protected by suitable reclamation works. On the other hand, the creation of superior efficiency of the herds is essential.

According to FAO (2012), the data of the presence of goats in Turkey asset share in the world in the past 40 years (1961-2011) were declined as 0.719 % from 7.073 % in the past 40 years. One reason of this is also the genetic erosion of kinds. For this reason, identification and control of genetic resources are great importance in terms of biochemical polymorphic features Turkey's livestock. Goat with 90% pregnancy rates is the most efficient type of domestic animal (Ince, 2010). Better quality breeding may be possible with the selection of high-yielding goats among the population (Kurnianto, 2009).

All over the world, Angora goats (*Capra hircus ancyrensis*) known as "Angora" goats homeland is Central Asia. This breed was grown in Turkey from the 13th century (Porter, 1996) until the middle of the 19th century. Breeding of Angora goats were began with 1 pairs of Angora goat and 12 kids in South Africa (Anonymous, 2013).

The importance of this breed utilized from meat, skin and rarely milk is mohair. A type of lint known as "Mohair" in the World is a valuable raw textile materials with long, soft and shiny fiber-like structure. Mohair produced abroad has not reached the same levels of produced in Turkey in terms of important features such as delicacy and softness. For this

reason, Turkey's role in the World market is very important at mohair.
In our country, the majority of the race has been spread to mainly in Ankara, Central Anatolian Plateau on especially in Anatolia. The purest samples collected on breed characteristics (Fig. 1a, b) are located in the Ankara region.

Figure 1. Angora Goats a) 1st herd b) 2nd herd

In order to improve the efficiency of our ruminants, define the genetic structure of populations and know the herds for the yield quality are necessary. On this case, it is heavily studied for making use of some blood parameters in order to obtain a new generation of high efficient. Biochemical parameters and introduction of genes and their mechanisms as well as detailed studies on the molecular level are very often used for the recognition of different biomolecules structures of herd.
Hair goat is another goat breed commonly found in Turkey. They are known "Black goat" or "Ordinary goat" among the people. These animals benefit from scrub areas, they are resistant to diseases and their adaptability are quite high.
Hair goat (Fig. 2a, b) is grown by Turkmens and Nomands as substantially domesticated animals and cover of the Nomand's tent is weaved from hair of these goats. Spread area of the Mediterranean Region and in the Antalya, Konya, Isparta triangle areas are mainly inhabited by Nomads. Especially rich marquis of vegetation, steep mountains and rugged construction of Mediterranean region are effective for goat breeding. Hair goat known as hot and cold-tolerant breed, its contribution to the local economy is very large and production of meat and hair are significant. Goat meat is higher preferred than the sheep meat by people living in the Antalya Region.

Figure 2. Hair goats a) 1st herd b) 2nd herd

Polymorphism studies made in goats hold common part of the genetic studies. This case means the differences between individuals in a population. Bushman and Schmid (1968) have drawn attention to the rare allele frequency in the population at least two alleles of a locus when defining the genetic polymorphism. Rare alleles relative frequency in the population should be at least 0.05 or 0.01 levels for a gene locus to be polymorphic.
Balanced polymorphism is an important term of polymorphism. This case is expressed as a heterozygote and homozygote mixture provided that with progeny mixture and opposite the selection pressure. Balanced populations are desirable in HWE. On the other hand, genes frequency of comprising population in the gene pool remains constant from insemination to fertilization. Also this type of populations is named as stable (balanced) population. Thus, HWE is achieved.
Hb which a large and complex protein is one of the polymorphic system that the most widely studied in vertebrates (Alphonsus et al., 2012). The presences of Hb in different structures were detected in the sheep bloods for the first time by Harris and Warrien (1955) and Cabannes and Serai (1955) (Dogrul 1985). Genetic variation of iron-binding protein was found for the first time in serum Tf.
In mammals, 90-95% of plasma Cu are found as Cp form which depending on α2-globulin (Burtis and Ashwood, 1999). There are significant positive correlations between the level of Cp and Cu levels of plasma, serum and whole blood (Herbert and Ravin, 1991).
GSH concentration in erythrocytes is controlled by one pair of autosomal allele gene. Genes which controlled by high levels of glutathione (GSH^H) are thought to be dominant versus to genes that controlled by low level of glutathione (GSH^h) (Rizzi et al., 1988).

MATERIALS AND METHODS

Sample collection phases of this study were done at Ankara-Ayas for Angora goat and Antalya-Akseki for Hair goat. Independently herds of goats have been determined in firstly. But similar care and feeding conditions are preferred for these goats in semi-intensive breeding system. Herds width were not allowed to be fewer than from 150 goats.

Totally, 120 goats in which 30 goats from each herds were studied on Angora and Hair goat breeds that native genetic resources grown in Turkey. A random selection among the other animals was made for adult female goats (2-4 years old) that appeared clinically healthy and showed the most obvious characteristics as phenotypic in the selected herds. Blood samples were taken at 8 o'clock in the morning from hungry goats which have done last feeding before one night.

The principle of Hb type assay is based on the separation of polymorph properties in the heredity structure of Hb with direct current power in starch gel plates (Soysal, 1983). Sample's Hb type in this study were studied in erythrocytes with continuous buffer system that using the horizontal starch gel electrophoresis system (Meyer, 1963; Braend, 1971). Also Hb genotypes were directly determined on the gel after than individual genotypes were noted. They were defined as the quick-moving type: Hb^A and the slow-moving type: Hb^B (Ustdal, 1976).

Serum samples were used for Tf analysis. Dashed SDS-PAGE method was adapted to BioRad Mini Protean Tetra Cell system for Tf system in these analyzes. And so, sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) (Zacharius et al., 1969; Racusen, 1979; Jay et al., 1990) was performed (Laemmli, 1970). Then, Tf types were read considering the electrophoretic rapids. Gel which carried samples was scanned and passed from the scanner and determined the genotype of each individual was noted in the computer environment. Bands of Tf were defined as the fastest-moving type: Tf^A, the slowest-moving: TF^C (Dogrul, 1985). All bands based on their fast were listed as Tf^A, Tf^B and Tf^C, from fast to slow.

Hb and Tf gene and the genotype frequencies were determined by direct counting method (Nei, 1987; Russell, 1992). Chi square ($\chi 2$) test was performed (Pembeci, 1978; Duzgunes et al., 1987; Yeh et al., 1997) for genetic equilibrium control of populations (importance of between the observed and expected genotype differences - HWE).

GSH levels in whole blood and Cp levels in serum were determined with spectrophotometrically. Absorbance of the colored product was based on P-phenylene diamine dichloride (PPD)-induced serum samples with acetate buffer (pH 5.2 and 37°C). Studied samples at 550 nm were analyzed for Cp levels (Ceron and Subiella- Martinez, 2004). Then according to the described method in the reference literature (Curzon and Vallet, 1960), Cp levels were calculated. GSH measurement was based on the analysis of the measured resultant yellow color as spectrophotometrically (Beutler et al., 1963; Rizzi et al., 1988). The amount of GSH in these colored compounds optical density at wavelength of 412 nm was evaluated by determining (Burtis and Ashwood, 1999). SAS 9.3 statistical analysis software package was used in GSH and Cp levels in the calculation as statistically.

RESULTS AND DISCUSSIONS

Three genotypes (AA, AB, BB) in Hb bands were obtained with starch gel electrophoresis in Angora and Hair goats. Frequencies of these genotypes and homologous / heterologous genotypes were presented in Table 1.

Difference between observed and expected gene frequencies in Angora goats was found to be non-significant at Hb electrophoresis. For this reason, The 1st herd, the 2nd herd and intra-breed population of Angora goats were in HWE in terms of Hb genotypes.

Difference between observed and expected gene frequencies of Hair goat in Hb electrophoresis were significant at the 1st herd ($P<0.05$), the 2nd herd ($P<0.01$) and intra-breed population ($P<0.0001$). HWE was found in this regard with respect to Hb genotypes (Fig. 3).

Table 1. Hb genotypes, allele frequencies and homologous / heterologous genotypes

		Angora Goat			Hair Goat		
		1st herd	2nd herd	Total	1st herd	2nd herd	Total
N / GENES		30	30	60	30	30	60
GENOTYPE	HbAA	14	13	27	12	17	29
		%	%	%	%	%	%
		46.67	43.33	45.00	40.00	56.67	48.33
	HbAB	12	15	27	8	6	14
		%	%	%	%	%	%
		40.00	50.00	45.00	26.67	20.00	23.33
	HbBB	4	2	6	10	7	17
		%	%	%	%	%	%
		13.33	6.67	10.00	33.33	23.33	28.33
FREQUENCY							
	HbA	40	41	81	32	40	72
		0.666	0.683	0.675	0.533	0.666	0.600
		7	3	0	3	7	0
	HbB	20	19	39	28	20	48
		0.333	0.316	0.325	0.466	0.333	0.400
		3	7	0	7	3	0
GEN	Hb AA,B B	18	15	33	22	24	46
		%	%	%	%	%	%
		60.00	50.00	55.00	73.33	80.00	76.67
	Hb AB	12	15	27	8	6	14
		%	%	%	%	%	%
		40.00	50.00	45.00	26.67	20.00	23.33

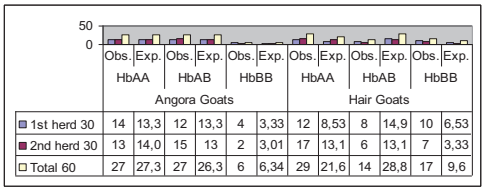


Figure 3. Observed and expected values of Hb frequencies in Angora and Hair goats

Six genotypes (AA, AB, AC, BB, BC, CC) of Tf were obtained in SDS-PAGE electrophoresis of Angora and Hair goat bloods. However, in Angora goats, AC genotype was not observed in the 1st herd. Homologous/heterologous genotypes and % frequencies of these genotypes were given in Table 2.

Observed and expected Tf gene frequencies differences were not significant in the electrophoresis of Hair goats. In this regard, the 1st herd, the 2nd herd and intra-breed population were in HWE in terms of Tf genotypes (Fig. 5).

Table 2. Tf genotypes, allele frequencies and homologous/ heterologous genotypes

		Angora Goat			Hair Goat		
		1st Herd	2nd herd	Total	1st Herd	2nd herd	Total
N / GENES		30	30	60	30	30	60
GENOTYPE	Tf AA	1	2	3	1	4	5
		%	%	%	%	%	%
		3.33	6.67	5.00	3.33	13.33	8.33
	TfAB	1	11	12	9	10	19
		%	%	%	%	%	%
		3.33	36.67	20.00	30.00	33.33	31.67
	TfAC	-	1	1	1	2	3
		%	%	%	%	%	%
		0.00	3.33	1.67	3.33	6.67	5.00
	TfBB	12	7	19	12	6	18
		%	%	%	%	%	%
		40.00	23.33	31.67	40.00	20.00	30.00
	TfBC	12	7	19	6	5	11
		%	%	%	%	%	%
		40.00	23.33	31.67	20.00	16.67	18.33
	TfCC	4	2	6	1	3	4
		%	%	%	%	%	%
		13.33	6.67	10.00	3.33	10.00	6.67
FREQUENCY	TfA	3	16	19	12	20	32
		0.050	0.266	0.158	0.200	0.333	0.266
		0	7	3	0		7
	TfB	37	32	69	39	27	66
		0.616	0.533	0.575	0.650	0.450	0.550
		7	3	0	0	0	0
	TfC	20	12	32	9	13	22
		0.333	0.200	0.266	0.150	0.216	0.183
		3	0	7	0	7	3
GEN	TfAA,B B CC TfAB AC BC	17	11	28	14	13	27
		%	%	%	%	%	%
		56.66	36.66	46.66	46.66	43.33	45.00
		13	19	32	16	17	33
		%	%	%	%	%	%
		43.33	63.33	53.33	53.33	56.66	55.00

Gen frequency differences of observed and expected in Tf electrophoresis were found significant ($P<0.01$) at the 1st herd of Angora goats. This herd in terms of Tf genotypes was not in the HWE. However, the 2nd herd and intra-breed population were not detected in HWE (Fig. 4).

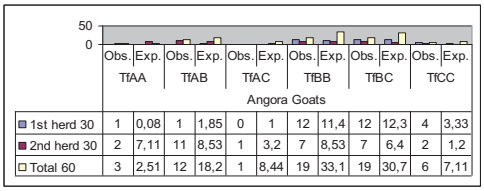
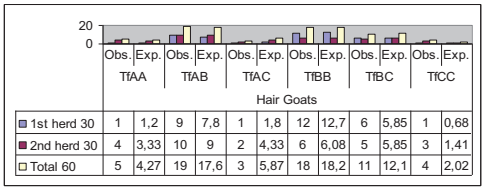


Figure 4. Observed and expected values of Tf frequencies in Angora goats.



1st herd 30	1	1,2	9	7,8	1	1,8	12	12,7	6	5,85	1	0,68
2nd herd 30	4	3,33	10	9	2	4,33	6	6,08	5	5,85	3	1,41
Total 60	5	4,27	19	17,6	3	5,87	18	18,2	11	12,1	4	2,02

Figure 5. Observed and expected values of Tf frequencies in Hair goats.

Significant differences (P<0.001) were detected in the 1st and the 2nd herds of Angora and Hair goats for analyzed Cp results. Fairly high level significant difference (P<0.0001) was found between breeds in terms of the Cp (Tab. 3).

Table 3. CP and GSH values in Angora and Hair goats.

		Angora Goat			Hair Goat		
		1st herd	2nd herd	Total	1st herd	2nd herd	Total
	N	30	30	60	30	30	60
CP	X	2.608	1.864	2.236	1.685	0.591	1.138
	±Sx	±0.14 9	±0.11 3	±0.10 4	±0.17 3	±0.07 0	±0.54 7
	Xmin	0.943	0.314	0.314	0.157	0.157	0.157
	Xmax	5.500	2.986	5.500	3.614	3.043	3.614
	P	<0.001			<0.001		
GSH	x±Sx	43.49 3±0.5	33.24 4±0.5	38.36 8±0.7	35.22 8±0.6	38.21 9±0.6	36.72 3±1.4
		94	93	86	29	90	92
	Xmin	37.33	28.80	28.80	29.33	32.26	29.33
	Xmax	48.53 3	39.46 7	48.53 3	42.66 7	46.13 3	46.13 3
	P	<0.001			<0.01		

Also scatter diagram of Cp values at the 1st and the 2nd herds were given in Fig. 6.

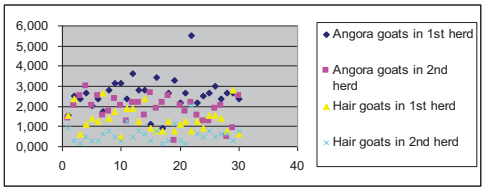


Figure 6. Cp scatter diagram

Also, scatter diagram of GSH levels was given for totally two herds from each breed in Fig. 7.

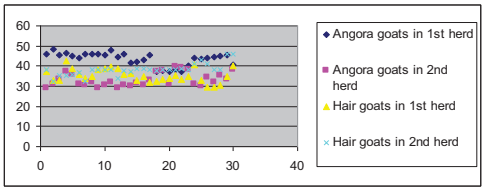


Figure 7. GSH scatter diagram

GSH levels were evaluated on the basis of herd. Goats below average were divided as GSH^H and the others were determined as GSH^h. These two groups were analyzed independently in themselves. The lowest GSH^h average (30.948 ± 0.268 mg/dl) was observed in the 2nd Angora goat herd. However the highest GSH^H average (39.903 ± 0.735 mg/dl) was found in the 1st herd of Angora goat (Tab. 4).

Table 4. GSH^h and GSH^H levels in Angora and Hair goats

		Angora Goat		Hair Goat	
		1st Herd	2nd herd	1st herd	2nd herd
N		30	30	30	30
GSH^h	X	39.903	30.948	32.879	35.980
	±Sx	±0.735	±0.268	±0.412	±0.589
	X_{min}	37.333	28.800	29.333	32.267
	X_{max}	43.467	33.067	34.667	38.133
GSH^H	X	45.572	36.689	38.555	40.644
	±Sx	±0.265	±0.595	±0.590	±0.851
	X_{min}	44.000	33.600	35.733	38.400
	X_{max}	48.533	39.467	42.667	46.133

In this study, 4 different polymorphic parameters, Hb, Tf, Cp and GSH were studied in Angora and Hair goats. Hb system was determined by 2 codominant alleles (Hb^A, Hb^B), when Tf system was controlled by 3 codominant alleles (Tf^A, Tf^B, Tf^C) in investigated goats. Also, Cp and GSH levels were determined in the expected physiological values.

A study on the 186 Hair goats was done in Antalya province by Karabag (2000). Accordingly, 126 HbAA and 40 HbAB genotypes were in his samples. Hb genotypes had been done for aimed at determining the Hb genotype frequencies of domestic Hair goats by Boztepe et al. (1993) in a different study. And Hb genotype variants have determined as HbAA: 0.50, HbAB: 0.48 HbBB: 0:02. In the same study, gene frequencies have been identified as Hb^A: 0.74 and Hb^B: 0:26.

Alphonsus et al. (2012) have come across Hb^C allele in goats reared in Abuja Nigeria in their study. 4 genotypes (AA, AB, BB, AC) managed by 3 allele genes (Hb^A, Hb^B, Hb^C) have been identified in the same study executed on 94 goats. But the HbBC and HbCC genotypes were not detected. Allele of the Hb^C was found at very low frequency as 0.0640 while HbAC genotypic frequency was 0.1227. Hb^A allele has been determined with the highest frequency of 0.6010.

In this study, Angora goats HB^A and Hb^B allele frequencies were calculated as 0.6667 and 0.3333 in the 1st herd, as 0.6833 and 0.3167 in the 2nd herd, respectively. Hb^A and Hb^B allele frequencies of Hair goats were detected as 0.5333 and 0.4667 in the 1st herd, as 0.6667 and 0.3333 in the 2nd herd, respectively. Two alleles were also determined in different study conducted on 54 Norduz goats by Aygun (2006) Also, 48% HbAA, 48% HbAB and 4% HbBB genotypes have been identified. Allele frequencies have been reported as 0.7222 and 0.2778 for Hb^A and Hb^B, respectively. Although there were different breeds, these results were similar to this study.

In this study, two allele genes (HB^A, HB^B) controlled by three hemoglobin genotype (AA, BB, AB) were determined. Three genotypes (AA, BB, AB) were also detected in the presence of both Angora and Hair goat herds. Common allele frequencies were less than 95%, so Hb system has been considered polymorphic in this study conducted in Angora and Hair goats.

Hair goats were not in HWE with regard to Hb genotype frequencies. We could be said to protect of stability of gene and genotype structures under the Panmixia conditions for these goats. Such as the used a small number of male goat could be effective on this case. On the other hand, small herd and used their own males could be caused to departing from genetic stability. It was sufficiently believed that population size and such case as chances factor could be effective on Angora goats.

TfAA, TfAB and TfBB genotypes in Norduz goats were detected by Aygun (2006). He had been identified as 0.787 and 0.213 for Tf^A and Tf^B in same study, respectively. Nozawa et al. (1978) had been detected Tf^A, Tf^B and Tf^C alleles in a different study which in Japanese goats. In the same study, Tf^A was reported as monomorphic. Tf^A in the Spanish domestic goat was identified as predominant by Tunon et al. (1987). Additionally, Tf^C was detected in only Negra Serrana breed by them.

Levels of Tf frequencies were detected as 0.0500 and 0.2667 for Tf^A, 0.6167 and 0.5333 for Tf^B, 0.3333 and 0.2000 for Tf^C in this study conducted in the 1st and the 2nd herds of Angora goats, respectively. Tf frequencies in the 1st and the 2nd herds of Hair goats were calculated as Tf^A: 0.2000 and 0.3333, Tf^B:0.6500 and 0.4500, Tf^C: 0.1500 and 0.2167, respectively. However, TfAC genotype could not be determined in the 1st herd of Angora goats. We have thought to be associated with scarcity of Tf^C allel in this breed. On the other hand, the effect of aleatory factor was not ignored for the selected animals.

In general, three allel genes (Tf^A, Tf^B, Tf^C) which controlled by the 6 Tf genotype (AA, AB, AC, BB, BC, CC) were determined in the goat herds. Presence of 6 genotypes was detected in both Hair goat herds. However, TfAC genotype was not determined in the 1st herd of Angora goat. Due to chance factor could be effective on this case. On the other hand, lack of one genotype was usual if we have thought that 6 genotypes detected in 30 goats.

Fewer male goats were probably used at researched materials. So, Tf genotype frequencies in populations and herds (the 1st herd of Angora goats, the 2nd herd of Hair goats) were not in HWE. Breeders have not applied any selection in investigated goat breeds. In general, the main reasons for departing from the genetic stability could be listed as migration, the effect of breeding systems, genetic mutations and absence of sufficient size of herd. Also, lots of high mating and using male goats with high reproductive performance and fertility could be leaded to some problems in terms of breeding. This condition might be brought genetic problems due to inbreeding such inadequate herd size and various chance factors were thought to be effective on the HWE.

Allele number of features which have been considered to be effective on analyses of blood characteristics with genetic variation (such as Hb and Tf ...). If the number of your samples is sufficient in study, homologous and heterozygous genotypes can obtain higher chances for determining by two allele genes (A, B) with three genotypes (AA, AB, BB) in different populations. Otherwise you may not have the chance to identify all of homologous and heterologous genes. Blood Hb and Tf electrophoresis were studied on 30 goats of each herd in this planned study. We have considered that obtained genotypes were enough for sufficient to define the whole

population. With more than expected number of genes alleles (3 and more) characteristics of the herd size might be more effective incoming research results. Otherwise, we would not have any chance for identify and describe the entire of population.

Investigated study, the average Cp values in Angora and Hair goats were detected as 2.236 ± 0.104 mg/dl and 1.138 ± 0.547 mg/dl on average, respectively. High level significant differences were detected in Angora and Hair goat herds when Cp levels were examined. It was thought to be associated with nutrition of goats and physiological states because blood samples were taken in February. Thus, the weak pasture composition might be effect on blood mineral levels.

Gurcan et al. (2011) have used Saanen X Malta crossbred goats in their erythrocyte GSH study. GSH^H and GSH^h allele gene frequencies have been reported as 0.43-0.57 in the same study by them. Otherwise in this study, average GSH levels were detected as 38,368 ± 0786 mg/dl in Angora goat herds and as 36,723 ± 1,492 mg/dl in Hair goat herds. Differences between Hair goat herds were found significant when GSH levels were examined on the basis of herds. On the other hand, high significant level difference was detected between two herds of Angora goats. This might be related with the result of different care and feeding conditions.

CONCLUSIONS

As a result, we can say that there was probably enough number of male goats for this study. About these goats may be said to be homogenous dispersion in herd and hold regular election especially for cases where the equilibrium provided. Other studies showed that numerical values obtained in polymorphic characters such as Cp and GSH were seen sufficient number of samples in the study. But, polymorphism studies with more numerous samples may be appropriate for multiple allele system examination.

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REFERENCES

Alphonsus C., Akpa G.N., Usman N., Barje P.P., Byanet O., 2012. Haemoglobin polymorphism and its distribution in smallholder goat herds of Abuja Nigeria, Global Journal of Molecular Science, 7, 1, 11-14.

Anonymous 2012. FAO. http//www.fao.org/docrep/017/i3028e/i3028e.pdf

Anonymous 2013. FAO. http//www.fao.org/docrep/018/i3107e/i3107e00.htm

Aygun T., 2006. Relationships between the polymorphism of blood proteins and some milk yield traits in Norduz goats. Yuzuncu Yil University, PhD Thesis, Van.Beutler E., Dubon O., Kelly B.M., 1963. Improved method for the determination of blood glutathione, Journal of Laboratory and Clinical Medicine, 61, 882-888.

Boztepe S., Ozbayat H.I., Kayis S.A., 1993. Blood potassium and hemoglobin polymorphism in Hair goat. Selcuk University Journal of Agricultural Science, 3, 5, 89-96, Konya.

Braend M., 1971. Haemoglobin varients in cattle, Animal Blood Groups and Biochemical Genetics, 2, 15-21.

Burtis C.A., Ashwood E.R., 1999. Tietz Testbook of Clinical Chemistry, 3rd Ed, WB Saunders Coumpany, Philadelphia.

Bushman H., Schmid D.O., 1968. Serum gruppen bei Tieren, Paul Parey, Berlin.

Cabannes H., Serain C., 1955. Etudes electrophoretigues des hemoglobines des amiferes domestiques d'Algerie, Comptes Rendus des Seances la Societe de Biologie et des ses Filiales, Jun;149, 11-12, 1193-1197.

Ceron J.J., Subiella-Martinez S., 2004. An automated spectrophotometric method for measuring canine ceruloplasmin, Veterinary Research, 35, 671-679.

Curzon G., Vallet L., 1960. The putrification of human ceruloplasmin, Biochemical Journal, 74, 279-287.

Dogrul F., 1985. Cesitli koyun irklarında transferrin ve hemoglobin tiplerinin dagılımı uzerine araştırma, Etlik Veteriner Mikrobiyoloji Dergisi, 5, 8-9, 61-75.

Duzgunes O., Elicin A., Akman N., 1987. Hayvan Islahi, Ankara Universitesi Ziraat Fakultesi Yayinlari, No, 1003, Ankara.

Gurcan E.K., Cobanoglu O., Kose M., 2011. Erythrocyte potassium and glutathione polymorphism determination in Saanen X Malta crosbred goats, African Journal of Biotechnology, 10, 38, 7534-7540.

Harris H., Warrien F.L., 1955. Occurance of electrophoretically distinct haemoglobins in ruminants, Biochemical Journal, 60, 29.

Herbert A., Ravin M.D., 1991. An inproved colorimetric enzymatic assay of ceruloplasmin, Journal of Laboratory and Clinical Medicine, 58, 1, 161-168.

Ince D., 2010. Reproduction performance of Saanen goats raised under extensive conditions, African Journal of Biology, 9, 8253-8256.

Jay G.D., Culp D.J., Jahnke M.R., 1990. Silver staining of extensively glycosylated proteins on sodium dodecyl sulfate-polyacrylamide gels, enhancement by carbohydrate-binding dyes, Analytical Biochemistry, 185, 324–330.

Karabag K., 2000. Antalya yoresi Kil kecilerinde biyokimyasal polimorfizm, Akdeniz Univ. Fen Bilimleri Enstitusu Yuksek Lisans Tezi, Antalya.

Kurnianto E., 2009. Pemuliaan Ternak, CV. Graha Ilmu, Yogyakarta.

Laemmli U.K., 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage, Nature, 227, 680-685.Meyer V.H., 1963. Genetic control of two pre albumins in pigs, Genetics, 48, 1059-1063.

Nei M., 1987. Molecular Evolutionary Genetics, Columbia University Press, New York.

Nozawa K., Shinjo A., Shotake T., 1978. Population genetics of farm animals, III. Blood-protein variation in the meat goats in Okinawa Islands of Japan, Z. Tierzüchtung, Züchtig Sbiol, 95,66-77, Japan.

Pembeci M., 1978. Ataturk Universitesi koyun populasyonlarinda kan potasyum seviyelerinin kalitimi ve verimle ilgileri, Doktora Tezi, Erzurum.Porter V., 1996. Goats of the World, Farming Press Miller Freeman Professional Ltd, United Kingdom.

Racusen D., 1979. Glycoprotein detection in polyacrylamide gel with thymol and sulfiric acid, Analytical Biochemistry, 30, 148.

Rizzi R., Caroli A., Bolla P., Acciaioli A., Pagnacco G., 1988. Variability of reduced glutathione levels in massese ewes and its effect on daily milk production, Journal of Dairy Research, 55, 345-353.

Russell P.J., 1992. Genetics, Harper Collins Publishers, New York.

Soysal M.I., 1983. Ataturk Universitesi koyun populasyonunun bazi kalitsal polimorfik kan proteinleri bakimindan genetik yapisi ve bu biyokimyasal karakterler ile cesitli verim ozellikleri arasindaki iliskiler, Ataturk Universitesi Fen Bilimleri Enstitusu Zootekni Anabilim Dali, Doktora Tezi, Erzurum.

Tunon M.J., Gonzalez P., Vallejo M., 1987. Blood biochemical polymorphism in Spanish goat breeds, Comparative Biochemistry and Physiology, 88B, 513-517.

Ustdal K.M., 1976. Turkiyedeki bazi yerli sigir irklarinda hemoglobin, transferin ve sut proteinlerinin biyokimyasal polimofizmi üzerinde arastirmalar, Ankara Universitesi Saglik Bilimleri Enstitusu, Doktora Tezi, Ankara.

Yeh F.C., Yang R.C., Boyle T.B.J., Ye Z.H., Mao X.J., 1997. POPGENE, The User-Friendly Shareware for Population Genetic Analysis, Molecular Biology and Biotechnology Centre, University of Alberta, Canada.

Zacharius R.M., Zell T.E., Morrison J.H., Woodlock J.J., 1969. Glycoprotein staining following electrophoresis on acrylamide gels, Analytical Biochemistry, Jul, 30, 1, 148–152.

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THE HAEMOGLOBIN, TRANSFERRIN, CERULOPLASMIN AND GLUTATHIONE POLYMORPHISM OF NATIVE GOAT BREEDS OF TURKEY, II- KILIS AND HONAMLI

Ayse Ozge DEMIR[1], Nihat MERT[2]

[1] University of Yuzuncu Yil, Faculty of Agriculture, Department of Animal Science, 65080, Van, Turkey, Phone: +90 538 654 61 61, Email: aodemir@yyu.edu.tr
[2] University of Yuzuncu Yil, Faculty of Veterinary Medicine, Department of Biochemistry, 65080, Van, Turkey, Phone: +90 532 376 13 18, Email: mertnihat@hotmail.com

Corresponding author email: aodemir@yyu.edu.tr

Abstract

In this research, Kilis and Honamli goats are used, which are specific local genetic resources of Turkey. The herds were independent, but they had similar care and nutrition circumstances. From each breed 30 samples were taken, in all 120 samples were collected. Erythrocyte, whole blood and serum samples were used for haemoglobin (Hb), glutathione (GSH) and transferrin (Tf), ceruloplasmin (Cp) analysis, respectively. In the analysis of these samples, Hb and Tf bands were determined by electrophoresis. However, Cp and GSH levels were analyzed by the spectrophotometer. 3 Hb phenotypes (AA, BB, AB) and 6 Tf phenotypes (AA, AB, AC, BB, BC, CC) were determined in this study. In addition, both the observed and the expected values of polymorphic characteristic for 2 characters were presented according to the Hardy-Weinberg Equilibrium (HWE). Cp levels were detected as 0.822 ± 0.055 mg/dl and 1.793 ± 0.109 mg/dl in Kilis and Honamli herds, respectively. Also GSH levels were detected as, 42,486 ± 1,034 mg/dl and 33.515 ± 0.345 mg/dl in these breeds, respectively. On the other hand, the high and low GSH levels (GSH^H and GSH^h) of herds were presented.

Key words*: electrophoresis, gene resource, goat, spectrophotometer.*

INTRODUCTION

Protecting the gene pool of breeds has a great importance, because there is a risk of genetic resources loss. In this case, the benefits of advanced breeding programs will be reduced. In studies about reclamation of livestock, the known characteristics of the population are used. The studies in order to determine the genetic quality have improved the quality in goats as in other types of genetic. Kurnianto (2009) reported that better quality breeding population through the selection of high yielding goats can be done with selection and in this way it may be possible to yield high quality animals.

Kilis goat, supply genes which are grown in Turkey (Fig. 1 a, b); occurred by crossbreeding of Hair goat with Aleppo goat originated Syrian. Kilis goats have the 25.6% of the total goat breeding presence in Turkey. This rate is quite high in terms of breeding. They are widely grown in the South-eastern Anatolia region and especially in Sanliurfa, Gaziantep, Kilis and Hatay which are borders of Syria. This area with hot weather is quite favourable for cultivation because of their vegetation (Kaymakci et al., 1997).

Figure 1. Kilis goats a) 1st herd b) 2nd herd

Honamli goats are a breed that spreading in the triangle from the lower slopes of the Taurus Mountains to the Mediterranean region and Antalya, Konya, Isparta provinces. One of purity properties of these animals is the distance between the horns on the forehead. This distance should be two centimetres for adult goats. This breed loves to play with its

caregivers. Honamli goats are highly active animals. Their eyes are large, vivid and bright.

Figure 2. Honamli goats a) 1st herd b) 2nd herd

Differences between individuals in a population were defined as polymorphism. Accordingly, alleles belong a population locus in the form of at least 5% differences are desired for balanced polymorphism by Goldstein and Schlotter (2000). These experiments are used to explain a various biochemical properties with different genetic forms and the morphological differences in chromosomes.

Polymorphism studies can be grouped under three main groups as different biochemical properties of the genetic forms (various proteins and blood group factors), the morphological differences in the chromosomes (chromosomal polymorphism) and differences in the DNA nucleotide sequence (DNA polymorphism).

Hb, one of the polymorphic characters in the blood, is frequently investigated in goats. This system is commonly controlled by two codominant alleles in the form of Hb^A and Hb^B. These two alleles have different types of electrophoretic mobility in electrophoretic analyses.

Tf availability often investigated in breeding is a polymorphic system. Tf supplies the phenotypes to be determined in the very early stages of life and allows them can be used in the indirect selection. Thus generation interval can be shortened. Especially in goats, the high correlation between Tf which in blood and mohair proceeds are noteworthy. It is possible to make a selection based on the result of the Tf polymorphism study for these proceed.

Orally administered copper (Cu), after being absorbed from the top of the stomach and intestines, enters into blood plasma and erythrocytes. After 24 hours from the absorbance, High amount of Cu are collected in Cp (Murray et al., 1990). Besides, Cp is involved in various antioxidative and cytoprotective activities and thus helps to maintain cell integrity, on the other hand, Cp which protein facilitates binding of Fe to Tf protein.

Types of erythrocyte GSH are under genetic control and they have also inherited property. GSH levels in the blood are fairly constant for all adults (Mert et al., 2003). Decrease in intracellular GSH levels lead to cell apoptosis via oxidative stress. Additionally, apoptosis for living is a great important condition related to cell death.

MATERIALS AND METHODS

In this study 30 animals from 2 herds (which two different breeds) with a total of 120 goats were used from Kilis and Honamli goats, indigenous genetic resources grown in Turkey. In study was gone to Kilis centre for Kilis goats and Konya-Seydisehir for Honamli goats and were studied with two different herds for avoiding similar blood results in the wake of kindred.

Blood samples were taken at 8 o'clock in the morning when they were hungry. The transferred bloods in purple tube with EDTA were used for Hb and GSH analysis. On the other hand, transferred serum samples to plastic dry tube were used for Tf and Cp analysis.

Polymorph properties in the starch gel plate, direct current power with the separation on the basis of Hb type determination (Soysal, 1983), a continuous buffer system using horizontal starch gel electrophores was made according to Meyer (1963) and Braend (1971) reported. Reading the Hb types were taken into account electrophoretic rate, the faster was defined as Hb^A and the slower was described as Hb^B (Ustdal, 1976).

Transferrin type analysis, the sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) (Zacharius et al., 1969; Racusen, 1978; Jay et al., 1990) discrete BioRad SDS-PAGE method was performed by adapting BioRad Miniprotean Tetra Cell system (Laemmli, 1970). Tf types were defined when they taken into their electrophoretic velocities. The fastest outgoing type was Tf^A and Tf^C was defined as the slow-moving (Dogrul, 1995).

Polyacrylamide gel, free functional groups which react with the acrylamide monomer

N,N'-methylene bisacrylamide-like through-linked with bifunctional compounds, are shaped to polymerize. A radiator must be used to determine the location of the protein bands on the gel under UV light.

Hb band types were directly determined on the gel at the study. The gel was photographed for Tf and the genotype of each individual was noted. Genetic variants in terms of both properties, gene and the genotype frequencies were determined by direct counting method (Nei, 1987; Russell, 1992). According to this method, related gen frequency are collected with more than two times of homozygous phenotypes and half of the number of heterozygous phenotypes. After then, the result is divided to the total number of individuals (Duzgunes et al., 1987; Nei, 1987).

Hb and Tf alleles in terms of population, in terms of Hb and Tf genetic stability control system (differences between expected and observed the importance of genotype-HWE) for identifying whether or not provided, the chi square ($\chi 2$) compliance test was used (Pembeci, 1978; Duzgunes et al., 1983; Yeh et al., 1997).

$$\chi^2 = \sum (\text{observed-expected})^2 / (\text{expected})$$

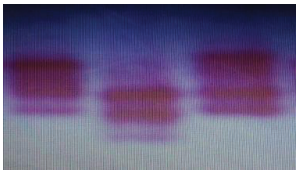
On a further analysis method, serum Cp levels, read in pH 5.2 and at 37 °C in acetate buffer, P-phenylene diamine dichloride (PPD) with serum samples formed by the colored product absorbance spectrometer at 550 nm were analysed (Ceron and Subiella-Martinez, 2004). Calculation of Cp was performed according to the method described in the literature reference (Curzon and Vallet, 1960).

Occurrence of the yellow color was measured spectrophotometrically at GSH analysis (Beutler et al., 1963; Rizzi et al., 1988). The amount of GSH in the sample, at a wavelength of 412 nm of the colored compound was assessed by determining the optical density (Burtis and Ashwood, 1999).

SAS 9.3 software package was used in the calculation of statistical analysis of Cp and GSH levels.

RESULTS AND DISCUSSIONS

Hb starch gel electrophoresis in Kilis goats, AA and AB genotypes obtained in the 1st herd, however the BB genotype was not observed. Three genotypes (AA, AB, BB) were obtained in the 2nd herd. The other hand, three genotypes (AA, AB, BB) in Honamli goats were obtained in Hb electrophoresis. Obtained bands image of Hb in starch gel electrophoresis was given in Fig. 3.



AA BB AB

Figure 3. Hb band types that appear in goat bloods

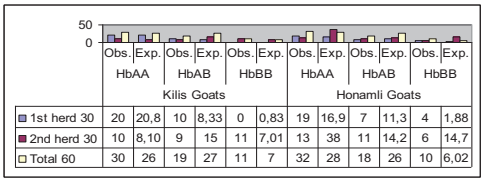
Belongings to genotypes frequencies % and homologous/heterologous genotypes were presented in Tab. 1.

Table 1. Hb genotypes, allele frequencies and homologous/ heterologous genotypes

		Kilis Goat			Honamli Goat		
		1st herd	2nd herd	Total	1st herd	2nd herd	Total
GENES \ N		30	30	60	30	30	60
GENOTYPE	HbAA	20 % 66.67	10 % 33.33	30 % 50.00	19 % 63.33	13 % 43.33	32 % 53.33
	HbAB	10 % 33.33	9 % 30.00	19 % 31.67	7 % 23.33	11 % 36.67	18 % 30.00
	HbBB	- % 0.00	11 % 36.67	11 % 18.33	4 % 13.33	6 % 20.00	10 % 16.67
FREQUENCY	Hb^A	50 0.833 3	29 0.483 3	79 0.658 3	45 0.750 0	37 0.616 7	82 0.683 3
	Hb^B	10 0.166 7	31 0.516 7	41 0.341 7	15 0.250 0	23 0.383 3	38 0.316 7
GEN	Hb AA,B B	20 % 66.67	21 % 70.00	41 % 68.33	23 % 76.67	19 % 63.33	42 % 70.00
	Hb AB	10 % 3.33	9 % 30.00	19 % 31.67	7 % 3.33	11 % 36.67	18 % 30.00

The 1st herd of Kilis goat was at HWE. In this regard, observed and expected gene frequencies difference at Hb electrophoresis was non-significant ($P>0.05$) in this herd. But the 2nd herd and intra population were not in HWE. Thus, P values were significant ($P<0.05$) in these. The situation was different for other breed. With reference to the 2nd herd of

Honamli goat was in HWE. In this regard, observed and expected gene frequencies difference at Hb electrophoresis was non-significant (P>0.05) in this herd. But the 1st herd and intra population were not in HWE. Thus, P values were significant (P<0.05) in these groups (Fig. 4).

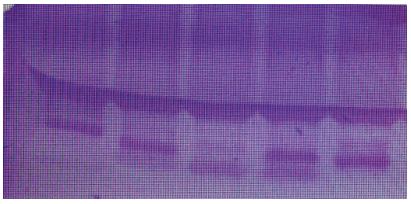


1st herd 30	20	20,8	10	8,33	0	0,83	19	16,9	7	11,3	4	1,88
2nd herd 30	10	8,10	9	15	11	7,01	13	38	11	14,2	6	14,7
Total 60	30	26	19	27	11	7	32	28	18	26	10	6,02

Figure 4. Observed and expected values of Hb frequencies in Kilis and Honamli goats

SDS-PAGE electrophoresis, 6 genotypes (AA, AB, AC, BB, BC, CC) were obtained from the 1st herd and 5 genotypes (AA, AB, AC, BB, BC) were detected from the 2nd herd in Kilis goats. Besides, SDS-PAGE electrophoresis of Tf, all 6 genotypes were obtained in Honamli goats.

Obtained bands image of Tf in starch gel electrophoresis was given in Fig. 5.



AA BB CC BC AB

Figure 5. Tf band types that appear in goat bloods

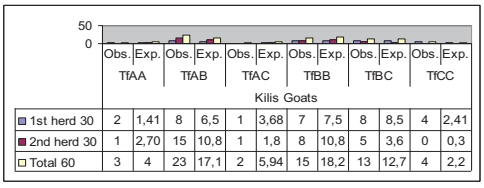
Belongings to these genotypes % frequencies and homologous/heterologous genotypes were presented in Table 2.

Non-significant difference (P>0.05) at observed and expected gene frequencies of Tf electrophoresis were in Kilis goats. So they were in HWE in terms of Tf genotypes (Figure 6).

In Honamli goats, significance of observed and expected Tf gene frequencies was important in the 1st herd (P<0.05), as it was not in HWE in terms of Tf genotypes, while others were in equilibrium (Fig. 7).

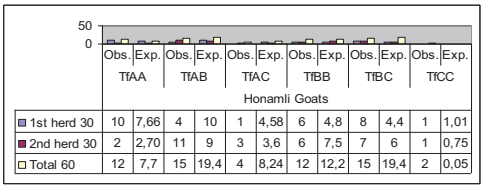
Table 2. Tf genotypes, allele frequencies and homologous/ heterologous genotypes

		Kilis Goat			Honamli Goat		
		1st Herd	2nd herd	Total	1st herd	2nd herd	Total
	N	30	30	60	30	30	60
GENOTYPE	Tf AA	2 % 6.67	1 % 3.33	3 % 5.00	10 % 33.33	2 % 6.67	12 % 20.00
	TfAB	8 % 26.67	15 % 50.00	23 % 38.33	4 % 13.33	11 % 36.67	15 % 25.00
	TfAC	1 % 3.33	1 % 3.33	2 % 3.33	1 % 3.33	3 % 10.00	4 % 6.67
	TfBB	7 % 23.33	58 % 26.67	15 % 25.00	6 % 20.00	6 % 20.00	12 % 20.00
	TfBC	8 % 26.67	5 % 16.67	13 % 21.67	8 % 26.67	7 % 23.33	15 % 5.00
	TfCC	4 % 13.33	- % 0.00	4 % 6.67	1 % 3.33	1 % 3.33	2 % 3.37
FREQUENCY	Tf^A	13 0.216 7	18 0.300 0	31 0.258 3	25 0.416 7	18 0.300 0	43 0.358 0
	Tf^B	30 0.500 0	36 0.600 0	66 0.550 0	24 0.400 0	30 0.500 0	54 0.450 0
	Tf^C	17 0.283 3	6 0.100 0	23 0.191 7	11 0.183 3	12 0.200 0	23 0.191 7
GEN	Tf AA,BB,CC	13 % 43.33	9 % 30.00	22 % 36.66	17 % 56.66	11 % 36.66	28 % 46.66
	Tf AB,AC,BC	17 % 56.66	21 % 70.00	38 % 63.33	13 % 43.33	19 % 63.33	32 % 53.33



1st herd 30	2	1,41	8	6,5	1	3,68	7	7,5	8	8,5	4	2,41
2nd herd 30	1	2,70	15	10,8	1	1,8	8	10,8	5	3,6	0	0,3
Total 60	3	4	23	17,1	2	5,94	15	18,2	13	12,7	4	2,2

Figure 6. Observed and expected values of Tf frequencies in Kilis goats



1st herd 30	10	7,66	4	10	1	4,58	6	4,8	8	4,4	1	1,01
2nd herd 30	2	2,70	11	9	3	3,6	6	7,5	7	6	1	0,75
Total 60	12	7,7	15	19,4	4	8,24	12	12,2	15	19,4	2	0,05

Figure 7. Observed and expected values of Tf frequencies in Honamli goats

There was non-significant difference (P>0.05) between Cp levels in Kilis goat herds. But on the contrary, high level significant difference (P<0.001) was detected between two Honamli herds. Analyzing Cp values of the breeds, the difference from other was fairly high level (P<0.0001) when two breeds were compared (Tab. 3). Cp level average of Honamli goats was significantly higher than Kilis goats.

There was non-significant difference (P>0.05) at GSH levels between Honamli goat herds. A low level significance (P<0.05) was detected in Kilis goats. In addition, fairly high level significant differences (P<0.0001) were found in herds and in the breed (Table 3).

Table 3. CP and GSH values in Kilis and Honamli goats

		Kilis Goat			Honamli Goat		
		1st Herd	2nd Herd	Total	1st herd	2nd herd	Total
	N	30	30	60	30	30	60
CP	X	0.770	0.874	0.822	2.121	1.356	1.739
	±Sx	±0.05	±0.09	±0.05	±0.16	±0.11	±0.10
		7	5	5	1	1	9
	X_{min}	0.157	0.157	0.157	0.786	0.471	0.471
	X_{max}	1.571	2.986	2.986	5.186	2.829	5.186
	P	NS*			<0.001		
GSH	X	41.45	43.52	42.48	32.92	34.10	33.51
	±Sx	3	0	6	8	2	5
		±0.51	±0.79	±1.03	±0.37	±0.72	±0.34
		2	7	4	9	6	5
	X_{min}	36.53	35.46	35.46	28.80	27.20	27.20
		3	7	6	0	0	0
	X_{max}	45.60	49.60	49.60	36.53	46.66	46.66
		0	0	0	3	7	6
	P	<0.05			NS*		

*Non Significant

The scatter diagram was in Fig. 8. According to study material forming the two goat breeds belonging to the 1st and the 2nd herd's CP values were given in there.

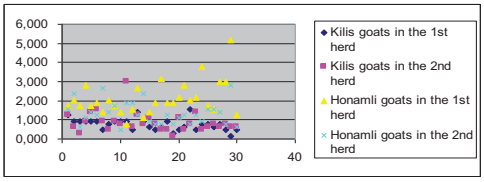


Figure 8. Cp scatter diagram

In Kilis and Honamli goats examined on the basis of GSH levels in herds, the goats above the average named as GSH^H and below average goats named as GSH^h. These two groups were analyzed independently in themselves. Value belonging to the arithmetic mean (X), standard error (Sx) and minimal-maximal values (Xmin-Xmax) were calculated. Results were given in mg/dl. The lowest GSH^h average was observed in the 1st Honamli goat herd (3.1219 ± 0.277 mg/dl); the highest GSH^H average was observed in the 2nd Kilis goat herd (4.7467 ± 0.402 mg/dl) (Table 4).

Table 4. GSH^h and GSH^H levels in Kilis and Honamli goats

		Kilis Goat		Honamli Goat	
		1st herd	2nd herd	1st herd	2nd herd
N		30	30	30	30
GSH^h	X	38.872	40.067	31.219	31.581
	±Sx	±0.440	±0.688	±0.277	±0.412
	X_{min}	36.533	35.467	28.800	27.200
	X_{max}	41.333	43.200	32.533	33.333
GSH^H	X	43.550	47.467	34.524	37.044
	±Sx	±0.327	±0.402	±0.335	±0.950
	X_{min}	41.600	44.533	33.067	34.667
	X_{max}	45.600	49.600	36.533	46.667

GSH scatter diagram of the two goat breeds' the 1st and the 2nd herds was given in Fig. 9.

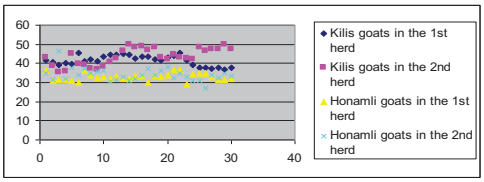


Figure 9. GSH scatter diagram

Obtaining maximum yield from living, raising resistance to various external factors and thus obtaining healthy and superior productive herds of animals is the main target from an economic standpoint.

Hb^A and Hb^B allele frequencies of Kilis goats were calculated as, 0.8333 and 0.1667 in the 1st herd, 0.4833 and 0.5167 in the 2nd herd, respectively. However in Honamli goats, Hb^A and Hb^B allele frequencies were detected as 0.7500 and 0.2500 in the 1st herd, 0.6167 and 0.3833 in the 2nd herd, respectively.

In the 1st and the 2nd herds of Kilis, Tf^A, Tf^B and Tf^C frequencies were calculated as 0.2167, 0.3000; 0.5000, 0.6000 and 0.2833, 0.1000, respectively. The other hand, Tf^A, Tf^B and Tf^C frequencies were detected as 0.4167, 0.3000; 0.4000, 0.5000; 0.1833, 0.2000 in the 1st and the 2nd herds of Honamli breed, respectively.

In this study, TfCC genotype could not be determined in the 2nd herd of Kilis breed. At that rate, only 1 of 4 from the studied herds was not obtained this genotype. The lack of Tf^C allele might be caused this situation. On the

other hand, especially the chance of choosing animals is considered as the main factor.

In this study Kilis and Honamli goat blood samples examined in terms of Hb locus and common allele frequencies were not exceeded 95%. So they have been considered as Hb polymorphic system. The other hand, results correspondingly examined in terms of Tf locus, common allele frequencies were not exceeded 95%. Also, Tf system Yuce ver. Bilgen's study (2004) have been reported a similar manner observed as in the polymorphic. Moreover, also coincides with the rare allele frequency was not less than 5% in this study when Hb and Tf were analyzed. It is desirable in such genetic studies.

In research material, frequencies of Hb genotype could not be provided HWE for herds and populations (Kilis goats: the 2nd herd, intra-population and Honamli goats: intra-population). Panmixi (random mating without selection) of genes and genotypes structure could be said that retains invariance under conditions. In this case the use of a small number of male goats for reproduction was known to be effective. When it comes to selection in goat breeds studied according to whether the reason for departing from the genetic stability of the herd was small and their causes can be connected to use the male. The reverse of this condition was detected in HWE for other populations and herds (Kilis goats: the 1st herd and Honamli goats: the 1st and the 2nd herds). Population size and such as chance factors could be effective on this desired conditions.

Tf results were obtained in analogy to Hb. In research material, Tf genotype frequencies for herds and populations which Kilis goats: the 2nd herd and Honamli goats: the 1st herd were not in the HWE. Genes and genotypes structures could be said that retains invariance under Panmixia conditions. In the case of a small number of male goats in reserched herds were likely inability to maintain the equilibrium. An investigated goat has no selection from breeds in practice. In this case, the main reasons of getting away for departing from the genetic equilibrium were effect of breeding systems, genetic mutations and inadequate herd size. Also, lots of high mating, fertility and reproductive performance of male goats can cause lead to problems in terms of polymorphic studies. This case brings genetic problems due to close kindship. On the other hand, HWE was provided in some herds and populations (Kilis goats: the 1st herd, the 2nd herd and intra-population and Honamli goats: the 2nd herd and intra-population). High level of chance factor can be caused on observed HWE. This equilibrium is desirable genetically.

Observed diversity of Hb and Tf genotypes were interpreted to animals have adapted to different environmental conditions. Additionally this can be adapted to the different environmental conditions of the various animals. If the genotype is very common in a region, environment X genotype interactions presence and indicate living things have a selective advantage. Disease resistance and yield characteristics on the other terms are important in the presence of homozygous and heterozygous genotypes. Also, impact of breed resistance and yield characteristics cannot be ignored.

The weak pasture could be caused significant differences between the obtained Cp results in this study. Furthermore, blood samples were taken in February; after pregnancy and in the first month of lactating period. On the other hand, pasture's weak mineral composition could be caused significant impact on blood levels. In addition, almost all of serum Cu in mammals and birds are in the structure of Cp.

Cobanoglu et al. (2011) have used a total of 15 goats to compare to genetic polymorphisms of Saanen, Malta and Hair goats. They figured out GSH levels on the basis of Emekci and Mert's study (2009) and found GSH^H and GSH^h alleles. Cobanoglu et al. (2011) have viewed the erythrocyte GSH polymorphism in Saanen and Malta goats. They have found GSH^h average as, 13.5 mg/dl and 13.0 mg/dl, respectively. But in Turkish Hair goats, average GSH^H level was measured as 8.9 mg/dl in same study. In these goats GSH^H levels were calculated as 44.6 mg/dl, 39.5 mg/dl and 48.5 mg/dl, respectively while there was a similar results with this study. GSH^h levels were significantly lower than the determined average values.

CONCLUSIONS

At the results of this study conducted on Kilis and Honamli goat herds, the polymorphism results were found to be useful in animal breeding and animal selection. Discussed polymorphism results may be beneficial in animal breeding for population polymorphic studies conducted in various goat breeds.

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REFERENCES

Beutler E., Dubon O., Kelly B.M., 1963. Improved method for the determination of blood glutathione. Journal of Laboratory and Clinical Medicine, 61, pp. 882-888Ceron J.J., Subiella- Martinez S., 2004. An automated spectrophotometric method for measuring canine ceruloplasmin. Veterinary Research, 35, 671-679.

Braend M., 1971. Haemoglobin varients in cattle. Animal Blood Groups and Biochemical Genetics, 2, 15-21.

Burtis C.A., Ashwood E.R., 1999. Tietz Testbook of Clinical Chemistry. 3rd ed, WB Saunders Coumpany, Philadelphia.

Cobanoglu O., Gurcan E.K., Pala A., 2011. Determination of erytrocyte potassium and glutathione polymorphism in Saanen, Maltase and Turkish Hair Goats. Journal of Animal and Veterinary Advances, 10, 14, 1817-1823.

Curzon G., Vallet L., 1960. The putrification of human ceruloplasmin. Biochemical Journal, 74, 279-287.

Dogrul F., 1995. Cesitli koyun irklarinda transferrin ve hemoglobin tiplerinin dagilimi uzerine arastirma. Etlik Veteriner Mikrobiyoloji Dergisi, 5, 8-9, 61-75.

Duzgunes O., Elicin A., Akman N., 1987. Hayvan Islahi. Ankara Universitesi Ziraat Fakultesi Yayinlari, No. 1003, Ankara.

Duzgunes O., Kesici T., Gurbuz F., 1983. Istatistik Metodlari (I). Ankara Universitesi Ziraat Fakultesi Yayinlari, 2. Baski, Ankara.

Emekci S., Mert H., 2009. Norduz kecilerinde hemoglobin, eritrosit potasyum ve glutatyon tiplerinin arastirilmasi. Yuzuncu Yil Universitesi Veteriner Fakultesi Dergisi, 20, 2, 23–26.

Goldstein D.B., Shlotter C., 2000. Microsatellites. 2nd ed.1-22, Oxford.

Jay G.D., Culp D.J., Jahnke M.R., 1990. Silver staining of extensively glycosylated proteins on sodium dodecyl sulfate-polyacrylamide gels, enhancement by carbohydrate-binding dyes. Analytical Biochemistry, 185, 324–330.

Laemmli U.K., 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage. Nature, 227, 680–685.

Mert N., Gunduz H., Akgunduz V., Akgunduz M., 2003. Merinos melezi koyunlarda bazi biyokimyasal kan parametreleri ile verim arasındaki iliskiler, I- Eritrosit potasyum ve glutatyon. Turkish Journal of Veterinary Animal Science, 27, 847-852.

Meyer V.H., 1963. Genetic control of two pre albumins in pigs. Genetics, 48, 1059-1063.

Murray R.K., Granner D.K., Mayes P.A., Rodwell V.W., 1990. Harper'in Biyokimyasi. Baris Kitabevi, Cevirenler: G. Mentes and B. Ersoz.

Nei M., 1987. Molecular Evolutionary Genetics. Columbia University Press, New York.

Kaymakci M., Askin Y., 1997. Keci Yetistiriciligi. Baran Ofset, Ankara.

Kurnianto E., 2009. Pemuliaan Ternak. CV. Graha Ilmu, Yogyakarta.

Pembeci M., 1978. Ataturk Universitesi koyun populasyonlarinda kan potasyum seviyelerinin kalitimi ve verimle ilgileri. Doktora Tezi, Erzurum.

Racusen D., 1978. Glycoprotein detection in polyacrylamide gel with thymol and sulfiric acid. Analytical Biochemistry, 30, 148.

Rizzi R., Caroli A., Bolla P., Acciaioli A., Pagnacco G., 1988. Variability of reduced glutathione levels in massese ewes and its effect on daily milk production. Journal of Dairy Research, 55, 345-353.

Russell P.J., 1992. Genetics. Harper Collins Publishers, New York.

Soysal M.I., 1983. Ataturk Universitesi koyun populasyonunun bazi kalitsal polimorfik kan proteinleri bakimindan genetik yapisi ve bu biyokimyasal karakterler ile cesitli verim özellikleri arasındaki iliskiler. Ataturk Universitesi Fen Bilimleri Enstitusu Zootekni Anabilim Dali, Doktora Tezi, Erzurum.

Ustdal K.M., 1976. Turkiyede' ki bazi yerli sigir irklarinda hemoglobin, transferin ve sut proteinlerinin biyokimyasal polimofizmi uzerinde arastirmalar. Ankara Universitesi Saglik Bilimleri Enstitusu, Doktora Tezi, Ankara.

Yeh F.C., Yang R.C., Boyle T.B.J., Ye Z.H., Mao J.X., 1997. POPGENE, The User-Friendly Shareware for Population Genetic Analysis. Molecular Biology and Biotechnology Centre, University of Alberta, Canada.

Yuce H., Bilgen G., 2004. Bornova tipi kecilerde kan proteinleri polimorfizmi ile bazı sut verim ozellikleri arasindaki iliskiler. Hayvansal Uretim, 45, 2 : 28-32.

Zacharius R.M., Zell T.E., MorrisonJ.H., Woodlock J.J., 1969. Glycoprotein staining following electrophoresis on acrylamide gels. Analytical Biochemistry, 1, 30, Jul., 148–152.

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GENOTYPE EFFECTS ON EGG QUALITY PARAMETERS

Armagan HAYIRLI[1], Guler YENICE[1], Feryaz HIRA[2], Ozgur KAYNAR[1], Sayda Yadira ARMENTA PEREZ[3], Fatih Sabit TIKIR[1], Funda KOCAK[1]

[1]Faculty of Veterinary Medicine, Ataturk University, Erzurum 25240, Turkey,
Phone: +90-442-231 5511, Fax: +90-442-231 5563
[2]Hiraogullari Poultry and Feed Mill Co., Erzincan 24100, Turkey
[3]Faculty of Science, Ataturk University, Erzurum 25240, Turkey

Corresponding author email: ahayirli_2000@yahoo.com

Abstract

This study was performed to compare morphological egg quality parameters in brown and white laying hen hybrids. Eggs (n=90 from Lohman Brown and n=90 from Lohman White at age of 42 weeks old) were obtained from a commercial poultry company raising laying hens in a multi-tier cage system. Egg quality parameters were analysed using one-way ANOVA considering statistical significance at P < 0.05. Except for shape and yolk indexes, other egg quality parameters did not differ by the hybrid (Table 1). Eggs from Lohman Brown had higher shape (77.74 vs. 74.53%) and yolk (4.19 vs. 3.97%) indexes than eggs from Lohman White. In summary, egg quality parameters for brown and white eggs are similar as long as hens are in the same age and are subjected to the same managerial production protocol.

Key words*: egg quality, genotype, conventional production.*

INTRODUCTION

Egg is one of the cheapest and most nutritious food sources for human consumption. Eggshell colour is mainly determined by the genotype. Consumer preference and marketing value for brown and white eggs vary by culture. Based on these farmers are thus eager to raise chickens that are suitable and lucrative for their regions. For instance, in France and UK brown eggs, whereas in Egypt and Japan white eggs are more preferable (Koizumi et al., 1993). There are no differences in taste as well as nutrient profile between white eggs and brown eggs. In market price differences are more related to the cost of egg production, being brown eggs are slightly more expensive than white eggs. This is absolutely not because brown eggs are superior to white eggs in terms of health related nutrient contents. In general brown chickens are heavier, consumes more feed, lay heavier eggs than white chickens (Bell, 1998a).

This experiment was conducted to compare physical characteristics and inner quality parameters of brown and white eggs.

MATERIALS AND METHODS

Eggs (n=90 from Lohman Brown and n=90 from Lohman White at age of 42 weeks old) were obtained from a commercial poultry company raising laying hens in a multi-tier cage system.

Egg quality parameters (Ergün et al., 1987) were:

Shape index (%) = (egg width, cm/egg length, cm)×100,

Shell strength (kg/cm^2) was determined by using machine with the spiral pressure system,

Shell thickness (mm×10^{-2}) was determined in 3 different parts by using a micrometer,

Albumen index (%) = (albumen height, mm/average of albumen length, mm and albumen width, mm)×100

Yolk index (%) = (yolk height, mm/yolk diameter, mm)×100,

Yolk colour was determined by using commercially available "yolk colour fan" according to the CIE standard colorimetric system (Yolk Colour Fan, the CIE standard

colorimetric system, F. Hoffman-La Roche Ltd., Basel, Switzerland), and

Haugh unit = 100×log (AH+ 7.57-1.7×$EW^{0.37}$), where AH = albumen height, mm and EW = egg weight, g.

Egg quality parameters were analysed using one-way ANOVA considering statistical significance at $P < 0.05$.

RESULTS AND DISCUSSIONS

Except for shape and yolk indexes, other egg quality parameters did not differ by the hybrid (Table 1). Eggs from Lohman Brown had higher shape (77.74 vs. 74.53%) and yolk (4.19 vs. 3.97%) indexes than eggs from Lohman White.

In comparison with white eggs, brown eggs were reported to have thinner shell thickness (0.01449 vs. 0.01535 inches), lower shell weight (8.7 vs. 9.4%), higher albumen weight (64.5 vs. 63.1%) and Haugh unit (85.1 vs. 81.9), and lower yolk (26.8 vs. 27.6%) (Bell, 1998b). Similiar inner quality parameters for brown and white eggs were also reported by Curtis et al. (1985).

These values are more related to egg weight than egg nutrient profile Curtis et al. (1986), which are affected by the the diet fed to chickens (Potts and Washburn, 1974).

Table 1. Comparison of egg quality parameters between Lohman Brown and Lohman White

Parameter	Brown egg	White egg	*P* > F
Egg weight, g	65.53±0.51	62.35±0.48	0.80
Eggshell weight, g	7.66±0.07	7.49±0.07	0.10
Eggshell weight, %	12.28±0.11	12.03±0.10	0.10
Eggshell stiffness, kg/cm^2	2.70±0.12	2.72±0.15	0.92
Eggshell thickness, mm	0.39±0.002	3.90±0.002	0.91
Shape index, %	77.74±0.24	74.53±0.25	0.0001
Yolk colour	10.36±0.09	10.17±0.08	0.13
Yolk index, %	4.19±0.03	3.97±0.02	0.0001
Albumen index, %	0.60±0.02	0.64±0.02	0.12
Haugh unit	70.10±0.89	71.95±0.93	0.18

CONCLUSIONS

In conclusion, egg quality parameters for brown and white eggs are similar as long as hens are in the same age and are subjected to the same nutritional and husbandry programmes.

REFERENCES

Bell D., 1998a. Comparing white and brown egg layer performance. http://animalscience.ucdavis.edu/Avian/cpl598.htm

Bell D., 1998b. Comparing white and brown egg layer performance - interior and exterior egg characteristics and composition. http://animalscience.ucdavis.edu/Avian/cpl598.htm

Curtis P.A., F.A. Gardner, D.B. Mellor, 1985. A comparison of selected quality and compositional characteristics of brown and white shell eggs. II. Interior quality. Poultry Sci. 64:302-306.

Curtis P.A., F.A. Gardner, D.B. Mellor, 1986. A comparison of selected quality and compositional characteristics of brown and white shell eggs. III. Composition and nutritional characteristics. Poultry Sci. 65:501-507.

Ergün A., S. Yalçın, I. Çolpan, T. Dikicioğlu, S. Yıldız, 1987. Utilization of vetch by laying hens. J. Fac. Vet. Med. Univ. Ankara 34:449-466.

Koizumi S., M. Nishino, M. Nagano, 1993. Studies on the consumer behavior for animal products, 13: An investigation on hen egg. http://agris.fao.org/aos/records/JP9301809

Potts P. L., K.W. Washburn, 1974. Shell evaluation of white and brown egg strains by deformation, breaking strength, shell thickness and specific gravity. 1. Relationship to egg characteristics. Poultry Sci. 53:1123-1128.

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THE USE OF BLOOD GROUPS AT INDIVIDUAL SELECTION OF CATTLE

Alexandra Konstandoglo, Valentin Focsha

Scientific and Practical Institute by Biotechnologies in Animal Husbandry
and Veterinary Medicine: MD 6525, v. Maximovca, dis. Anenii Noi, Republic of Moldova

Corresponding author email: aconstandoglo@yahoo.com

Abstract

This article presents the results of research, whose purpose was to use alleles AEB-locus of blood groups with individual selection of cattle in animal herds of STE "Maximovca". For individual fixing have been selected 2 bull-producer - Academician 767 and Kiperush 79/395360, which are improvers on a complex of traits with the category $A_2Б_2$. Productivity of their mothers was 9331 kg of milk, with fat content 4.90% and 10915 kg of milk with 4,42 percent of fat, respectively. At bull Academician 767 allele of EAB locus - $B_2O_1/G_2Y_2E'_1Q'$, at bull Kiperush 79 - $G_2Y_2E'_1Q'/G''$. Heifers (mother of the future bulls) belonged to the lines Rozeyf Siteyshna 2671510, Montvik Chifteyna 95679, Pawnee Farm Arlinda Chifa 1427381, of which 47% - marked with allele B_2O_1, 20,0% - with allele $G_2Y_2E'_1Q'$ 'and 35,4% of the animals are marked with other alleles. Genetic distance between the lines, which include dockable bulls and heifers, is large, thus avoiding a future inbreeding.

Key words*: genetic markers, selection, alleles, sire, cows, heifer.*

INTRODUCTION

Genetic marking in the breeding of cattle is based on the integrated use of modern zootechnical and molecular genetic methods in the assessment of the genetic structure of herds, lines, groups and individual animals. As molecular-genetic markers use a variety of allele pool AEB and AEC loci of blood groups and others., that is, the traditional markers of I-generation (Bukarov et al., 2004). In the current system of dairy cattle selection on the alleles of father demonstrates the reliability of the selection of future "improvers" of their sons at the level of 5% (Popov, 1995). But selection of heifers in the breeding herd with the alleles of blood groups approaches to the selection on yield of mothers.

It should be noted that the selection on blood groups should be used to increase the accuracy of prediction of breeding values in combination with other methods of selection, and can not replace them, if only because the antigenic characteristics of the bull does not give plenty of choice, because at bulls prevail individual alleles and antigens. Secondly, currently certified animal on blood groups are gradually replacing more informative methods for studying DNA polymorphism.

For the implementation of individual selection of bulls for breeding stock need the following information: a) a database of blood groups on bull-producer and all bloodstock inventory of potential fathers and mothers of bulls and cows; b) description of herds over the frequencies of antigens, genes and genotypes.

One of the genetic methods, widely used in the Republic of Moldova in the selection of cattle is immunogenetics. Necessarily were subjected for examination of the origin of reconstruction bulls intended for cultivation in elevere and subsequent manning of enterprises in artificial insemination, bull-producing cows, reconstruction young animals and their mothers.

Since 2005, plans for individual fixing for animals of the Moldavian type black and white cattle in a herd of STE "Maximovca" compiled using genetic markers of AEB-locus of blood groups.

The aim of this work is the use the alleles of AEB-locus at individual selection of cattle.

MATERIALS AND METHODS

The materials have served the data on the study of blood groups bulls-produced, as well as serological tests of cows and heifers with expertise authenticity of origin.

In plans of the individual fixing on group of bull-producing cows and heifers in a herd of cattle of STE "Maximovca" attention was

drawn to: a linear membership expectant mothers and to select bulls-produced, breeding category, milk productivity of their mothers, the alleles at AEB locus. Are taken into account genetic distances between the lines of bulls (Focsha V., Alexandra Constandoglo, 2006), which were used in a herd of: STR - Seyling Trayyun Rokita 252803 (n=159); VBA - Vis Back Aidiala 1013415 (n=88); PFAC - Pawnee Farm Arlinda Chifa 1427381 (n=63); RS - Reflection Sovering 198998 (n=62); OA - Osborndeyl Aivengo 1189870 (n=51); MC - Montvik Chifteyna 95679 (n=29); RS - Rozeyf Siteyshna 267150 (n=22): SST - Sanisayd Stendaut Tween 1428104 (n=19); PA - Paklamar Astronaut 1458744 (n=13); UI - Ues Ideal (n=16).

RESULTS AND DISCUSSIONS

As a result, of comprehensive analysis of the breeding and genetic parameters for artificial insemination of heifers were selected 2 bull-produced: Academician 767 and Kiperush 79/395360 (table 1).

Table1.Characteristics of bulls, assigned to heifers

Indices	Bulls-produced	
	Academician 767	Kiperush 79
Breed	Holsteins	Holsteins
Genealogical line	Vis Back Aidiala	Pawnee Farm Arlinda Chifa
Breeding category	A_2B_2	A_2B_2
Place of birth	Canada	
A number of progenitor	4th	2nd
Productivity mother milk: yield, kg fat, %	9331 4,90	10915 4,10
Productivity father's mother: yield, kg fat, %	11713 4,20	11485 3,60
Alleles of AEB-locus	$\mathbf{B_2O_1}/G_2Y_2E'_1Q'$	$\mathbf{G_2Y_2E'_1Q'}/G''$

The bull-producer Academician 767 - line Vis Back Aidiala 1013415, Holstein breed is improver on a complex of traits with the category A_2B_2. Productivity of the mother for the highest lactation was 9331 kg of milk, with fat content 4,90% and the mother of his father - 11713 kg and 4,20%, respectively Allele of AEB locus - $\mathbf{B_2O_1}/G_2Y_2E'_1Q''$.

The bull-producer Kiperush 79-395360 - belongs to the line of Pawnee Farm Arlinda Chifa 1427381 and is the grandson of progenitor line. Yield of his mother was 10915 kg of milk with 4.42 percent of fat, and his mother's of father, respectively 11485 kg and 3.60%. Allele of AEB locus is $\mathbf{G_2Y_2E'_1Q'}/G''$. It should be noted that mothers of bulls used in terms of selection, combined such features as richly-dairy and high fat content of milk, especially at the mother of the bull Academic productivity which was 9331 kg of milk with fat content 4.90%, which is very important for fixing sign of butterfat Holsteins offspring.

It should be noted that in our studies of animals in different herds (Focsha et al., 2003, 2005) it was found that the cow-bearer of alleles B_2O_1 and $G_2Y_2E'_1Q'$ have combinability high milk yield and milk fat and significantly superior to cows-bearer of other alleles. Therefore, our emphasis was mainly on the allele $G_2Y_2E'_1Q'$ and B_2O_1.

In studies (Grindina and Romanenko, 2011) observed the dynamics of allele frequencies carrying a high milk production. Thus, the frequency of allele $G_2Y_2E'_1Q'$ for the entire sample ranged from 10% in 2008 to 18% in 2009, similar results were obtained in studies (Efymenko et al., 2009), where the daughter of the bull Montfrech 91779 were marked with allele $G_2Y_2E'_1Q'$ with a high milk yield.

Selection was carried out and taking into account the genetic distances between the lines of bulls used in the herd of STE "Maximovca" for a long time (Focsha et al., 2006). Figure 1 shows a diagram of genetic distances between the lines indicating the affiliation of bulls to a particular line.

Thus, the line Rozeyf Siteyshna and Montvik Chifteyna, which include the mother of the future of bulls are in different clusters (Fig.2).

Relative to the lines, which include dockable bulls, it should be noted that the line Vis Back Aidiala (Academic 767) is located on the dendrogram apart, and the line Pawnee Farm Arlinda Chifa (Kiperush 79) clustered with the line Sanisayd Stendaut Tween.

The results of cluster analysis showed that the above lines, which are lockable bulls, and also heifers (Table 2, fragment of one of the planes of individual selection), substantially separated from each other, that is the genetic distance between them is large, which will avoid future inbreeding.

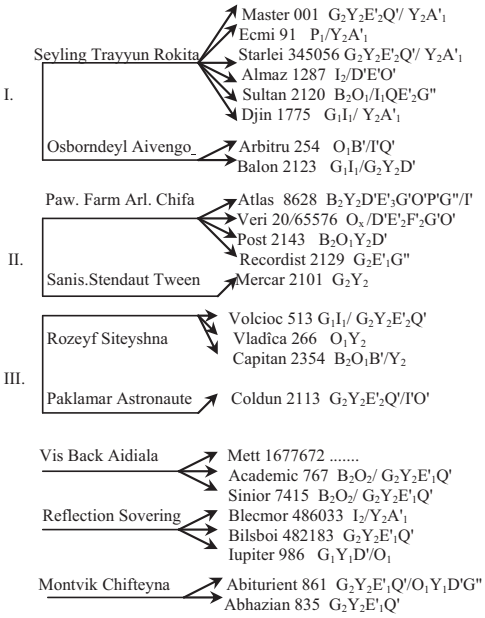

Figure 1. Scheme of genetic distances between the lines of bulls

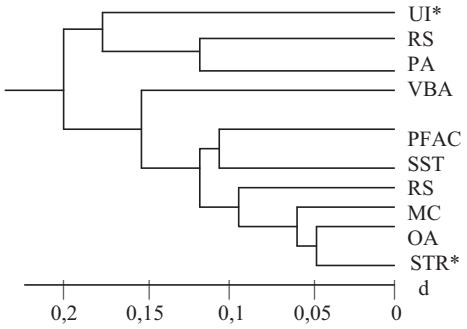

Figure 2. Micro-phylogeny of genetic relationships between the lines bulls-produced in a herd of of STE "Maximovca"

Note: *STR - Seyling Trayyun Rokita; OA - Osborndeyl Aivengo; MC - Montvik Chifteyna; RS-Reflection Sovering; SST - Sanisayd Stendaut Tween; PFAC - Pawnee Farm Arlinda Chifa; VBA - Vis Back Aidiala; PA - Paklamar Astronaut; RS - Rozeyf Siteyshna; UI - Ues Ideal.

Table 2. Individual fixation of bulls per heifers

No. of heifers	The allele of AEB-system of heifers	Mather		Fixed bulls-producer	The allele of AEB-system of bulls
		No. of heifers	productivity		
Line Vis Back Aidiala					
1	B_2O_2B'	718	1-4899-3,62	Academician 767	$B_2O_2/G_2Y_2E'_1Q'$
11	B_2O_1	1085	1-6536-3,72	Kiperush 79	$G_2Y_2E'_1Q'/G"$
114	$O_1T_1Y_1$	432	1-3157-4,08	Academician 767	$B_2O_2/G_2Y_2E'_1Q'$
116	P_2Y_2	674	3-4613-3,59	Academician 767	$B_2O_2/G_2Y_2E'_1Q'$
Line Pawnee Farm Arlinda Chifa					
3	B_2O_2/Q	1037	1-6566-4,08	Academician 767	$B_2O_2/G_2Y_2E'_1Q'$
5	$B_2O_2/G_1I_1I'B"$	1055	1-6664-3,99	Academician 767	$B_2O_2/G_2Y_2E'_1Q'$
7	$B_2O_2/G_2Y_2E'_1Q'$	959	1-4945-4,09	Academician 767	$B_2O_2/G_2Y_2E'_1Q'$
9	G_2Y_2O'/O_2	979	1-6830-4,04	Kiperush 79	$G_2Y_2E'_1Q'/G"$
15	B_2O_1/G_2G'	1201	1-6753-4,08	Academician 767	$B_2O_2/G_2Y_2E'_1Q'$
Line Montvik Chifteyna					
106	$O_1/Y_2D'G'O'$	710	1-3294-3,52	Academician 767	$B_2O_2/G_2Y_2E'_1Q'$
108	G_1I_1	404	1-5695-4,07	Academician 767	$B_2O_2/G_2Y_2E'_1Q'$
110	P_2Y_2	246	2-3607-3,70	Academician 767	$B_2O_2/G_2Y_2E'_1Q'$
118	$G_2Y_2E'_1Q'/O_1$	243	3-4653-3,86	Kiperush 79	$G_2Y_2E'_1Q'/G"$
122	$G_2Y_2E'_1Q'/P_2B'E'_3$	744	3-5081-4,58	Kiperush 79	$G_2Y_2E'_1Q'/G"$
Line Rozeyf Siteyshn					
17	$B_2O_1/I_1QY_2E'_2Y'$	712	1-5933-3,97	Academician 767	$B_2O_2/G_2Y_2E'_1Q'$

Heifers (the mother of the future bulls) belonged to the lines Rozeyf Siteyshna 2671510, Montvik Chifteyna 95679, Pawnee Farm Arlinda Chifa 1427381, of which 47% - marked with allele B_2O_2, 20.0% - with allele $G_2Y_2E'_1Q'$ and 35.4% of the animals marked with other alleles.

CONCLUSIONS

In the selection takes into account genetic markers of bulls-produced and broodstock, all animals belong to genetically distant lines.

Screening and selection of bulls-produced and broodstock is aimed at increasing the

proportion of animals in a herd with these genotypes, which in the future will help increase milk production and milk fat.

REFERENCES

Bukarov N.G., Lebedev E.J., Kaneev A.Z., Morozov I., 2004. Actual problems of genetic research in breeding dairy cattle. "Genetics guarantee of success breeding." VNIIplem with.. nr.1 (80), 12

Efymenko M.J., Podoba B.E., Biryukova O.D., 2009. The role of genetic markers in the genomic selection. Materials Intl. Scientific Conference "Advances in genetics, breeding and reproduction of farm animals" June 9-11, part 2: Sankt. Petersburg, 78-82.

Focsha V., Konstandoglo A., 2003. Using of genetic markers to select cattle. International scientific conference "70 years of State Agrarian University of Moldova" October 7 to 8, Chisinau, 24-25

Focsha V., Konstandoglo A., Smirnov E., Ciubatico V., 2005. Monitoring of alelofund of cattle herd in the experimental section of INZMV. Scientific papers, Livestock and bioengineering. Animal, Vol.13, 138-142

Focsha V., Alexandra Konstandoglo, 2006. The genetic structure of the lines used in herd of experimental section of Institute of Animal Husbandry and Veterinary Medicine, Moldova. Scientific, Animal series. Publishing "Ion Ionescu de la Brad" Iasi, Vol.49, 256-262

Gridina S.L., Romanenko G.A., 2011. Immunogenetic studies in the selection of the Ural Black Pied cattle. Agrarian bulletin Urals, №1 (80), 37.

Popov N.A., 1995. The frequency of occurrence of genes of blood groups and some aspects of selection dairy cattle. Tr. Kostroma MR, Conf. anniversaries, 65-70.

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A LOCAL LIVESTOCK PROTECTION DOG TYPE RAISED IN COKELEZ MOUNTAIN REGION IN DENIZLI PROVINCE OF TURKEY

Orhan Yilmaz[1], Mehmet Ertugrul[2]

[1]Ardahan University, Vocational High School of Technical Sciences, 75000, Ardahan, Turkey
[2]Ankara University, Faculty of Agriculture, Department of Animal Science, 06110, Ankara
Tel: +90-4782112687/5135, Fax: +90-4782112937

Corresponding author email: zileliorhan@gmail.com

Abstract

This study was conducted to define some morphological characteristics of a local type of Turkish Kangal (Karabas) Shepherd Dogs raised in Denizli province by comparing them with certain other breeds from other regions of Turkey, USA and UK. To this end, a total of 48 (39 males and 9 females) dogs were analyzed with the Minitab 16 statistical software program using ANOVA and Student's T-Test. Descriptive statistics were for withers height 78.7 ± 0.59, height at rump78.4 ± 0.60, body length87.6 ± 1.14, heart girth circumferences 91.2 ± 0.86, chest depth36.4 ± 0.63, cannon circumferences 15.3 ± 0.20and tail length 51.0 ± 0.51 cm, respectively. The overall results of the study demonstrated that Turkish Kangal (Karabas) Shepherd Dogs raised in Denizli province had a very close resemblance to dogs raised in the UK and USA, but that they were larger than the dogs raised in other regions of Turkey. It could be because of better life conditions or higher genotypic capacity.

Key words*: Genetic resource, Karabas shepherd dog, morphological trait.*

INTRODUCTION

It is believed by scientist that the domestic dog (*Canis familiaris*) is the first domesticated animal (Clutton-Brock, 1995). Belli has found some rock carving figures in the village of Calli, in the district of Kagizman, in Kars province. Those figures showed that dog was used as a hunting tool to hunt deer and/or wild goats in that region (Yilmaz, 2007[b]).

The Republic of Turkey is like a bridge between Asia and Europe geographically, thus lots of civilizations either lived or passed through Turkey in ancient times. Hence Turkey has a wide array of domestic animal species such as cattle, water buffalos, camels, horses, donkeys, sheep, goat, dogs, cats, rabbit, bees and poultry including lots of breeds (Wilson et al., 2011; Yilmaz and Ertugrul, 2012[c]; Yilmaz and Wilson, 201;, Yilmaz et al., 2012[a-e])

In Turkey there are about 11 dog breeds and five of those are livestock protection dogs. Turkish Kangal (Karabas) Shepherd, Turkish Akbash Shepherd, Kars (Caucasian) Shepherd, Koyun, and Karaman Dogs are native livestock protection dog breeds of Turkey (Yilmaz and Ertugrul, 2011[a-e]; Yilmaz, 2012; Yilmaz and Ertugrul, 2012[a-g]). Turkish Kangal (Karabas) Shepherd is an elegant livestock protection dog breed which is bred by Turks for centuries (Broadhead, 2003; Yilmaz, 2006; Yilmaz, 2007[a]).

The province of Denizli is located in southwest of Turkey and in east of Aegean Region. The province has a passage character among three geographical regions of Aegean, Central Anatolia and Mediterranean. The population of the province is about 0.94 million. Denizli is rich about freshwaters therefore agriculture is a crutial sector in the province (www.denizli.gov.tr, 2012).

A number of studies have been carried out on Turkish Kangal (Karabas) Shepherd Dogs as seen in Table 1. A PhD study was carried out by Kirmizi (1991) on 86 Turkish and 249 German Shepherd Dogs raised at Gemlik Military Veterinary School and Education Centre Commandership (GAVOK) between 1982 and 1990. Yildiz et al. (1993) worked on head sizes of Turkish and German Shepherd Dogs raised at GAVOK. Ozbeyaz (1994) studied the body traits of 59 Kangal Dogs raised at GAVOK. Gonul (1996) carried out a study to determine body traits and training

performance of 202 Turkish and 464 German Shepherd Dogs raised at GAVOK. Tepeli (1996) made a PhD study to determine body traits, growth rate and reproductive performance of 57 Turkish Kangal Shepherd Dogs raised at Research Centre of Veterinary Faculty in Selcuk University. Ozcan and Altinel (1997) worked on some morphological traits of 45 Kangal and 63 German Shepherd Dogs raised at GAVOK. Altuner (1998) prepared a PhD thesis to determine reproductive performance, survival rate, growth and body traits of 32 adult and 167 juvenile Kangal Dogs raised at Ulas Agricultural Management Institution in Sivas province. Tepeli and Cetin (2003) carried out a study on head traits of Kangal and Akbash Shepherd Dogs. In this study 33 Kangal and 30 Akbash Dogs were measured for four head traits. Daskiran (2007) studied to define some morphological traits on 38 Kangal Dogs.

The goal of this study is to define some body measurements of local Kangal (Karabas) livestock protection dogs raised in Denizli Province by comparing with livestock protection dogs raised in different regions of Turkey, USA and UK.

MATERIALS AND METHODS

Experimental animals

The Kangal (Karabas) livestock protection dogs in the study were surveyed in September 2012 in the Denizli province (37º46'N; 29º04'E) (www.googleearth.com). A total of 48 dogs, 39 males and 9 females, were studied. The dogs were aged from1to15 years, and divided into three age groups: 1-2 years, 3-4 years, and 5-15 years. In the first group there were 14 males and 2 females; in the second group there were 15 males and 7 females; and in the third group there were only 10 males. The ages of the dogs were determined from the information given by their owners.

Measurements

The sampled dogs were measured for withers height (WH), height at rump (HR), body length (BL) and chest depth (CD) by using a measuring stick calibrated in centimetres. Other linear measures such as hearth girth circumferences (HGC), cannon circumferences (CC) and tail length (TL) were measured by using a graduated plastic tape (Yilmaz, 2007[a]).

Statistical analysis

The data obtained were analyzed using the Minitab 16 statistical software program. Descriptive statistics for body dimensions were analyzed using ANOVA and Student's T-Test that also determined the impact of sex, age and coat colour group on the response variables of WH, HR, BL, HGC, CD, CC, and TL (Anonymous, 2011).

RESULTS AND DISCUSSIONS

As seen in Table 2, between male and female dogs there were significant differences for morphological traits of WH, HR, and BL ($P<0.01$) and HGC ($P<0.05$). For all results, significant or not, male dogs yielded higher values than females. The coat colour effect was not significant for all morphological traits.

For the age factor there were significant differences among age groups for the traits of WH ($P<0.01$), HR, and CD($P<0.05$). The age group of 5-15 years old had the higher values than the other two groups.

The phenotypic correlation values displayed in the Table 3 showed that most of the observed values were affected by selected factors. The highest values were found between WH and HR ($r = 0.92$) ($P<0.01$). Other high values were found between WH and HGC ($r = 0.68$), HR and HGC (0.63) WH and CD (0.51) and WH and CW ($r = 0.50$) ($P<0.01$), which were higher than $r = 0.50$ ($P<0.01$). The correlations of CD-BL, and HR-CD, HGC-CD, WH-BL and HR-BL also yielded higher values than $r = 0.70$ ($P<0.01$). The lowest and the only negative correlation value ($r = -0.05$) were found between BL-CD ($P<0.05$). Other low correlation values were found between BL-CC ($r = 0.11$) and BL-CW ($r = 0.19$).

Table 1. Some morphological traits on Turkish Kangal (Karabas) dogs

BREED AND SOURCE	WH (cm)	HR (cm)	BL (cm)	HGC (cm)	CD (cm)	CC (cm)	TL (cm)
Kirmizi (1991)	68(♂) 62.9(♀)	-	71.5(♂) 67.4(♀)	-	-	14.7(♂) 14.0(♀)	
Yildiz et al. (1993)	-	-	-	-	-		
Ozbeyaz (1994)	69.1(♂) 62.4(♀)	71(♂) 64(♀)	-	82.1(♂) 73.9(♀)	-		
Gonul (1996)	63		71.2	82.3-85.8(♂) 74.8-80.2(♀)	21.1	13.5-14.8(♂) 12.1-14.3(♀)	
Tepeli (1996)	68.9	70.4	63.8	82.6(♂) 78.0(♀)	24.7(♂) 23.0(♀)	13.5(♂) 13.0(♀)	54.5(♂) 51.5(♀)
Ozcanand Altinel (1998)	-	-	-	-	28.8(♂) 26.8(♀)	13.8(♂) 12.7(♀)	46.8(♂) 43.7(♀)
Altuner (1998)	-	-	-	-	-		
Tepeli and Cetin (2003)	-	-	-	-	-		
Daskiran (2007)	71.7(♂) 65.2(♀)	72.1(♂) 64.5(♀)	71.1(♂) 66.2(♀)	-	-		
Yilmaz (2007[a])	75.9(♂) 73.3(♀)	74.9(♂) 72.2(♀)	86.4(♂) 81.9(♀)	87.2(♂) 84.9(♀)	31.9(♂) 31.2(♀)	13.4(♂) 13.1(♀)	48.3(♂) 47.2(♀)
(www.akdc.com.uk, 2011)	74-81(♂) 71-79(♀)	-	-	-	-		
(www.ukcdogs.com, 2011)	74-81(♂, 71-79(♀)	-	-	-	-		

Table 2.Descriptive statistics and comparison results of some morphological characteristics of Turkish Kangal (Karabas) Dogs for different sexes and ages

Traits	Overall (n=48)	Sex		Age (Year)		
		Male (n=39)	Female (n=9)	1-2 (n=16)	3-4(n=22)	5-15(n=10)
	$\bar{X} \pm S_{\bar{X}}$	$\bar{X} \pm S_{\bar{X}}$	$\bar{X} \pm S_{\bar{X}}$	$\bar{X} \pm S_{\bar{X}}$	$\bar{X} \pm S_{\bar{X}}$	$\bar{X} \pm S_{\bar{X}}$
WH (cm)	78.7 ± 0.59	79.4A± 3.62	78.7B ± 0.59	77.3A ± 3.98	78.0A ± 3.32	82.3B ± 3.83
HR (cm)	78.4 ± 0.38	79.1A± 3.72	75.1B± 4.54	76.9a ± 4.07	78.0b ± 3.61	81.5c ± 4.12
BL (cm)	87.6 ± 1.14	88.5A± 6.84	83.6B± 10.93	84.5 ± 8.81	88.2 ± 7.77	91.2 ± 4.61
HGC(cm)	91.2 ± 0.86	92.1a± 5.42	87.1b± 6.85	90.0 ± 6.96	90.8 ± 5.31	93.9 ± 5.34
CD (cm)	36.4 ± 0.63	36.8 ± 4.55	34.9 ± 3.22	36.5b ± 3.32	34.9a ± 3.97	39.7c ± 5.23
CC (cm)	15.3 ± 0.20	15.6 ± 1.19	14.0 ± 1.30	15.3 ± 1.40	15.0 ± 1.34	15.9 ± 1.17
TL (cm)	51.0 ± 0.51	51.7 ± 3.37	47.9 ± 2.71	51.6 ± 2.47	49.8 ± 3.90	52.5 ± 3.75

a, b, c = P<0.05; A, B = P<0.01

* There were no significant differences between means which had not letters of the alphabet in factor groups.

Table 3. Phenotypical correlation coefficients (r) between body measurements in dogs

Traits	WH	HR	BL	HGC	CD	CC
HR	0.92**					
BL	0.47**	0.47**				
HGC	0.68**	0.63**	0.32*			
CD	0.51**	0.48**	-0.05	0.48**		
CW	0.50**	0.41**	0.19	0.41**	0.45**	
CC	0.22**	0.40**	0.11	0.34*	0.49**	0.38**

*P<0.05, **P<0.01

According to the results obtained in this research, Denizli dogs were large-size livestock protection dogs. The results showed that dogs from Denizli were the largest dog group in Turkish Kangal (Karabas) Shepherd dogs by comparing with other studies. Related with the trait of WH the result obtained agreed with only results from the UK and USA Kennel Clubs. The values of WH in this study were lower than the values reported by other researchers of Kirmizi (1991), Yildiz et al. (1993), Ozbeyaz (1994), Gonul (1996), Tepeli (1996), Ozcan and Altinel (1998), Altuner (1998), Tepeli and Cetin (2003), Daskiran (2007) and Yilmaz (2007).

For the traits of HR, BL, HGC, CD and CC all the values reported by Kirmizi (1991), Yildiz et al. (1993), Ozbeyaz (1994), Gonul (1996), Tepeli (1996), Ozcan and Altinel (1998), Altuner (1998), Tepeli and Cetin (2003), Daskiran (2007) and Yilmaz (2007) were lower than the values of this study. It could be concluded that for the traits of HR, BL, HGC, CD and CC, dogs raised in the other regions of Turkey were lower than dogs raised in Denizli province.The value for TL obtained in this study was lower than results reported by Tepeli (1996), but higher than results reported by Ozcan and Altinel (1998) and Yilmaz (2007).

CONCLUSIONS

The results of the current study showthat Turkish Kangal (Karabas) Shepherd Dogs raised in Denizli Province have a very close resemblance to the dogs raised in the UK and USA. It could also be concluded that the Turkish Kangal (Karabas) Shepherd Dog raised in Denizli was larger than the dogs raised in other regions of Turkey. The overall results of the current study show that the Turkish Kangal Dogs raised in Denizli region are the largest dog group in Turkey and also are similar in size dogs raised in USA and UK. It can be said that could be because of better life conditions or higher genotypic capacity. The dogs raised in Denizli region can be examined more by scientists.

REFERENCES

Altuner A., 1998. Reproductive performance, survival rate, growth and body traits on Kangal Dogs. PhD Thesis (Unpublished). Ankara University, Ankara.

Anonymous, 2011. Minitab 15 Computer Program.

Broadhead P., 2003. The Karabash Kangal Shepherd Dogs. I[st] International Symposium of Kangal Dog.11.July.2003, Sivas, Turkey.

Clutton-Brock J., 1995. Origins of the dog: domestication and early history. In: Serpell J, editor. The domestic dog, its evolution, behaviour and interactions with people. Cambridge: Cambridge University Press., 7–20.

Daskiran I., 2007. Body Weight and Some Morphological Characteristics of Kangal Dogs. Journal of Animal and Veterinary Advances 6 (3): 368-370.

Gonul N., 1996. The body traits and comparative training performances of Turkish Shepherd Dogs and German Shepherd Dogs raised in GAVOK. PhD Thesis.(Unpublished).Uludag University, Bursa.

Kirmizi E., 1991. Comparisons Between Turkish and German Shepherd Dog Breeds Growth and Survival Rates, Reproductive Performance and Body Traits. PhD Thesis.(Unpublished), Istanbul University, 114.

Ozbeyaz C., 1994. Some morphologic traits in Kangal Dogs.Lalahan Hayvancilik Arastirma Enstitusu Dergisi, 34 (1-2): 38-46.

Ozcan M., Altınel A., 1997.Some morphologic traits in Kangal and German Shepherd Dogs. Journal of Istanbul Univ. Veterinary Faculty, 23(2): 413-422.

Tepeli C., 1996. Determination of Growth, Some Body Measurements and Reproductive Traits of Kangal Turkish Shepherd Dogs. PhD Thesis.(Unpublished), Selcuk University, Konya, 70.

Tepeli C., Cetin, O., 2003. A Research on Head Measurements of Turkish Shepherd and German Shepherd Dogs. Selcuk Univ. Veteriner Fakültesi, Konya.

Wilson R.T., Yilmaz O., Ertuğrul M., 2011. The Domestic Livestock Resources of Turkey: Pig Veterinary Journal**,** 66: 26-30.

Yildiz B., Yilmaz O, Serbest A., Kirbiyik H., 1993. A Research on Head Measurements of Turkish Shepherd and German Shepherd Dogs. Uludag University Veterinary Faculty, 12 (1): 35-37.

Yilmaz O., 2006. Breeds of Livestock Protection Dogs. PhD seminar (unpublished). Ankara University, Turkey.

Yilmaz O., 2007[a]. Some Morphological Characteristics of Kangal Dogs Raised in Various Regions of Turkey. PhD thesis (unpublished). University of Ankara, Turkey.

Yilmaz O., 2007[b]. Turkish Kangal (Karabash) Shepherd Dog. Impress Printing Comp. Ankara, Turkey.

Yilmaz O., Ertugrul M., 2011[a]. Some Morphological Traits of the Zagar (erect-ear) Dog in Turkey. Igdir University Journal of Institute of Science and Technology, 1(2): 107-112.

Yilmaz O., Ertugrul M., 2011[b]. Spread Story of Kangal (Karabash) Shepherd Dogs in The World. Igdir

University Journal of Institute of Science and Technology, 1(3): 117-120.

Yilmaz O., Ertugrul M., 2011[c]. Some morphological characteristics of Turkish Tazi (Sighthound). Journal of Animal and Plant Sciences, 21(4): 794-799.

Yilmaz O., Ertuğrul M., 2011[d]. Some morphological characteristics of Turkish Tazi (Sighthound). 2-3.June.2011, 21st International Scientific Conference of Union of Scientist in Stara Zagora, Bulgaria.

Yilmaz O., Ertuğrul M., 2011[e]. Some morphological traits of the Tarsus Fork-nose dog in Turkey. 2-3.June.2011, 21st International Scientific Conference of Union of Scientist in Stara Zagora, Bulgaria.

Yilmaz O., 2012. Determination of Kars (Caucasian) Shepherd Dog Raised in Turkey. Canadian Journal of PureandAppliedScience, 6 (3):2127-2130.

Yilmaz O., Ertuğrul M., 2012[a]. Some Morphological Characteristics of the Tarsus Fork-noseDog in Turkey. Bulgarian Journal of Agricultural Science, Bulgaria 18(1): 111-115.

Yilmaz O., Ertuğrul M., 2012[b]. Determination of the Rize Koyun (sheep) dog in Turkey. Canadian Journal of AppliedSciences, 2(1): 216-221.

Yilmaz O., Ertuğrul M., 2012[c]. Native Dogs Breeds and Types of Turkey. Igdir University Journal of Institute of Science andTechnology, 2(1): 99-106.

Yilmaz O., Ertuğrul M., 2012[d]. Determination of Akbash Shepherd Dog raised in Turkey. Bitlis Eren University Journal of Institute of Science and Technology, 2(1): 7-10.

Yilmaz O., Ertuğrul M., 2012[e]. Determination of Akbaş Shepherd Dog raised in Turkey. 7-8 June 2012, 22st International Scientific Conference of Union of Scientist in Stara Zagora, Bulgaria.

Yilmaz O., Ertuğrul M., 2012[f]. Some Phenotypic Traits of Turkish Kangal (Karabash) Dogs Raised in Europe. V-th International Symposium of Livestock Production of U. Ss. Cyril and Methodius, Institute of Animal Science, 5-7 September 2012, Skopje, Macedonia.

Yilmaz O., Ertugrul M., 2012. Determination of Zerdava Dog raised in Turkey. Journal of Veterinary Advances, 2(9): 462-468.

Yilmaz O., Wilson R.T., 2012. The Domestic Livestock Resources of Turkey: Economic and Social Role, Species and Breeds, Conservation Measures and Policy Issues. Livestock Research for Rural Development, Vol. 24, Article 157.

Yilmaz O., Ertuğrul M., Wilson R.T., 2012[a]. Domestic Livestock Resources of Turkey: Water Buffalo. Tropic Animal Health and Production Journal, 44 (4): 707-714.

Yilmaz O., Ertuğrul M., Wilson R.T., 2012[b]. The domestic livestock resources of Turkey: breed descriptions and status of guard and hunting dogs. 63rd Annual Meeting of the EAAP, 27-31 August 2012, Bratislava, Slovakia.

Yilmaz O., Boztepe S., Ertuğrul M., 2012[c]. The Domesticated Donkey: II – Types and Breeds. Canadian Journal of Applied Science, 2(2): 267-286.

Yilmaz O., Boztepe S., Ertuğrul M., 2012[d]. Phenotypic Characteristics of Turkish Mules. International Journal of Agriculture and Biology, 14: 450–452.

Yilmaz O., Akın O., Yener S.M., Ertugrul M, Wilson R.T., 2012[e]. The Domestic Livestock Resources of Turkey Cattle: Local Breeds and Types and Their Conservation Status. Animal Genetic Resources, 50: 65-74.

www.akdc.com.uk (accessed on 07.05.2011)

www.denizli.gov.tr (accessed on 25.09.2012)

www.googleearth.com (accessed on 07.05.2011)

www.ukcdogs.com (accessed on 07.05.2011)

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THE CHARACTERISTIC OF PROTEOGLYCANS AS NATURALIZE PRODUCT OF SHRIMP WASTE EXTRACT WITH ION SULFATE ON BROILER

Abun[1], Tuti WIDJASTUTI[1], Kiki HAETAMI[2]

[1]Faculty of Animal Husbandry, Universitas Padjadjaran,
[2]Faculty of Fisheries and Marine Science, Universitas Padjadjaran,
Jalan Raya Bandung-Sumedang KM. 21, 45363, Sumedang, Indonesia,
Tel.: +6222 7798241, Fax: +6222 7798212

Corresponding author email: abun_hasbuna.fapet @ yahoo.co.id

Abstract

The naturalization process of shrimp waste extract into proteoglycans with different types of binder was an attempt of making material that is expected to provide benefits to increase the productivity of livestock. This new material (proteoglycans) formed through a reaction mechanism that is highly dependent on the origin and additional components that serve as receptors (inhibitory) or activator (trigger) formation/ occurrence of adhesions (chelating) molecule at the core chain. Research has been carried out in the Laboratory of Animal Nutrition Poultry, Non-Ruminant and Feed Industry, and Laboratory of Feed Chemistry Faculty of Animal Husbandry, University of Padjadjaran, Jatinangor-Sumedang. The experimental design used was completely randomized design (6 X 4), with six treatments on chemical and biological extract binding with 3 types of binders on chemical and 3 types binders of biological extraction of shrimp waste, which was repeated four times. Treatment effect was tested statistically by analysis of variance, and differences between treatments were tested by Duncan's Multiple Range Test. The study was done in two stages, namely the stage of preparation (manufacture proteoglycans with the addition of three types of binders sulfate ions), and the stage of biological test. Testing products through value measurement digestibility and hematological of blood broiler. The results showed that the liquid of shrimp waste extraction (either through chemical or biological processes) can be used as a source of manufacture of proteoglycans and as a feed supplement for monogastric. The characteristic of proteoglycans product based of digestibility value with the high quality was occure both chemical and biological extract binding with potassium sulphate; the medium quality Proteoglycans product was chemical and biological processes with ammonium sulfate binder, and with sodium hidrosulphate binder. While less good quality was for chemical extract with sodium hidrosulphate binder. The number of erythrocytes, leukocytes and hematocrit of blood broilers were normaly for all types of products.

Key words: *Shrimp waste, binder, digestibility, hematological, broiler.*

INTRODUCTION

The naturalization process of shrimp waste extract into proteoglycans with different types of binder was an attempt of making material/ material that is expected to provide benefits to increase the productivity of livestock. New material (proteoglycans) formed through a reaction mechanism that is highly dependent on the origin and additional components which will serve as receptors (inhibitory) or activator (trigger) formation or occurrence of adhesions (chelating) molecule at the core chain. In the otherwise the naturalization process of liquid waste once an environmental management efforts or handling of waste which if left untreated can have a negative impact on the environment. This can occur due to denaturation of the protein components or molecules contained in the liquid waste and pollution as a result of the decay process.

Among the fisheries products developed by the government was the development and post-harvest management of shrimp

farming for export. Waste from these activities in the form of shrimp shells. The waste management efforts carried out by using it as raw material for industrial chitosan. Chitosan needed in many industries, such as pharmaceuticals, textiles, and food. With the increasing demand for chitosan, and the rise of industrial manufacture of chitosan, the greater the volume of liquid waste generated from the chitosan extraction process. This chitosan liquid waste extracts rich in dissolved nutrients, including amino-glycans gluko-uronat and acid-forming potential as an ingredient/ constituent proteoglycans. In addition, chitosan liquid waste extract in a state that is not biologically active as it has undergone a process of biological or chemical denaturation. If such things were allowed to happen denaturation sustained negative impact on the environment.

In order to overcome these two efforts naturalize liquid waste of shrimp waste extraction for chitosan by adding a binder which is expected to be a binder nutrients contained in the waste, and perform the polymerization reaction is terminated by the esterification reaction to stop the process/ polymerization reaction. This study aims to find and characterize the products of the process of denaturation (liquid waste) into proteoglycans with binder containing sulfate ions and its influence on digestibility, metabolizable energy, and hematological blood.

MATERIALS AND METHODS

The study was carried out experimentally in the laboratory with the following stages:

1) Preparation of product (scale up) chitosan extract either chemically or biologically.
2) Preparation of proteoglycans extracted chitosan materials, selected products from the best optimization results.
3) Testing of biological products of the three types of binders proteoglycans sulfate ions that have been characterized physically and chemically, through the measurement of dry matter digestibility and protein products of proteoglycans, and blood hematological values in broiler.

$$D = 100\ \% - 100 \left\{ \frac{\%\ IR}{\%\ IF} \ X \ \frac{\%\ NF}{\%\ NR} \right\}$$

(Schneider and Flatt, 1975)

Information:
D = Digestibility; IR = indicators in ration; IF = indicators in feces; NR = nutrien in ration; NF = nutrien in feces.

4) Measured parameters include :
   a) the content of dry matter and crude protein products of proteoglycans (%);
   b) the lignin content of proteoglycans products (%);
   c) the content of dry matter and crude protein feces (%);
   d) the lignin content of feces (%);
   e) the number of erythrocytes (mm/dL);
   f) the number of leukocytes (mm/dL);
   g) hematocrit value (%).

The experimental design used was completely randomized design (6 X 4), with six treatments on chemical and biological extract binding with 3 types of binders on chemical and 3 types binders of biological extraction of shrimp waste, which was repeated four times. Treatment effect was tested statistically by using analysis of variance, and differences between the treatment effect was tested by Duncan's Multiple Range Test.

RESULTS AND DISCUSSIONS

1) Digestibility of Proteoglycans in Broiler

The potential value of nutritional proteoglycans products from shrimp waste

extract can be determined by chemical analysis. The true value of the missing pieces is shown after feedstuffs digested, absorbed and metabolized (Tillman et al., 1991). The results showed that treatment significantly (P<0.05) to the value of the ration digestibility.

Table 1. Digestibility Mean Value Products Proteoglycans in Each Treatment

Treatment	Dry matter digestibility	Protein digestibility
	.....(%).....	
PgKA	66,17 ^{D}	65,38 ^{D}
PgKP	72,30 ^{A}	73,36 ^{A}
PgKS	58,60 ^{E}	56,44 ^{E}
PgBA	66,12 ^{D}	67,35 ^{C}
PgBP	70,38 ^{B}	71,66 ^{B}
PgBS	68,41 ^{C}	67,17 ^{C}

Note: PgKA = Proteoglycans chemical process with ammonium sulfate binder
PgKP = Proteoglycans chemical process with potassium sulfate binder
PgKS = Proteoglycans chemical process with sodium hydro sulphate binder
PgBA = Proteoglycans biological processes with ammonium sulfate binder
PgBP = Proteoglycans biological process with potassium sulfate binder

Table 1 shows that the dry matter digestibility value in the treatment of binder ammonium sulfate ($(NH_4)_2SO_4$) extracted from either chemically or biologically both showed no significant difference (P>0.05), but significantly (P<0.05) more higher compared sodium hydro sulphate binder, and lower than potassium sulfate and sodium hydrosulfate binders of biological extraction. The use of sodium sulfate binder had lowest dry matter digestibility. The protein digestibility values in proteoglycan products with ammonium hidroksisulfat binder types were not significantly (P>0.05) with sodium hidroxisulfat, but significantly (P<0.05) higher compared with the treatment PgKS and PgKA.

Dry matter digestibility in addition to indicate the portion of nutrients in feed that can be ingested. Reid (1973) argues that there are three categories of quality feed ingredients based digestibility level, ie low quality with digestibility values in the range of 50-60%, with the quality being digestibility values in the range of 60-70%, and high quality with digestibility values above 70%. From the results of this study indicate that potassium sulfate proteoglycans of the type, has a high quality in terms of digestibility. This is understandable because the potassium ion (K) absorption and instrumental help maintain osmotic pressure. K ion permeability of the cell membrane also maintains so traffic in and out of nutrients. In the terms of its characteristics as a constituent amino acid, alkaline products tend to be more profitable. This is because many types of amino acids essential, which is alkaline, such as lysine. The fermentation product was generally acidic. Given that the products tend to be alkaline then it will quickly help neutralize a friendly at the gut, which is more profitable.

Use of proteoglycans bioprocess products (from extraction of Chemicals) PgKP protein digestibility values higher than PgBP. Digestibility value differences caused by the differences in the amount of dissolved nutrients while shrimp waste extraction more, so when naturalization, nutrients much more bound with potassium sulphate ions.

Proteoglycans bioprocess products PgKS had lowest digestibility value. According Kompiang and Ilyas (1983) and Wahju (1997), the difference of digestibility values caused by the differences types of

feed will be processed, including its suitability for broiler hydrolyzed by digestive enzymes. The use of sodium sulfate binder hidroxisulfat ions tends to have a lower protein digestibility than ammonium sulphate and potassium sulphate. This is because the pH of the product is lower PgKS proteoglycans (inclined acid) than the other two types of binders. Results of the first year of the study showed that the pH of the end product of proteoglycans from the $NaHSO_4$ binder was 6, while the results naturalization with ammonium sulfate at pH 7 and pH potassium results at 10. Lehninger (1992) studied that an amino acid formed in pH 4 was aspartic and glutamic acid, while the amino acid lysine which was alkaline (pH 10.5), arginine (pH 12.5), cystine (pH 8.4), and tyrosine (pH 10.5). These amino acids alkaline are more essential requirement for monogastric than aspartate and glutamate.

2) Hematological Value

Blood hematological values of broiler were obtained as products of proteoglycans influence can be seen in Table 2.

Table 2. Mean blood hematological Value of Each Treatment

Treatment	Erythrocytes	Leukocytes	Hematocrit
	(x 10^6 /mm^3)	(x 10^3 /mm^3)	.....(%).....
PgKA	2.37 ^{A}	17.56 ^{A}	31.25 ^{AB}
PgKP	2.46 ^{A}	19.29 ^{A}	33.50 A
PgKS	2.12 ^{B}	15.46 ^{A}	27.50 ^{B}
PgBA	2.38 ^{A}	18.15 ^{A}	31.75 ^{A}
PgBP	2.44 A	19.68 ^{A}	33.00 ^{A}
PgBS	2.39 ^{A}	18.44 ^{A}	32.00 ^{A}

Based on Table 2, the average number of erythrocytes in this study were within the normal range (2.12 to 2.46 x 10^6 piece/mm^3). According to Smith (1987) the number of normal erythrocytes in broilers by 2.0 to 3.2 x 10^6 butir/mm^3. Means the broilers were not impaired in their blood due to physiological systems throughout the normal range flats. The number of erythrocytes per mm^3 of blood varies according to species and also between individuals within a species. According to Swenson (1977), the number of erythrocytes is influenced by several factors, including age, sex, diet quality, disease and environmental temperature.

Protein and minerals contained in liquid waste extraction of shrimp waste was easily digested by broilers. Increased digestion and absorption of nutrients will affect the metabolic processes in the body to be smooth. According Anggorodi (1994) that the metabolic processes that will affect the current living cells including blood cells. Anggorodi (1994) starting that the protein can be used to repair damaged cells and tissues in the body, the metabolic process to be smooth and improve growth. With a smooth affect erythrocyte metabolism that has durability with longer life, thereby reducing the number of damaged cells and affect the overall number of erythrocytes.

Leukocyte counts of broilers in this study were within the normal range in the amount of 15.46 to 19.68 x 10^3 butir/mm^3. According to Smith (1987) normal leukocyte count in broilers at 16-40 x 10^3 butir/mm^3. According Frandson (1993), the number of white blood cells is much less than the red blood cells. Brown and Dellman (1989), adding the number of erythrocytes and leukocytes far below vary depending on the type of animal. Fluctuations in the number of leukocytes in each individual fairly large in certain

conditions such as: stress, physiological activity, nutrition, age, and others. Frandson (1993) stated that the increase in the number of leukocytes is generally a sign of infection or injury.

The results of this study illustrate that the product proteoglycans contain enough nutrients for protein and mineral requirements in each treatment so it would affect the number of leukocytes that uniform so as to increase endurance. According to Swenson (1977), leukocytes play a role in strengthening the immune system of various diseases and wound infections. Proteoglycans treatment effect was not significant on the number of leukocytes, means the product can be used as building blocks of animal feed protein sources in broiler chickens. Diet containing enough protein for broilers can affect a performance, health, and endurance. The high hematocrit value is due to the tendency of high red cell count. According to Swenson (1977) hematocrit value has a positive relationship with the number of erythrocytes. Frandson (1993), adding that the hematocrit value is the percentage of blood that consists of red blood cells (erythrocytes).

CONCLUSIONS

The wastewater chitosan extraction from shrimp shells (either through chemical or biological processes) can be used as a source of manufacture of proteoglycans and can be used as a feed supplement in broiler rations. Proteoglycans product quality characteristics of various types of binders with testing blood hematological values and digestibility of broilers was as follows:

a. Digestibility

1. Better quality (value > 70%): Proteoglycans chemical and biological processes with potassium sulfate binder.
2. Medium quality (60-70% value): Proteoglycans chemical and biological processes with ammonium sulfate binder, and the binder Proteoglycans biological process with sodium hydro sulphate.
3. Less good quality (value < 60%): Proteoglycans chemical process with sodium hydro sulphate binders.

b. Hematologic blood

1. The normal amount of blood erythrocytes (range 2.12 to 2.46 x 106 butir/mm^3) for all types of products.
2. Amount of normal blood leukocytes (range 15.46 to 19.68 x 103 butir/mm^3) for all types of products.
3. Abnormal blood hematocrit value (range from 27.50 to 33.50%) for all types of products.

Suggestion

1. Liquid waste from the extraction of chitosan can be utilized in the manufacture of proteoglycans with binders suggested using potassium hidrosulfat.
2. Products of proteoglycans can be used as a feed supplement in broiler rations to increase the absorption of nutrients and maintain the health of livestock.

REFERENCES

Anggorodi R. 1994. Various Poultry Nutrition. Gramedia Pustaka Utama, Jakarta, 55-59.

Brown A., Dellman I., 1989. Histology Veteriner 1. Third Edition. UI Press, Jakarta.

Frandson R.D., 1993. Anatomy and Physiology Livestock. Edition-4. Gajah Mada University Press.Yogyakarta.

Kompiang I.P., Ilyas S., 1983. Fish Silage: Pengolahan, Pengguna, dan Prospeknya di Indonesia. Jurnal Litbang Pertanian. Balai Penelitian Ternak Ciawi, Bogor.

Lehniger, Terjemahan Maggy Thenawidjaya, 1991. Dasar-dasar Biokimia. Erlangga. Jakarta

Reid J.M., 1973. Thiamine Deficient in Broiler J. Nutrition, 110:139-144.

Schneider B.H., W.P. Flatt, 1975. The Evaluation of Feeds Through Digestibility Experiment. The University of Georgia Press, New York.

Smith J.B., 1987. Pemeliharaan, Pembiakan dan Penggunaan Hewan Percobaan di Daerah Tropis. UI Press, Jakarta.

Swenson M.J., 1977. Blood Circulation and Cardiovascular System , in Dukes Physiology of Domestic Animals. Ad.Cornell. Univ.Press Ithaca. London : 574-587 .

Tillman A.D., H. Hartadi, S. Reksohadiprodjo, S. Prawirokusumo, S. Lebdosoekojo, 1991. Basic Animal Feed Science. Gadjah Mada University Press, Yogyakarta.

Wahju J., 1997. Poultry Nutrition. Edition-4. Gadjah Mada University Press, Yogyakarta.

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IN VITRO ALTERATIONS IN RUMINAL PARAMETERS BY *MEGASPHAERA ELSDENII* INOCULATION ON SUBACUTE RUMINAL ACIDOSIS (SARA)

Mustafa Selcuk ALATAS, Huzur Derya UMUCALILAR

Department of Animal Nutrition and Nutritional Disorders, Faculty of Veterinary Medicine, Selcuk University, Konya 42100, Turkey

Corresponding author email: selcukalatas@gmail.com

Abstract

SARA is a common and serious problem in well-managed and intensive dairy herds or beef cattle operations, because of triggering other metabolic disorders and causing lactation-fertility losses. It is a metabolic disease in dairy cattle that occurs during early and mid-lactation and has traditionally been characterized by low rumen pH, but lactic acid does not accumulate as in acute lactic acid acidosis. Managing the disease, rather than eliminating it, has been suggested in high-producing dairy herds. SARA was induced in vitro to appraise the effectiveness of Megasphaera elsdenii inoculation. Rumen fluid was collected from 2 ruminally cannulated Holstein heifers. Medium was prepared by mixing macromineral (200 ml), micromineral (0.1 ml), buffer (200 ml), reduction (40 ml) and resazurin (1 ml) solutions as well as distilled water (400 ml). The media was then added with a test diet consisting (g/kg) of 550-soluble starch, 260-glucose, 60-cellulose, 70-cellobiose and 60-tripticase, at levels of 10, 15, 20, 25, 30, 40, 50, 70 and 100 g/l. After determining its level causing SARA as reflected by pH (~5.8) in preliminary experimentation, the substrate (test diet, 25 g/l) were incubated with presence of 0, 10^5, 10^6, and 10^7 cfu M. elsdenii per ml at 39°C for 24 h. Rumen parameters were analyzed by 2-way ANOVA. It is determined that most of the tested parameters are not influenced after inoculation of M. elsdenii, but the level of NH3-N (P<0.002) and Butyric acid (P<0.006) in rumen fluid, are observed to increase with the growth of bacterium level. When the bacterium is inoculated in the level of 7 cfu ml^{-1}, it is reached to the highest level of butyric acid (20.29 mM). It is showed that the existent evidence is similar to other studies. In conclusion, addition of M. elsdenii into media, one of the predominant lactate-utilizing bacteria failed to reverse SARA in vitro.

Key words: *in vitro, subacute ruminal acidosis (SARA), Megasphaera elsdenii, rumen fermentation.*

INTRODUCTION

Subacute ruminal acidosis (SARA) is a common and serious problem in well-managed and intensive dairy herds or beef cattle operations, because of triggering other metabolic disorders and causing lactation-fertility losses. *Megasphera elsdenii* is one of the bacteria that are presented in the rumen of high-grain fed cattle (McDaniel et., 2009; Klieve et al., 2003). *Megasphaera elsdenii* is utilized by about 97% of lactate that was generated from starch fermentation (Counotte et al., 1981; Piknova et al., 2004). It is confirmed by both *in vitr*o and *in vivo* studies that rumen pH and acidity can be regulated by the increase in the population of lactate-utilizing bacteria like *M.elsdenii,* so that acidosis may be prevented (Greening et al., 1991; Robinson et al., 1992; Kung and Hession, 1995; Wiryawan and Broker, 1995; Henning et al., 2010). *M. elsdenii* is reported to reduce adaptation period by 5-7 days to high-grain diet when introduced gradually (Klieve et al., 2003).

This study is performed to determine the effect of *M. elsdenii* on the rumen fermentation at *in vitro* SARA conditions.

MATERIALS AND METHODS

Prior to morning feeding, rumen fluids were collected from 2 ruminally cannulated Holstein heifers. Medium was prepared by mixing macromineral (200 ml), micromineral (0.1 ml), buffer (200 ml), reduction (40 ml) and resazurin (1 ml) solutions as well as distilled water (400 ml). In pressure-resistant Pyrex tubes, different amounts (10, 15, 20, 25, 30, 40, 50, 70 and 100 g/l) of test diet (550 g soluble

starch + 260 g glucose + 60 g cellulose + 70 g cellobiose + 60 g tripticase), was mixed with 20 ml rumen fluid and 30 ml buffer at 39°C for 20 h. pH and lactic acid concentration were determined (Sung et al., 2004) to assess amounts of substrates necessary to induce acidosis *in vitro*. After determining its level causing SARA as reflected by pH (~5.8) in preliminary experimentation, the substrate (test diet, 25 g/l) was incubated with presence of 0, 10^5, 10^6, and 10^7cfu *M. elsdenii* per ml at 39°C for 24 h. Gas production, VFA, lactic acid, and NH_3-N, and pH were measured at 2, 4, 6, 8, 10, 12, and 24 h relative to incubation. Amount of gas was calculated based on pressure, which was determined by digital manometer (with sensitivity of 0.2%; Keller Leo 1, Switzerland), in 100 ml bottle (Lopez et al., 2007).

The linear model included the effect of substrate, day, and sampling time as well as their interaction in data analysis using one-was ANOVA (SPSS 16.0.0, 2007). Significance was declared at $P<0.05$.

RESULTS AND DISCUSSIONS

All rumen response variables are summarized in Table 1. When the results of variable concentrations of *M. elsdenii* inoculation subsequent to SARA that is provided by test diet was analyzed; it is observed that 3 different inoculated bacteria concentrations had no effect on pH, lactate accumulation and gas formation levels. It was determined that ammonia (N-NH_3) concentration of *in vitro* media was higher (8.05 mmol/l) with M. *elsdenii* inoculation at 7 cfu ml^{-1} level ($P<0.002$).

Although pH, N-NH_3, lactate accumulation and gas formation levels of the samples that were obtained at the 4, 8, 12 and 24 hours of incubation were altered ($P<0.0001$), no interaction was observed between different bacteria concentration levels and time for pH, ammonia concentration, lactate accumulation and gas formation levels.

It was detected that bacteria inoculation was effective on butyrate concentration ($P<0.006$), highest butyrate concentration level was obtained by the bacteria inoculation at 7 cfu/ml^{-1} level. It was observed that bacteria inoculation had no effect on other volatile fatty acids (VFA). Total VFA concentration varied due to time, total VFA concentration increased as the incubation time prolonged. There was no interaction between time and bacteria in terms of VFA parameters.

Different results may be obtained from *in vitro* studies in comparison with *in vivo* studies because fermentation end products don't be absorbed and accumulate in the media (Menke et al., 1979). When the results of three different bacteria inoculation dosage after SARA that was obtained by test diet was analyzed, it was observed that most of the analyzed parameters hadn't been affected by *M. elsdenii* inoculation. However, ammonia and butyrate concentration of ruminal fluid increased by bacteria concentration elevation.

Table 1. Responses of rumen parameters to addition of *M. elsdenii* into media containing test diet.

Trt	Response variables											
Bacteria cfu ml^{-1}	pH	N-NH_3 (mM)	Lactate (mM)	Gas (ml)	Ac (%)	Pr (%)	Bu (%)	Isobu (%)	Va (%)	Isova (%)	ΣVFA (mM)	Ac:Pr
0	5.81	6.70	0.37	240.00	57.74	18.83	17.94	1.38	2.55	1.57	1.29	3.12
5	5.78	6.76	0.36	238.90	57.68	18.06	17.77	1.45	3.45	1.67	1.31	4.07
6	5.77	7.26	0.32	245.94	58.51	17.53	18.54	1.28	2.59	1.55	1.42	3.39
7	5.81	8.05	0.38	244.23	56.67	17.40	20.29	1.35	2.72	1.56	1.33	3.34
SEM	0.07	0.37	0.08	13.39	0.62	0.46	0.55	0.24	0.36	0.13	0.06	0.39
ANOVA												
B	0.9	0.002	0.36	0.97	0.26	0.1	0.006	0.97	0.27	0.93	0.41	0.38
T	0.0001	0.0001	0.0001	0.0001	0.13	0.78	0.17	0.89	0.08	0.61	0.0001	0.37
B*T	1	0.87	0.85	1	0.52	0.43	0.77	0.31	0.5	0.45	0.34	0.24

Trt: Treatment, B: Bacteria, T: Time

It is reported that inoculation of *M. elsdenii* to the *in vitro* fermentation media had reduced propionate formation and increased butyrate levels (Marounek et al., 1989; Slyter et al., 1992; Kung et al., 1995). Correlatively, bacteria inoculation caused an increase in butyrate formation in this study, too.

As a result of amino acid deamination that *Megasphaera elsdenii* participates in (Bladen et al., 1961; Russell et al., 1988; Rychlik et al., 2002), sometimes more ammonia than bacteria can utilize may be generated in rumen (Leng and Nolan, 1984). Accordingly, it is determined by *in vitro* experiment that *M. elsdenii* inoculation is effective on ammonia level ($P<0.002$).

CONCLUSIONS

M. elsdenii inoculation did not affect fermentation parameters in media containing test diet. Different bacteria levels had no effect on pH, lactate accumulation and gas formation levels; this is considered to be a result of unfavorable conditions for bacteria due to fermentation end products accumulation in media because of not being absorbed.

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REFERENCES

Bladen H.A., Bryant M.P., Doetsch R.N., 1961. A Study of Bacterial Species from the Rumen Which Produce Ammonia from Protein Hydrolyzate. Appl.Microbiol. 9:2, 175-180.

Counotte G.H.M., Prins R.A., Janssen R.H.A.M., Debie M.J.A., 1981. Role of *Megasphaera elsdenii* in the fermentation of DL- [2-'3C] lactate in the rumen of dairy cattle. Appl. Environ. Microbiol. 42: 649–655.

Greening R.C., Smolenski W.J., Bell R.L., Barsuhn K., Johson M.M., Robinson J.A., 1991. Effect of inoculation of *Megasphaera elsdenii* strain 407A (UC 12497) on ruminal pH and organic acids in beef cattle. J. Anim.Sci. 69 (1): 518 (Abstr.).

Henning P.H., Horn C.H., Leeuwa K.J., Meissnera H.H., Hagg F.M., 2010. Effect of ruminal administration of the lactate-utilizing strain *Megasphaera elsdenii* (Me) NCIMB 41125 on abrupt or gradual transition from forage to concentrate diets. Anim. Feed Sci.Technol. 157: 20–29.

Klieve A.V., Hennessy D., Ouwerkerk D., Forster R.J., Mackie R.I., Attwood G.T., 2003. Establishing populations of *Megasphaera elsdenii* YE 34 and *Butyrivibrio fibrisolvens* YE 44 in the rumen of cattle fed high grain diets. J. Appl. Microbiol., 95, 621-630.

Kung L. Jr., Hession A.O., 1995. Preventing in vitro lactate accumulation in ruminal fermentations by inoculation with *Megasphaera elsdenii*. J. Anim.Sci. 73: 250–256.

Leng R.A., Nolan J.V., 1984. Nitrogen metabolism in the rumen. J. Dairy Sci. 67: 1072–1089.

Lopez S., Dhanoa M.S., Dijkstra J., Bannink A., Kebreab E., France J., 2007. Some methodological and analytical considerations regarding application of the gas production technique. Anim. Feed Sci.Technol. 135: 139–156.

Marounek M., Fliegrova K., Bartos S., 1989. Metabolism and some characteristics of ruminal strains of *Megaspha eraelsdenii*. Appl. Environ. Microbiol. 55: 1570-1573.

McDaniel M.R., Higgins J.J., Heidenreich J.M., Shelor M.K., Parsons G.L., Henning P.H., Drouillard J.S., 2009. Effects of *Megasphaera elsdenii* on Ruminal pH, Ruminal Concentrations of Organic Acids, and Bacterial Genomes Following a Grain Challenge. Beef Cattle Research. Kansas State University Agricultural Experiment Station and Cooperative Extension Service, 62-65.

Menke K.H., Raab L., Salewski A., Steingass H., Fritz D., Schneider W., 1979. The estimation of digestibility and metabolizable energy content of ruminant feedingstuffs from the gas production when they are incubated with rumen liquor in vitro. J. Agric.Sci.Camb. 93: 217-222.

Piknova M., Filova M., Javorsky P., Pristas P., 2004. Different restriction and modification phenotypes in ruminal lactate-utilizing bacteria. FEMS Microbiology Letters 236: 91–95.

Robinson J.A., Smolenski W.J., Greening R.C., Ogilvie M.L., Bell R.L., Barsuhn K., Peters J.P., 1992. Prevention of acute acidosis and enhancement of feed intake in the bovine by *Megasphaera elsdenii* 407A. J. Anim.Sci. 70 (1), 310.

Russell J.B., Strobel H.J., Chen G.J., 1988. Enrichment and isolation of a ruminal bacterium with a very high specific activity of ammonia production. Appl. Environ. Microbiol. 54 (4): 872-877.

Rychlik J.L., Lavera R., Russell J.B., 2002. Amino acid deamination by *Megasphaera elsdenii* starins, Current Microbiol. 45: 340-345.

SPSS for Windows. Released 16.0 Sep 13, 2007 Copy right (c.SPSS Inc. 1989–2007).

Slyter L.L., Tung R.S., Kung Jr L., 1992. Effect of monensin and lysocellin on growth and fermentation by pure cultures of ruminal bacteria. J. Appl.Anim. Res. 1: l.

Wiryawan K.G., Broker J.D., 1995. Probiotic control of lactate accumulation in acutely grain-fed sheep. Aust. J. Agric. Res. 46: 1555-68.

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CAUSES OF MILK FAT DEPRESSION IN DAIRY COWS

Mustafa Selcuk ALATAS[1], O Baris CITIL[2], Oguzhan KAHRAMAN[1], Abdullah OZBILGIN[1]

[1]Department of Animal Nutrition and Nutritional Disorders, Faculty of Veterinary Medicine, Selcuk University, Konya 42100, Turkey
[2]Department of Veterinary, KarapinarAydoganlar Vocational School of Higher Education, Selcuk University, Karapinar/Konya, Turkey

Corresponding author email: selcukalatas@gmail.com

Abstract

Fat is typically the most variable component in milk, and is affected by many physiological and environmental factors. In dairy cattle, both the concentration and composition of milk fat are influenced by the diet. Concentration is reduced by feeding diets that contain large proportions of readily-fermentable carbohydrates (starch) and unsaturated fat. Conversely, the percentage of fat in milk can be increased by feeding rumen-inert fats.
Milk fat depression is due to changes in rumen biohydrogenation of unsaturated fatty acids and the passage of specific intermediates of biohydrogenation out of the rumen (e.g. trans-10, cis-12 conjugated linoleic acid) that subsequently reduce milk fat synthesis in the mammary gland. Low milk fat tests typically occur as a result of several concurrent diet or management factors rather than as a result of a single factor. Low rumen pH is a key change in the rumen environment that may lead to flux of fatty acids through alternate pathways of ruminalbiohydrogenation. In this situation, high concentrate/low forage diets or dietary supplements of plant oils or fish oils cause a dramatic decline in milk fat secretion, whereas yields of milk and other milk components remain unchanged. If the feeding of unsaturated fats reduces the numbers or activity of fiber-digesting bacteria in the rumen, then feed intake can decrease, milk production can decrease, and milk fat concentration can decrease.
This review contains the information about the milk fat depression which is caused by improper feeding. And also its huge economic losses in farm animals.

Key words*: Milk Fat Depression, Dairy Cattle, Concentrate Diet, Biohydrogenation.*

1. INTRODUCTION

Fat is the major energy component in milk and accounts for many of the physical properties, manufacturing characteristics, and organoleptic qualities of milk and milk products. Dairy producers have long been interested in milk fat in ruminants because of its economic value, and research has been directed toward understanding the biosynthesis of milk fat and the factors that influence its quantity and fatty acid composition.
Several factors can contribute to change the proportion and total amount of fat in the milk. The most common include genetic makeup, environment, health, season of the year, and physiological state; but fat content and composition of milk can be markedly affected by diet. The fat content in milk can be altered positively or negatively by dietary changes. Synthesis of milk fat is an energy demanding process, but also represents a significant portion of the economic and nutritional value of dairy products.
Milk fat depression (MFD) is classically observed in ruminants fed highly fermentable diets or diets high in plant oils. Varying levels of MFD are commonly experienced today in both intensively and extensively managed dairy herds, and this represents a level of milk fat production below the genetic potential of the cow. Milk fat depression is also a useful variable for evaluating herd management. In many cases onset of diet-induced MFD is an indication of modified ruminal fermentation and in more pronounced cases this can be associated with ruminal acidosis and reduced efficiency. Therefore, maintaining optimal milk fat synthesis has value beyond the milk fat sold. Although we know extensively the cause of MFD we continue to experience MFD because of the high-energy requirements of cows and

the desire to maintain optimal milk production. Numerous dietary factors commonly interact to cause MFD, making prediction difficult.

2. MILK FAT SYNTHESIS

Milk fat is a major component in milk, which is played an important role in supplying energy and accounts for many physical properties and manufacturing characteristics of milk and milk products (Bauman and Griinari, 2001). The content of lipid present in cow milk is usually about 4% (Garton, 1963). In milk fat, the most predominant lipid class (more than 95%) is TAG, followed by approximately 2% of diacylglycerol (DAG), while other lipids include small amounts of phospholipids and cholesterol, about 1 and 0.5%, respectively, and a very small fraction of free FA (about 0.1%) (Jensen and Newberg, 1995). In addition, trace amounts of ether lipids, hydrocarbons, fat-soluble vitamins, flavor compounds and compounds introduced by the feed are present in milk fat (Parodi, 2004).

Milk fat is mainly composed of triacylglycerol (TAG) with 3 fatty acids (FA) esterified into the glycerol-3-phosphate backbone. Fatty acids are classified according to carbon chain length and saturation. Based on chain length, FA are grouped as short-chain FA (4-8 carbons), medium-chain FA (10-14 carbons), and long-chain FA (16 and more carbons). Fatty acids are also classified by desaturation, including saturated FA (no double bond), monounsaturated FA (one double bond), and polyunsaturated FA (more than one double bond).

There are two sources of FA for milk fat synthesis, the *de novo* FA synthesis in mammary epithelial cells and preformed FA uptake from blood circulation derived from either diet or mobilized body fat (Barber et al., 1997). To synthesize milk fat, many enzymatic activities are involved in the pathways, including FA activation, transport, desaturation, TAG synthesis, milk fat globule formation and secretion (Clegg et al., 2001).

3. THE MILK FAT SYNDROME

Most of these theories postulated that limitations in substrate supply for milk fat synthesis caused MFD, generally based on changes in absorbed metabolites as a consequence of alterations in ruminal fermentation. For example, the alterations in the ruminal environment typically include decreased pH and decreased acetate to propionate molar ratio (Bauman and Griinari, 2001). This formed the basis for one of the most widely known substrate supply limitation theories that proposed that acetate supply was limiting milk fat synthesis. However, the reduced ratio of acetate to propionate with highly fermentable diets is predominantly due to increased ruminal production of propionate (Bauman and Griinari, 2001, 2003), and ruminal infusion of acetate to cows during MFD has only a marginal impact on milk fat yield (Davis and Brown, 1970). Overall, several decades of research has tested numerous theories based on substrate limitations and found little to no evidence in their support (Bauman and Griinari, 2003; Bauman et al., 2011; Shingfield and Griinari, 2007).

Davis and Brown (1970) recognized that trans-C18:1 fatty acids (FA) were increased in milk fat of cows with low-milk fat syndrome. They suggested that these trans-FA originated from incomplete ruminalbiohydrogenation of unsaturated FA and might contribute to the development of MFD. Subsequent studies have demonstrated a clear relationship between trans-FA and MFD (Bauman and Griinari, 2003; Bauman et al., 2011; Shingfield and Griinari, 2007). Investigations over the past dozen years have clearly established that diet-induced MFD is associated with rumen production of unique FA from ruminal metabolism of dietary polyunsaturated fatty acids (PUFA). Referred to as the *biohydrogenation theory*, the basis for diet-induced MFD relates to an inhibition of mammary lipid synthesis by specific FA that are intermediates in the biohydrogenation of dietary PUFA, and these are only produced under certain conditions of altered ruminal fermentation (Bauman and Griinari, 2003). Trans-10, cis-12 conjugated linoleic acid (CLA) was the first of these to be recognized and it has been extensively investigated at the whole animal and molecular level (Bauman et al., 2011).

4. DIETARY RISK FACTORS FOR MILK FAT DEPRESSION

Milk fat depression has been associated with a reduction in the acetate to propionate ratio and increased insülin (Byers and Schelling, 1988; Bauman and Griinari, 2003), and the production of trans-octadecenoic acids in the rumen (Griinari et al., 1998; Bauman and Griinari, 2003). Induction of SARA by adding grain pellets or by adding alfalfa pellets to the diet reduced both milk fat and the acetate to propionate ratio in the rumen fluid, but the decrease in this ration was due to an increase in propionate and not due to a decrease in acetate (Gozho et al., 2006; Fairfield et al., 2007; Khafipoor et al., 2007). Griinari et al. (1997) found that a hyperinsulinemic–euglycemic clamp did not depress milk fat. An increase in insulin decreases lipolysis (Bauman and Griinari, 2003). This might explain why stage of lactation and energy balance could affect the milk fat depression, as the contribution that body fat makes to milk fat is much greater in cows in a negative energy balance compared to cows in a positive energy balance. The results obtained by Griinari et al. (1998) support the theory that a low rumen pH caused by feeding a low fiber diet results in incomplete biohydrogenation of fatty acids and increases in trans-octadecenoic acids, and especially the trans-10 isomer of transoctadecenoic acid, that cause milk fat depression. It is not yet understood why experimentally induced SARA increases milk protein, but an increase in rumen digestible organic matter, which increases microbial protein synthesis in the rumen (NRC, 2001) might play a role.

4.1. Diet Fermentability

The microbial population is driven by the substrate available and by the rumen environment and is directly dependent on the concentration of starch and NDF and the rates and extent of ruminal digestion. Maximizing fermentablity is important for energy intake, but care should be given to minimizing subacuteruminal acidosis. Milk fat depression more commonly occurs with corn silage compared to haylage based rations and with more rapidly digested starch sources such as high moisture corn compared to dry ground corn. Providing multiple sources of starch and fiber with overlapping rates of digestion is the safest approach. Additionally, sugar substituted for dietary starch reduces risk without loss of digestibility (Mullins and Bradford, 2010).

Low milk fat is commonly associated with subacute and acute ruminal acidosis, but MFD is frequently observed without a reduction in rumen pH (Harvatine and Allen, 2006a). Rumen pH is dependent on the VFA profile, rate of production, and rate of absorption; buffer secretion; and presence of dietary buffers and varies by approximately 1 to 1.2 pH units over the day (Allen, 1997). It appears that the microbial shift causing MFD occurs before changes in rumen pH are apparent, but may be related to more subtle changes such as the timing of low pH.

4.2. Diet Polyunsaturated Fatty Acids

Unsaturated FA have a dual impact on ruminalbiohydrogenation in that they modify the microbial population and increase the amount of substrate that must be biohydrogenated. It is important to know the total amount of unsaturated fat and also the source, since this dictates the FA profile and rate of ruminal availability. Fish oil has the greatest impact, but is not commonly found in excessive amounts in diets. Cotton, soy, corn, and many other plant oils are high in linoleic acid and incorporation of these grains, oils, and their byproducts increases the risk of MFD. The concept of Rumen Unsaturated Fatty Acid Load (**RUFAL**; Jenkins, 2011) is a simple and insightful calculation that is complemented by consideration of the fat source. There are significant differences in the rate of ruminal availability, for instance cottonseed and whole roasted soybeans are expected to have a much slower release of FA in rumen than distillers grains, ground sources, or oil supplements. Fat is commonly supplemented to increase diet energy density and many protected fat supplements are available. Supplements that are high in saturated fat (palmitic and stearic) do not increase the risk of MFD; however calcium salts of FA are available in the rumen and can reduce milk fat (Harvatine and Allen, 2006b; Lundy et al., 2004). The calcium salt

slows the release of unsaturated fat in the rumen and does reduce the impact of these oils compared to free oil, but does not provide a high level of rumen inertness. The impact of calcium salts depends on the profile of the fat supplement and interaction with other factors. For instance, we have observed in two experiments that calcium salts of palm FA reduced milk fat in high producing cows, but not in low producing cows; presumably because of differences in intake, passage rate, and rumen environment (Harvatine and Allen, 2006a; Rico and Harvatine, 2011).

4.3. Rumen Modifiers

Many supplements have a large impact on the rumen microbial population. Monensin is the most common rumen modifier associated with MFD (Jenkins, 2011). However, it is only a risk factor and can be safely used in many diets. Other rumen modifiers may reduce risk, although their effectiveness generally has not been specifically tested. For example, there may be some potential for 2-hydroxy-4 (methylthio) butanoic acid (**HMB**) to modify milk fat yield (St-Pierre and Sylvester, 2005); although its role in rumen biohydrogenation has not been specifically investigated. Additionally, a direct fed microbial product was shown to stabilize rumen biohydrogenation during a high diet fermentability challenge (Longuski et al., 2009).

4.4. Feeding Strategies

Slug feeding grain is commonly associated with sub-clinical rumen acidosis and MFD. Many assume that TMR feeding eliminates this issue since every bite has the same nutrient composition. However, the rate of intake of fermentable organic matter is very variable over the day due to sorting and variable rates of intake. Generally, cows sort for more fermentable feed particles early in the day, but also consume feed at approximately a three times higher rate after delivery of fresh feed. We recently compared feeding cows once per day or in four equal meals every six hours (Rottman et al., 2011). The frequent feeding treatment decreased the concentration of alternate biohydrogenation FA and increased milk fat yield and concentration. This experimental treatment highlights the potential to increase milk fat through management of feed delivery.

5. HOW TO PREDICT THE OCCURENCE OF MILK FAT DEPRESSION

The complexity of predicting dietary fermentability and associative effects makes prediction of MFD difficult. It is arguably impossible to balance a diet that maximizes milk yield and energy intake without incorporation of numerous risk factors. Ruminant nutrition is best practiced as a continuous experiment that monitors cow response to diet modification (Allen, 2011). It is important to monitor nutrient concentrations and model predicted benchmarks that are applicable to your region and logical based on previous experience with similar diets. However, even with the best feed analysis, software, and experience the interaction of diet ingredients and effectiveness of the diet is best determined by the cow and observed by titration and observation.

Diet fermentability is much more extensively handled by feed analysis and software prediction than dietary fat. Dietary FA have typically been consolidated in ration balancing and simply reported as total ether extract or fat concentration. More recently the FA profile of feedstuffs has been included in feed libraries and a more detailed approach of FA nutrition has been taken (Moate et al., 2004). Effectively utilizing this information in diet formulation represents a challenge because of rumen alterations of dietary FA and the fact that individual FA isomers differ in their biological effect. Thus, based on the current understanding of bioactive FA, effective models must predict ruminal outflow of individual FA, including specific trans-FA isomers. Secondly, the metabolism of FA by rumen bacteria is extremely dynamic and difficult to integrate into prediction algorithms. Ruminal FA models must account for dietary associative effects that modify the predominant pathways and rates of ruminalbiohydrogenation; thereby altering the

pattern of FA outflow. This may require a mechanistic rather than empirical approach to adequately model. Book values are expected to accurately represent the FA profile of forages and grains and testing of individual lots should not be required for most feedstuffs. However, more variability exists in byproducts, which may require frequent testing of FA concentration and profile depending on the byproduct and source. An understanding and quantification of all factors that induce altered ruminal fermentation is not currently available and development of prediction equations that consider dietary risk factors will require further experimentation and more advanced modeling.

6. CONCLUSIONS

Milk fat depression results from an interaction between ruminal fermentation processes and mammary tissue metabolism. MFD continues to be a real-world condition that reduces the efficiency and productivity of dairy cows, but understanding its fundamental basis will allow for effective management and intervention strategies. Management of the risk factors associated with MFD is required to reach both milk and milk fat yield goals. The time course of induction and recovery can be utilized to both identify contributing factors and set expectations for recovery. Lastly, the seasonal and circadian pattern of milk fat synthesis explains variation observed between summer and winter and between milkings and should be considered in monitoring and setting production goal.

REFERENCES

Allen, M. S. 1997. Relationship between fermentation acid production in the rumen and the requirement for physically effective fiber. J. Dairy Sci. 80:1447-1462.

Allen, M. S. 2011. Mind over models.Pages 29-44.*In:* Proc. 20th Ann. Tri-State Dairy Nutr. Conf., Fort Wayne, IN.

Barber, M.C.,Clegg, R.A., Travers, M.T., Vernon, R.G., 1997. Lipid metabolism in the lactating mammary gland. Biochim. Biophys. Acta 1347, 101–126.

Bauman, D. E., and J. M. Griinari. 2001. Regulation and nutritional manipulation of milk fat: low-fat milk syndrome. Livest. Prod. Sci. 70:15-29.

Bauman, D. E., and J. M. Griinari. 2003. Nutritional regulation of milk fat synthesis. Ann. Rev. Nutr. 23:203-227.

Bauman, D. E., K. J. Harvatine, and A. L. Lock. 2011. Nutrigenomics, rumen-derived bioactive fatty acids, and the regulation of milk fat synthesis. Ann. Rev. Nutr. 31:299-319.

Byers, F.M.,Schelling, G.T., 1988. Lipids in ruminantnutrition. In: Church, D.C. (Ed.), The Ruminant Animal, Digestive Physiology and Nutrition. Prentice Hall, Englewood Cliffs, NJ, pp. 298–312.

Clegg, R.A.,Barber, M.C., Pooley, L., Ernens, L., Larondelle, Y., Travers, M.T. 2001. Milkfat synthesis and secretion: molecular and cellular aspects. Livstk. Prod. Sci. 70, 3–14.

Davis, C. L., and R. E. Brown. 1970. Low-fat milk syndrome. Pages 545-565.*In:* Physiology of Digestion and Metabolism in the Ruminant. A. T. Phillipson, ed. Oriel Press, Newcastle upon Tyne, UK.

Fairfield, A.M.,Plaizier, J.C., Duffield, T.F., Lindinger, M.I., Bagg, R., Dick, P., McBride, B.W., 2007. Effects of a prepartum administration of a monensincontrolled release capsule on rumen pH, feedintake, and milk production of transition dairy cows. Journal of DairyScience 90, 937–945.

Garton, G. A. 1963. The composition and biosynthesis of milk lipids. Journal of lipid research 4:237-254.

Gozho, G.N.,Krause, D.O., Plaizier, J.C., 2006. Effects of gradual adaptation to concentrate and subsequentinduction of subacuteruminalacidosis in steers on ruminallipopolysaccharide and acute phase proteins. Journal of DairyScience 89, 4404–4413.

Griinari, J.M.,McGuire, M.A., Dwyer, D.A., Bauman, D.E., Palmquist, D.L., 1997. Role of insulin in theregulation of milkfatsynthesis in dairycows. J DairySci 80, 1076–1084.

Griinari, J.M.,Dwyer, D.A., McGuire, M.A., Bauman, D.E., Palmquist, D.L., Nurmela, K.V., 1998. trans-Octadecenoicacids and milk fat depression in lactating dairy cows. Journal of DairyScience 81, 1251– 1261.

Harvatine, K. J., and M. S. Allen. 2006a. Effects of fatty acid supplements on milk yield and energy balance of lactating dairy cows. J. Dairy Sci. 89:1081-1091.

Harvatine, K. J., and M. S. Allen. 2006b. Fat supplements affect fractional rates of ruminal fatty acid biohydrogenation and passage in dairy cows. J. Nutr. 136:677-685.

Jenkins, T. C. 2011. Managing the rumen environment to control milk fat depression.Pages 31-37.*In:* Penn State Dairy Nutr. Workshop, Grantville, PA.

Jensen, R. G. and D. S. Newberg. 1995. Bovine milk lipids. Handbook of milk composition. Academic Press, San Diego, CA.

Khafipoor, E.,Krause, D.O., Plaizier, J.C., 2007. Induction of subacuteruminalacidosis (SARA) by replacingalfalfa hay with alfalfapellets does not stimulate inflammatory response in lactating dairy cows. J Anim Sci 85 (Suppl. 1), 654.

Longuski, R. A., Y. Ying, and M. S. Allen. 2009. Yeast culture supplementation prevented milk fat depression by a short-term dietary challenge with fermentable starch. J. Dairy Sci. 92:160-167.

Lundy, F. P., E. Block, W. C. Bridges, Jr., J. A. Bertrand, and T. C. Jenkins. 2004. Ruminal biohydrogenationin Holstein cows fed soybean fatty acids as amides or calcium salts. J. Dairy Sci. 87:1038-1046.

Moate, P. J., W. Chalupa, T. C. Jenkins, and R. C. Boston. 2004. A model to describe ruminal metabolism and intestinal absorption of long chain fatty acids. Anim. Feed Sci. Tech. 112:79-105.

Mullins, C. R., and B. J. Bradford. 2010. Effects of a molasses-coated cottonseed product on diet digestibility, performance, and milk fatty acid profile of lactating dairy cattle. J. Dairy Sci. 93:3128-3135.

National Research Council, 2001. National Research Council. Nutrient Requirements of DairyCattle. 7th rev. ed. National Academies Press, Washington, DC.

Parodi, P. 2004. Milk fat in human nutrition. Australian J. Dairy Technol. 59:3-59.

Rico, D. E., and K. J. Harvatine. 2011. Effect of a high palmitic acid fat supplement on ruminal fermentation and milk production in high- and low-producing dairy cows. J. Dairy Sci. 94(E-Suppl. 1): 202(Abstr.).

Rottman, L. W., Y. Ying, and K. J. Harvatine. 2011. Effect of timing of feed intake on circadian pattern of milk synthesis. J. Dairy Sci. 94(E-Suppl. 1): 750(Abstr.).

Shingfield, K. J., and J. M. Griinari. 2007. Role of biohydrogenation intermediates in milk fat depression. Eur. J. Lipid Sci. Technol. 109:799-816.

St-Pierre, N. R., and J. T. Sylvester. 2005. Effects of 2-hydroxy-4-(methylthio) butanoic acid (HMB) and its isopropyl ester on milk production and composition by Holstein cows. J. Dairy Sci. 88:2487-2497.

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EFFECT OF PROTECTED ARGININE SUPPLEMENTATION TO RATION OF AWASSI LAMB ON THE CHEMICAL AND PHYSICAL ANALYSIS OF CARCASSES MEAT

Amera Mohammad Saleh Al-Rubeii[1], Ali A. H. D. Al-Badri [2], Sadeq A. Taha[3]

[1]Department of Animal Resources, College of Agriculture, University of Baghdad, Baghdad, Iraq
[2]Department of Animal production, College of Agriculture, University of Wasit.
[3]State Board of Agric. Res., Ministry of Agriculture, Baghdad, Iraq

Corresponding author email: alrubeii@yahoo.co.uk

Abstract
The objective of this study was to investigate the effect of supplementation protected arginine to the Awassi lambs diets on the chemical and physical analysis of carcasses meat. Twenty five male Awassi lambs ages 3 - 3.5 months and averaged 24,924 kg live body weight were used . Lambs were distributed randomly into five similar group (5 lambs for each) and individually housed, and assigned to five experimental diets by amount of addition of protected arginine . T1 is the (control diet) neither added protected arginine, T2 treatment were lambs fed ***on*** *5g / day of protected arginine, T3 treatment were lambs fed diet control 5 g / cut of arginine twice a week (on eating a three-day break), T4 treatment were lambs fed diet control 7 g / day of arginine and T5 treatment were lambs fed diet control 7g / cut of arginine is the same way as the T3 treatment. All the lambs were assigned in homogeneous batches by the administrative, veterinary and nutritional rules along the experimental period (84 days). Fifteen lambs were slaughtered (3 lambs from each treatment), then carcasses chilled were for 24h at 2°C. Thereafter, several measured were taken in including some chemical and physical analysis. The results of chemical compassion of the leg and rack cuts were obtained treatment T5 was superior (P<0.01) higher than other treatments to record the highest percentage of moisture, protein and lowest percentage in fat. The lamb of T5 treatment was superior (P<0.01) in the results of chemical composition than the other treatments to recorded the highest percentage of moisture, protein, pH, water holding capacity and the lowest percentage of fat for each Longissimus dorsi and Semimembranosus muscles an compared with the other treatment . The lamb of T5 treatment was recorded the lowest percentage of drip loss and cooking loss than the other treatments. It can be concluded from this study that adding the protected arginine to the diets of Awassi lambs improved the quality characteristics of carcasses meat and structure compound by additive of7g protected arginine.*

Key words: *protected arginine, chemical and physical analysis, meat, Awassi lambs.*

INTRODUCTION

The quality of nutrition one of the important reasons that have a direct impact on the productivity of agricultural animals, especially the Iraqi sheep because of the low quantity and quality of forage available and the lack of certain nutrients, especially those related to the green forage and natural pastures, or low levels in the diet provided to the animal it may direct impact on the lower animal productivity agricultural, so attention must be paid to diversity and improved through additions unconventional to diets in order to improve their nutritional value, and access to animals for maximum production level. And this has been confirmed by research and modern technologies in the nutrition science, immunology and endocrinology that the use of nutritional elements such as chromium element and the amino acid arginine has play an important role in regulating the growth, reproduction and immunity in farm animals (Barb, 1991; Cunningham-Rundles, 1993; AL-Dabbas et al., 2008.) The studies were indicated in the nutrition science, immunology, endocrinology and organic chemistry that there are specific nutrients play an important role in regulating the growth and immune function and regulation metabolism (Cunningham-Rundles, 1993). Several studies were indicated that amino acid arginine has

promoting role on the biological and physiological activity in the animal body and on the secretion of growth hormone (Flynn et al., 2002), and prolactin (Rakoff et al., 1973), insulin and glycogen (Palmer et al., 1975), also affects on the immune system and the secretion of hormones, reproductive as well as, absorption of nitrogen and reduce the ammonia toxicity in the tissues (Madden, 1988) and regulate metabolism and immune response (Fu et al., 2005; Morris, 2006; Li et al., 2007) .But few studies that indicated the effect of adding the amino acid arginine protected to the diets in growth rates and the quantity and quality characteristics of the meat produced from the lambs carcasses. Therefore, the objective of this study was to investigate the effect of supplementation protected arginine to the Awassi lambs diets on the chemical and physical analysis of carcasses meat.

MATERIALS AND METHODS

Twenty five male Awassi lambs ages 3 - 3.5 months and averaged 24.924 kg live body weight were used. The study carried out in plant breeding and improvement of sheep and goats of the Ministry of Agriculture / the Authority of the Agricultural Research in Baghdad. Lambs were distributed randomly into five equal groups (5 lambs for each) and individually housed, and assigned to the five experimental diets contenting amount of different protected arginine. Treatment T1 diet (control) canting non protected arginine, treatment (T2) canting 5 g/day, treatment (T3) were lambs fed diet 5 g/day with cut of arginine twice a week (on eating a three-day break), treatment (T4) canting 7 g/day and treatment (T5) were lambs fed diet control 7 g/day with cutting of arginine in the same way as the T3 treatment. All lambs were fed Alfalfa hay ad libitum as a basal diet. Lambs were introduced live body weight (3%) of concentration diet and the diets were offered once daily in the morning. Allowances were recalculated every week according to live weight. All the lambs were assigned in homogeneous batches by the administrative, veterinary and nutritional rules along the experimental period (84 days). Formulation and chemical composition of the experimental diets are shown in Table 1. The diets were contained on 14.82% crude protein and 11.10 MJ/kg DM. At the end of feeding trial, the lambs were slaughtered after over night with draw of feed. Slaughter was performed according to local Muslim practice. Fifteen lambs were Slaughtered (3 lambs from each treatment). Carcasses were weighed and chilled for 24 h at 4°C weighted again and cut into left and right sides, after removing the fat tail from the carcasses. The left side was cut into standardized wholesale cuts (Forrest et al., 1975). The cuts were weighed separately, the chemical analysis for the rack and leg were determined according to according to AOAC (2000). The right side was used to muscles dissection according to the procedures Butterfield et al. (1983) from pelvic limb (SM=*Semimembranosus*) and abdominal wall (LD=*Longissimus dorsi*) the surfaces of muscles are cleaned of all fat and connective tissues and then weight it, the chemical analysis for the LD and SM were determined according to according to AOAC (2000).

The ultimate pH of the muscles LD and SM were determine according to the procedure of Rashid et al. (1983), water holding capacity (WHC) was determined according to Dolatowski and Stasiak (1998), thaw loss and cooking loss were determined according to Denhertog - Meischke et al. (1997), Purchas and Barton (1976), respectively data was statistically analyzed using Completely Randomized Design Model (CRD) procedure by (SAS, 2001). Duncan's multiple range test was used to determine the significance of differences between treatments means.

Table 1. Formulation and chemical composition of concentrate diets

Ingredients	%	C.P %	C.F %	E.E %	NFE %	ASH %	OM %	DM %	M.E MJ/KG DM
Barley	34	3.77	2.72	0.54	22.34	1.82	29.38	31.19	3.88
Yellow corn	20	1.86	0.62	0.78	13.74	1.09	17.00	18.09	3.35
Wheat bran	30	3.57	4.38	1.13	16.41	1.72	25.49	27.21	3.29
Soybean meal	14	5.62	0.71	0.27	5.04	0.95	11.63	12.59	1.50
Salt	1.3	0	0	0	0	1.30	0	1.30	0
Calcium carbonate	0.7	0	0	0	0	0.70	0	0.70	0
Total	100	14.82	8.43	2,72	57.53	7.58	83.50	91.07	10.11

*ME (MJ/ kg DM) = 0.012 CP +0.031 EE+0.005 CF +0.014 NFE (MAFF, 1977)

RESULTS AND DISCUSSIONS

Chemical analysis of leg

The results of chemical analysis of the leg showed the presence of high significant differences ($P<0.01$) among the treatments (Table 2). All treatments added amino acid arginine protected had superiority than control treatment, and the fourth treatment T4 was recorded the highest moisture, protein and lower fat percentage was (58.47, 18.65 and 20.56%, respectively) as compared than the lowest percentages of moisture, protein and a higher percentage of fat in the control treatment was (55.40, 15.66 and 26.71%, respectively). The higher percentage of ash (1.71%) was recorded in treatment fifth addition (T5) as compared with other treatments, while the lowest percentage of ash in the control treatment (1.60%).

Chemical analysis of rack cut

From the results of the chemical analysis of the rack cut (Table 2) observed high significant ($P<0.01$) differences in treatments added amino acid arginine protected than control treatment. The fourth treatment T4 was recorded the highest moisture, protein and lower fat percentage was (58.10, 17.98 and 21.60%, respectively) as compared than the lowest percentages of moisture, protein and a higher percentage of fat in the control treatment was 54.86, 15.12 and 27.79%, respectively. The ash content was observed from the results the second treatment (T2) was recorded higher ash percentage (1.75%), as compared with the other treatments, while the lowest percentage in the control treatment was (1.58%). In light of what came clear that the addition of arginine protected led to improved the quality characteristics of the meat and the fourth treatment (which added to her 7 g arginine) was recorded higher moisture and protein percentages and lower fat percentage as compared with the other treatments. and these results reinforce what we got in the previous results (Al-Badri et al., 2010 a) who showed that increased meat percentage and decreased fat percentage and increased muscles weight and decreased fat deposition in the carcasses and this indicates to improved the efficiency of the muscle production than fat deposition in the carcasses.

Table 2. The effect of protected arginine addition to diets on the chemical analysis of leg and rack of Awassi lambs carcasses (Mean ± standard error).

Cut	Chemical analysis	Treatments				
		T1	T2	T3	T4	T5
Leg	moisture	55.40± 0.13 d	58.20± 0.05 ab	57.73± 0.11 c	58.74± 0.11a	57.88± 0.14 bc
	protein	15.66± 0.04 d	18.52± 0.09 a	17.59± 0.05 c	18.65± 0.12 a	17.96± 0.12 b
	fat	26.71± 0.07 a	21.13± 0.06 d	22.25± 0.09 b	20.56± 0.08 c	21.60± 0.05 c
	ash	1.60± 0.02 d	1.70± 0.02 a	1.67± 0.03 ab	1.65± 0.02 ab	1.71± 0.02a
Rack	moisture	54.86± 0.10 d	57.79± 0.04 b	57.30± 0.07 c	58.10± 0.09a	57.55± 0.07 bc
	protein	15.12± 0.10 d	17.68± 0.03b	17.40± 0.07 c	17.98± 0.06 a	17.75± 0.03 b
	fat	27.79± 0.05a	21.93± 0.06 d	22.75± 0.07 b	21.60± 0.03 e	22.20± 0.05 c
	ash	1.58± 0.01c	1.75± 0.02a	1.65± 0.02 abc	1.60± 0.00bc	1.70± 0.05ab

Means± SE within the same row having unlike letters (a-d) are significantly different among treatments (P<0.01). Control (T1), 5 g/day of protected arginine (T2), 5 g/day, cut of arginine twice a week (on eating a three-day break) (T3), 7 g/day of arginine (T4) and 7 g/day cut of arginine twice a week (on eating a three-day break) (T5).

Chemical analysis of *Longissimus dorsi* Muscle (LD)

The results in the table (3) revealed that effect significant differences for addition to the protected arginine in the chemical analysis of the LD muscle. The moisture percentage was increased in the addition treatments with a highly significant difference (P<0.01) compared with the control treatment. The highest percentage of moisture (70.98%) was found in the fifth treatment T5 and the lowest (69.80%) percentage of moisture in the control treatment. It is noticed that adding arginine led to increase the percentage of protein in the addition treatments with a highly significant difference (P<0.01) compared with the control treatment. The fourth treatment T4 recorded the highest (23.22%) percentage of protein while, the lowest percentage of protein (22.27%) was recorded in the control treatment. These results confirm data referred to previously about increasing the percentages of meat and decreasing the percentages of fat in the main cuts and whole half carcass and the full effect of the positive effect of the arginine to add to the diets of lambs this indicates improve the efficiency of the production of muscle to fat deposition in the carcass account (Al-Badri et al., 2010 b). On the other hand, the percentages of fat were decreased significantly in all addition treatments as compared with the control treatment. The highest percentage (5.55%) of fat in the LD muscle was found in the control treatment, while the fifth treatment T5 (which added to her 7 g arginine) was recorded the lowest (4.04%) fat percentage with a significant difference among the other treatments. It was clear from the results the no significant differences in the percentages of ash among treatments.

Chemical analysis of the *Semimembranosus* Muscle (SM)

The results of the chemical analysis of SM muscle are shown in the table 3. It appears that the addition amino acid arginine treatments were superior (P<0.01) in the chemical

analysis of SM muscle as compared to the control treatment .It has been observed from the results that all addition arginine treatments were superior significantly (P<0.01) in the percentage of moisture, the highest percentage of moisture (73.60%) in the fifth treatment T5 with significant difference than other treatments, while the control treatment was recorded the lowest (72.58%) percentage of moisture. The results showed significant differences in the percentage of muscle protein among treatments, the highest (21.46%) percentage of protein in the fifth treatment T5. However, the control treatment had the lowest (20.62%) percentage of protein. Data indicated that differences among treatments were significant (P<0.01) in the percentages of fat (Table 3). The percentage of fat was decrease significantly (P<0.01) in all addition arginine treatments than control treatment. The highest (4.38%) percentage of fat was recorded in the control treatment while the fifth treatment T5 had the lowest (3.12%) percentage of fat with significant difference than other treatments. The ash percentage was observed from the results that second treatment (T2) was recorded higher ash percentage (1.71%), as compared with the other treatments, while the lowest percentage in the control treatment was (1.60%) and these results confirm what we got in the previous results (Al-Badri et al., 2010 b) about increasing the percentage of meat and decreasing of fat percentage and increased muscle weight and decreased of fat deposition in the carcass and this indicates to improvement in the efficiency of muscle production and decreasing of fat deposition in the carcasses.

Table 3. The effect of addition of arginine protected to diets on the chemical analysis of *Longissimus dorsi* (LD) and *Semimembranosus* (SM) of Awassi lambs carcasses (Mean ± standard error).

Muscle	chemical analysis	Treatments				
		T1	T2	T3	T4	T5
LD	Moisture	69.80± 0.05 d	70.60± 0.02c	70.52± 0.04c	70.80± 0.02b	70.98± 0.04 a
	protein	22.27± 0.02d	22.95± 0.05 b	22.71± 0.04 c	23.22± 0.02a	23.00± 0.05 b
	fat	5.55± 0.02 a	4.50± 0.06b	4.63± 0.01c	4.19± 0.03d	4.05± 0.02 e
	Ash	1.63± 0.01 a	1.70± 0.05 a	1.70± 0.01a	1.61± 0.01a	1.60± 0.05a
SM	Moisture	72.58± 0.04 d	73.05± 0.04 c	72.93± 0.09 c	73.31± 0.02b	73.60± 0.04a
	protein	20.62± 0.03d	21.21± 0.04b	20.96± 0.03c	21.30± 0.02b	21.46± 0.03a
	fat	4.38± 0.02 a	3.51± 0.02c	3.80± 0.02b	3.35± 0.02d	3.12± 0.02e
	Ash	1.60± 0.02 b	1.71± 0.01a	1.70± 0.02a	1.65± 0.02ab	1.61± 0.00b

Means±SE within the same row having unlike letters (a-e) are significantly different among treatments (P<0.01). Control (T1), 5 g/day of protected arginine (T2), 5 g/day cut of arginine twice a week (on eating a three-day break) (T3), 7 g/day of arginine (T4) and 7 g/day cut of arginine twice a week (on eating a three-day break) (T5).

Chemical and Physical Tests

pH and Water Holding Capacity (WHC)

The effect of addition arginine treatments on pH and WHC are summarized in Table 4. Statistical analysis indicated that pH and WHC were affected significantly ($P<0.01$) by addition arginine as compared with the control treatment. Add arginine effect in raising the pH significantly, the higher value (5.82) of pH was recorded in the fifth treatment T5 in LD muscle while the lower value (5.60) of pH was recorded in the control treatment LD muscle. Similar a tendency was obvious towards an increase the pH values in SM muscle by addition arginine as compared with the control treatment (Table 4). The results showed superiority of the fifth treatment (T5) to raise the pH value (5.74) with a significant difference than the other treatments.

Regarding the results of the water holding capacity (WHC), it appears that addition arginine treatments were superior ($P<0.01$) in WHC percentage as compared with the control treatment (Table 4). The highest (63.25%) WHC percentage in the fifth treatment (T5), while the lowest (62.95%) WHC percentage in the control treatment .In the same boat results of water holding capacity appeared in SM, data showed that WHC tended to increase with addition arginine treatments as compared with the control treatment. The fifth treatment (T5) had recorded the highest value (63.25%) of WHC than other treatments, while the control treatment had recorded the lowest value (60.55%) of WHC, and this may be due to high moisture and protein percentages and reduce the amount of fat in meat and that has contributed to increased water retention within the muscle and thus raise the meat's ability to holding the water and reflected that on raising the pH value in meat of the effect of addition amino acid arginine to diets lambs. All addition treatments were improved the chemical characteristics, especially the fifth treatment. In the absence of the studies who show the role of add arginine on the chemical composition of the lambs meat, it can be used the study conducted on rats made by Fu et al. (2005) who showed that the addition of arginine to food by 1.51% for a period of 10 weeks determinate the low results ($P<0.01$) for each of the abdominal fat weight (45%) and subcutaneous fat (25%) and a significant decrease ($P<0.05$) for each of the triglyceride (23%), Free Fatty Acid (27%) and percentage of fat (22-24%) in blood serum. Tan et al. (2008) also reported that, when feeding the pigs was on arginine by 1%, the treatment of arginine improved muscle content (*Longissimus dorsi*) of protein and glycogen, respectively an increase (4.8 0.42%).

Table 4. The effect of protected arginine addition to diets on the pH and water holding capacity (WHC) of *Longissimus dorsi* (LD) and *Semimembranosus* (SM) of Awassi lambs carcasses (Mean ± standard error)

Muscle	Test	Treatments				
		T1	T2	T3	T4	T5
LD	drip loss %	2.35± 0.05 a	2.09± 0.05b	2.17± 0.04 b	1.85± 0.02 c	1.70± 0.05 d
	cooking loss%	27.19± 0.22a	25.14± 0.12b	25.42± 0.15b	23.45± 0.14c	23.25± 0.10c
SM	drip loss %	2.67± 0.05a	2.20± 0.01b	2.26± 0.05b	1.98± 0.07c	1.80± 0.03d
	cooking loss%	28.11± 0.20a	26.70± 0.22b	26.54± 0.18b	26.15± 0.11c	25.97± 0.15c

Means± SE within the same row having unlike letters (a-d) are significantly different among treatments ($P<0.01$). Control (T1), 5 g/day of protected arginine (T2), 5 g/day cut of arginine twice a week (on eating a three-day break) (T3), 7 g/day of arginine (T4) and 7 g/day cut of arginine twice a week (on eating a three-day break) (T5).

Physical Tests Drip loss and Cooking loss

The results presented in Table 5 show that all the addition treatments exhibited low (P<0.01) drip loss and cooking loss percentages as compared with control treatment. The fifth treatment (T5) had the lowest percentages in drip loss (1.70%) and cooking loss (23.25%) while, the control treatment had the highest percentages in drip loss (2.35%) and cooking loss (27.19%) in LD muscle. Similar a tendency was obvious towards an decrease (P<0.01) the drip loss and cooking loss percentages in SM muscle by addition arginine as compared with the control treatment (Table 4). The fifth treatment (T5) had the lowest percentages in drip loss (1.80%) and cooking loss (25.97%) while, the control treatment had the highest percentages in drip loss (2.67%) and cooking loss (28.11%) in SM muscle. That is probably due to the mode of action of addition of arginine in increasing moisture bind, pH and WHC, and hence increases ability of meat tissue to retain water and reduce moisture loss during storage and cooking (Al-Rubeii et al., 2008).

Table 5. The effect of addition of arginine protected to diets on the drip loss and cooking loss percentages % (WHC) of *Longissimus dorsi* (LD) and *Semimembranosus* (SM) of Awassi lambs carcasses (Mean ± standard error).

Muscle	Test	Treatments				
		T1	T2	T3	T4	T5
LD	pH	5.60± 0.01 c	5.76± 0.02ab	5.73± 0.01b	5.80± 0.02ab	5.82± 0.03a
	WHC %	62.95± 0.10d	64.52± 0.05c	64.40± 0.05 c	64.89± 0.01b	65.11± 0.07a
SM	pH	5.58± 0.01d	5.65± 0.02ab	5.63± 0.01cd	5.71± 0.02ab	5.74± 0.02a
	WHC %	60.55± 0.03d	62.75± 0.02c	62.67± 0.04c	63.07± 0.06b	63.25± 0.05a

Means± SE within the same row having unlike letters (a-d) are significantly different among treatments (P<0.01). Control (T1), 5 g/day of protected arginine (T2), 5 g/day cut of arginine twice a week (on eating a three-day break) (T3), 7 g/day of arginine (T4) and 7 g/day cut of arginine twice a week (on eating a three-day break) (T5).

CONCLUSIONS

It is concluded that adding the protected arginine to the diets of Awassi lambs improved the efficiency of meat production, as well as quality and quantity characteristics of their carcasses and structure compound by additive of 7 g protected arginine, and this may give approve to the critical role of these additives in improving growth performance.

REFERENCES

Al-Badri A. A. H. D., A. M. S. Al-Rubeii, S. A. Taha, 2010a. Effect of Arginine Supplementation to Ration of Awassi Lamb on Muscles Weight, Bones, Carcass Fat Partitioning And Distribution. Egyption J. of Applied Sciences, Vol. 25, 4:196-211.

Al-Badri A. A. H. D., A. M. S. Al-Rubeii and S. A. Taha.2010b. Effect of Arginine Supplementation to Ration of Awassi Lamb in Quantity Characteristics of Meat of Their Carcasses. Egyption J. Nutrition and Feeds Vol. 13, 2:285-300.

AL-Dabbas, F. M., A. H. Hamra and F. T. Awawdeh, 2008. The effect of arginine supplementation on some blood parameters, ovulation rate and concentration of estrogen and progesterone in female Awassi sheep. Pakistan J. Biological Science 11 (20):2389-2394.

Al-Rubeii, A.M.S., Hamodi, S.J., Al-Hamdani H.K., 2008. The Effects of using rosemary to improve quality characteristics and sensing of minced cold poultry meat. (Accepted for publication in J. King Faisal University, 9).

A.O.A.C., 2000. Official Methods of Analysis. 17th Ed. Helrich K. Pub. By Association of Official Analytical Chemists, Washington, DC.

Barb C. R., R.R. Kraeling, J.B. Barrett, G.B. Rampacek, R.M. Camphell, T.F. Mowles. 1991. Serum glucose and free fatty acid modulate growth hormone and luteninizing hormone secretion in the pig. Proc. Soc. Exp. Med., 198:636.

Butterfield R.M., J.,Zamora, J.M. Thompson, J. Williams, 1983. Changes in body composition relative to weight and maturity in large and small strains of Australian Merino rams. Ll. Individual muscle groups. Anim.Prod. 36, 165-174.

Cunningham-Rundles S., 1993. Preface. In: M. Decker (Ed.) Nutrient Modulation of the Immune Response. p III. Marcel Dekker, New York.

DenHertog-Meischke M.J.A., F.J.M. Smulderes, Vanloglestijn Vanknapen F., 1997. The effect of electrical stimulation on the water holding capacity and protein denaturation of two bovine muscles. J. Anim. Sci., 75: 118-124.

Dolatowski J.Z., Stasiak, D.M., 1998. The effect of low frequency and intensity ultrasound on pre-rigor meat on structure and functional parameters of freezing and thawed beef Semimembranosus muscle. Proc. 44th Int. Cong. Meat Sci. Technol. Barcelona, Spain.

Flynn N.E., C.J. Meininger, T.E. Haynes, G. Wu, 2002. The metabolic basis of arginine nutrition and pharmacotherapy. Biomed. Pharmacother., 56 : 427-438.

Forrest J.C., Aberle E.D., Hedrick H.B., Judge, M.D., Merkel, P.A., 1975. Principles of meat science, Schweigret, B. S. Ed., Freeman, W. H. and Company, San Francisco

Fu W.J., T.E. Haynes, R. Hu Kohli, R. Hu, J. B. Shi, W.T.E.Spencer, R.J. Carroll, C.J. Meininger, G. Wu, 2005. Dietary L-arginine supplementation reduces fat mass in Zucker diabetic fatty rats. J. Nutr., 135 : 714-721 .

Li P., Yin, Y. L., Li, D. F., Kim, S. W., Wu, G., 2007. Amino acids and immune function. Br. J. Nutr., 98 : 237-252 .

Madden H.P.R., T.,H.L. Breslin, G. Wasserkrug, A. Barbul, 1988. Stimulation of T cell Immunity By arginine Enhances Survival in Peritonitis .J. Surg. Res. 44 : 658-663.

MAFF. 1975. Ministry of Agric . Fisheries and food dept, of Agric, and fisheries for Scotland. energy allowances and feed systems for ruminants. Technical Bulletin. 33 : First published.

Morris J.R., 2006. Arginine: beyond protein. Am. J. Clin. Nutr., 83 : 508S-512S .

Palmer J.P., Walter R.M., Ensinck J.W., 1975. Arginine-stimulated acute phase of insulin and glucagon secretion. I. In normal man. Diabetes., 24 : 735-740 .

Purchas R. W., R.A. Barton, 1976. The tenderness of meat of several breeds of cattle raised under New Zealand pastoral condition.New Zealand J. Agric. Res. 19 : 421-428.

Rakoff J.S., T.M. Siler, Y.N. Sinha, S.S. Yen, 1973. Prolactin and growth hormone release in response to sequential stimulation by arginine and synthetic TRF. J. Clin Endocrinol Metab., 37 : 641-644.

Rashid N.H., Henrickson R.L., Asghor A., Claypool P.L., 1983. Evaluation of certain electrical parameters for stimulating lamb carcasses. J. Food Sci. 48 : 10-14.

SAS, 2001. SAS User's Guid: Statistics (Version6.0).SAS Inst.Inc.Cary.NC. USA.

Tan B., Li X.G., Kong G., Huang R ., Ruan Z., Yao K., Deng Z., Xie M., Shinzato L., Yin Y., Wu G., 2008. Dietary L-arginine supplementation enhances the immune status in early-weaned piglets. Springer-Verlag. DOI 10.1007/s00726-008-0155-1.

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STUDIES ON THE USE OF MILK AND MILK PRODUCTS ATHLETES DIET

BĂRBUICĂ IULIUS SORIN

University of Agronomic Sciences and Veterinary Medicine of Bucharest, 59 Mărăşti Blvd, District 1, 011464, Bucharest, Romania, Phone: +4021 3182564, Fax: +4021 3182567, e-mail: sorin.barbuica@yahoo.com

Corresponding author email: sorin.barbuica@yahoo.com

Abstract

In order to ensure the energy, plastic and catalytic needs, the human consumes food. The consumption of milk and cheese leads to good physical development and also increases the physical resistance, which is also essential in sport activities. The food is a product made of nutrients. Milk and dairy products are also categories of nutritious foods. This paper presents a study on the nutritional value of the milk and dairy products, the advantages and disadvantages of their use in athletes' nutrition and the ration required.

Key words*: milk, dairy products, athletes' nutrition, ration.*

INTRODUCTION

The food is a product consisting of nutrients called trophines. Trophines (substances well-defined from the chemical point of view and indispensable to the humans) are: proteins, lipids, carbohydrates, minerals, vitamins and water(Craciun,1996). In human body, trophines have an important role to maintain its vital functions, to improve and restore cells (plastic role) and to provide the necessary energy needed for the work (energetic role) (Alexandrescu,1994). Depending on their compounds and on their biological value, foods are divided into several groups. Milk and dairy products belong to one of these groups. Milk and cheese combined with cereals may cover the full nutritional needs of an adult.

MATERIALS AND METHODS

The research presented on this paper was concluded by bibliographic study and by experimental method. Butter and cream had been excluded from milk and dairy products group because they are part of fats category.

RESULTS AND DISCUSSIONS

This paper evaluates the sport performances in relation to milk and dairy products nutrients consumption. Butter and cream had been excluded from milk and dairy products group because they are part of fats category. Based on the study will determine nutritional values and the athlete's diet. Milk and cheese should form 15% of the caloric intake (Craciun, 1996).

Nutritional value.

Milk and cheese are the most important source of calcium. Because of this quality it have mineralizing action on children and anti decalcifying action on adults due to their calcium, phosphorus and vitamin D content, which contributes to bone magnesium, sodium and iron salts retention (Hodosan, 2014). Also due to its calcium citrate, potassium, magnesium, and alkaline miliequivalents contents, the milk is the only food of animal origin to be recommended to be given to athletes even after the effort stops (Petrescu, 2002).

Milk and dairy products are a source of protein rich in essential amino acids. They are the cheapest protein animal (casein and lactalbumine) and also have a high digestive utilization coefficient (90-96%), which increases their plastic role. Therefore, milk and cheese consumption is indicated during growing. Being rich in essential amino acids, the proteins in this food category complement

the proteins in food made out of grain (corn, wheat and their derivatives) (Hodosan, 2004). Therefore, polenta or pasta is recommended to be consumed with milk and cheese in order to increase their nutritional value. Milk and its derivatives can also replace meat: whole milk contains all the vitamins as meat contains, but in different proportions. Example: 35 g of cheese or 0.250 l of milk can replace 50 g of meat (Table 1) (Craciun, 1996).

Table 1. The content of vitamins and minerals in milk and dairy products per 100 g of food used in the athletes' diet

Food	Vitamins per 100 grams of food				Minerals in milligrams per 100 grams of food				
	Carotene Y	B1 Y	A (U.I.)	D (U.I)	K	Na	Ca	Fe	P
Cow"s milk	35	45	150	3-4	160	50	125	0,05	90
Cheese	20	30	50	-	120	30	250	0,5	180
Cottage chesse	0	50	1200	20-40	150	2	500	0,6	400
Butter	700	-	3500	50	16	6	15	0,2	25
Sour cream	500	25	2000	35	95	30	70	0,2	60

Whole milk contains all the vitamins but in different proportions. It is rich in vitamins A (retinol), B2 (riboflavin), K (phylloquinone), B2 (pantothenic acid), relatively rich in vitamin D (cholecalciferol), B6 (pyridoxine), B12 (cyanocobalamin), but low in vitamins B1 (thiamine) and C (ascorbic acid). Acid dairy products (sour milk, yogurt, kefir, etc.) are richer in B group vitamins than fresh milk. Also, acid dairy content of lactose help intestinal microbial flora to thrive and to synthesize vitamins of group B. Fat cheeses are also a good source of A and B2 vitamins (Nistor, 2015).

Milk and cheese are also an important source of energy. It ranges from 70 calories per 100 ml. milk, up to 300-400 calories per 100 g of fat cheese.

Athlete rations on milk and milk products

In order to achieve performance and weight control (Table 2), food rations can be combined in athletes' diet by taking into consideration the caloric value of each food compound in relation to the needs of sport branches (Petrescu, 2002).

Table 2. Calories consumption per sports branch

Gymnastics	4500 calories
Boxing, wrestling	4500-5000 calories
Climbing competition	5000 calories
Sports games	4400-4600 calories
Cycling backgroung	6000 calories
Speed swimming	4500 calories
Swimming endurance	5000-5500 calories

Determining the rations was accomplished by taken into consideration a caloric intake of 5000 cal / 24h. This calculation was done for an athlete with an average weight (70 kg) (Table 3) (Alexandrescu, 1994).

Table 3. Average quantities of milk and dairy products that are part of the athlete's ration (protein content, fat, carbohydrate and calories)

Food	Quantity/week	Proteins	Lipids	Carbohydrates	Calories
Cow's milk	7 days x 300 g=2100 g	70	70	96	1340
Cheese	3 days x 100 g=300 g	42	4	12	252
Cottage cheese	3 days x 50 g=150 g	35	38	-	497
Butter	7 days x 50 g=350 g	3	294	1	2754
Sour cream	2 days x 100 g=200 g	6	40	7	426

Milk, taking into consideration its nutritive contribution, may be considered a complete food (Figure 1).

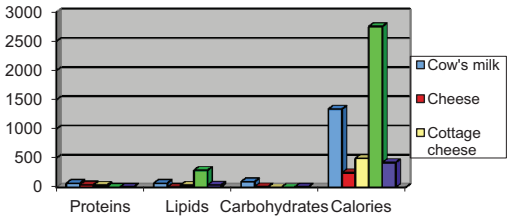

Figure 1. The contents of proteins, fat, carbohydrate and caloric value of the athlete's ration for dairy and milk products

Daily ratio of an athlet should contain about 500 ml of milk and 50 g cheese which, in addition to their nutritional value, will prevent muscle cramps occurrence. Drinking buttermilk or 200-250 ml yogurt is better than consuming soft drinks or beer or wine. Also, cheese, in addition to its nutritional value, help balance the intestinal flora in case of a mixed diet or in case of having a predominantly vegetable diet. However, fermented cheese should not be part of athletes' diet.

CONCLUSIONS

Milk and cheese are the most important source of calcium. Also, they are a source of rich in essential amino acids proteins.

Whole milk contains all the vitamins but in different proportions. It is also an important source of energy.

REFERENCES

Alexandrescu C., 1994. Raţia alimentară a sportivului, Editura Didactică şi Pedagogică, Bucureşti.

Craciun M., 1996. Alimentaţia sportivului, Editura Didactică şi Pedagogică, Bucureşti.

Hodoşan C., 2014. Chimie anorganică, Editura Pim, Iaşi.

Hodoşan C., 2004. Metode fizico-chimice de analiză generală aplicate în industria alimentară, Editura Pim, Iaşi.

Nistor L., 2015. Agricultura generală, Editura Pim, Iaşi.

Petrescu M., 2002. Regimul şi alimentaţia sportivului în perioada de antrenament şi concursuri, Editura Didactică şi Pedagogică, Bucureşti.

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STUDY ON THE USE OF CEREAL AND VEGETABLE IN THE DIET OF ATHLETES

BĂRBUICĂ IULIUS SORIN

University of Agronomic Sciences and Veterinary Medicine of Bucharest, 59 Mărăşti Blvd, District 1, 011464, Bucharest, Romania, Phone: +4021 3182564, Fax: +4021 3182567, e-mail: sorin.barbuica@yahoo.com

Corresponding author email: sorin.barbuica@yahoo.com

Abstract

Normal human diet, as well as the athlete's diet, should include all the nutrients such as proteins, lipids, carbohydrates, water, mineral salts and vitamins. Cereals, along with tubers, roots and wild fruits, were the first human food. This study presents the nutritional value, the nutritious advantages and disadvantages and also the necessary ration of cereal and vegetables in the diet of athletes.

Key words*: athletes' nutrition , cereal products, dried vegetables.*

INTRODUCTION

Rational diet is the diet that fulfils the nutrients quantity and quality needs of human body considering the physiological features, the effort and environmental conditions in which the effort takes place(Alexandrescu,1994). It contains plastic, energy and catalytic substances needed. The state of nutritional balance is the state in which the athlete's body should be found (Craciun, 1996).

MATERIALS AND METHODS

Research method is based on bibliographic study. The most used cereals in human food are: wheat, rice, maize, millet, rye, barley and oats. The most common dried vegetables are beans, peas and lentils. All these must provide 40% of the caloric value of athletes' food ration.

RESULTS AND DISCUSSIONS

The results of the research presents the nutritional values of grain products and vegetables for food rations in athletes' food and also the advantages and disadvantages of using these food groups (Craciun, 1996).

A. Nutritional value

Carbohydrate's main role in the body is to produce energy (Hodosan, 2014). Cereal products are the most important source of energy material, as the starch may provide as much as 70-80% of human carbohydrates needs (Hodosan, 2014). The proteins have a plastic role in the body by forming and growing new cells and by replacing the damaged tissues. Proteins in connection with some enzymes have catalytic action in some reactions of the body. Cereals and dried vegetables can be a source of vegetable proteins. The protide content varies from 7 to 16% for cereals and from 20 to 26% for dried vegetables, reaching up to 32-34% in soy (Taras, 2012). These proteins have a much lower nutritional value than the ones of animal origin because they contain a lower quantity of essential amino acids. Soy proteins have a more balanced content in essential amino acids (Table 1) (Nistor, 2015).

Vitamins (Table 1) are part of the substances acting as enzymes that facilitate various chemical reactions especially those like redox catalysts. The cereals and the dried legumes represent an important source of B vitamins and E vitamin. These vitamins are mainly concentrated in the shell grain (bran) and therefore the vitamin content of extraction flour is reduced. This decrease in vitamins is due baking the bread.

Table 1. The content of vitamins and minerals products derived from cereals and dried vegetables per 100 g food used in the athletes' diet

Food	Vitamins per 100 grams of food				Minerals in miligrams per 100grams of food				
	Carotene Y	B1 Y	PP mg	B6 mg	K	Na	Ca	Fe	P
Rice	30	40	1,0	0,2	200	30	15	0,5	150
Semolina	100	150	2	-	180	25	20	1,5	120
Wheat flour	200	150	5	0,6	350	40	35	3	400
White bread	-	100	1	0,5	120	360	12	1,5	120
Brown bread	-	250	2	0,25	190	400	28	2,5	200
Biscuits	-	60	-	-	90	350	15	0,5	150
Pasta	60	120	1	0,2	140	100	22	1,5	110
Beans	100	700	2,5	0,55	1500	60	110	6	400
Peas	150	600	3	0,45	1000	35	80	5	300

Since bread is the food which mainly covers the humans needs of thiamine (B1), the flour extraction of which bread is made on plays a more significant role. From this point of view black bread is the most appropriate. Even that dried vegetables contain significant amounts of thiamine, their contribution to human needs of thiamine is reduced due to the fact that dried vegetables are consumed in smaller amounts than cereals.

The mineral elements (Table 1) are part of trophines (food substances) with plastic role (calcium, phosphorus, sodium, potassium, iron) and catalytic role (copper salts, iodine, cobalt, iron) (Nistor, 2015). They are indispensable to the organism in maintaining of health and life. Cereals and dried legumes are a source of minerals.

Cereal products contain phosphorus = 200-400 mg %, potassium = 150-350 mg %, magnesium = 50-160 mg %, while dried vegetables contain potassium = 700-1500 mg %, iron and some minerals such as copper and manganese, but they are poor in calcium and sodium (Taras, 2012). Hence, cereals and dried vegetables are not a source of calcium. Consumed in large quantities they can turn insoluble some part of calcium brought in from other foods. This action extends to other minerals.

B. Ration

The food value that composes the ratio is estimated by its content and by its caloric effect per 100 g. Therefore, the diet should contain carbohydrates, lipids and minerals. It was found that calories needs in different sports branches are about 5500 calories/24 hours (Table 2) (Alexandrescu, 1994).

These calories are presented in Table 3 (Alexandrescu, 1994).

Table 2. Grams of energy necessary for an athlete in a sport branch consuming about 5500 calories / 24 hours

	Calories	Grams	
12% Proteins	660	160	Animals origin: 60 %=96 g
			Plant origin: 40%=64 g
30 % Lipids	1650	180	Animals origin: 70%=126 g
			Vegetal origin: 30%=54 g
60% Carbohydrates	3190	790	Polysaccharides: 65%=514 g
			Mono and disaccharides: 35%=276 g

Table 3. Foods of plant origin part of the athlete's ration (protein content, fat, carbohydrate and caloric value)

Food	Quantity/week	Proteins	Lipids	Carbohydrates	Calories
Brown bread	7 days x 250 g = 1750 g	148	26	375	3762
White bread	7 days x 250 g = 1750 g	140	18	875	4340
Pasta	4 days x 50 g =200 g	26	2	252	732
Rice	4 days x 50 g =200 g	8	2	135	610
Biscuits	4 days x 50 g = 200 g	20	20	144	860
Semolina	4 days x 50 g = 200 g	9	-	152	704
Beans, dried peas	4 days x 50 g = 200 g	10	31	324	1280
Wheat flour	7 days x 400 g = 300 g	41	6	252	1250

The average daily amount required by an athlete diet, for different derived food categories belonging to this food group, is made of: bread 500-600 grams; 70-80 pasta grams; flour 50 grams; rice 50 grams; semolina 50 grams; 50 biscuits grams; dry beans or peas 100 grams (Figure 1).

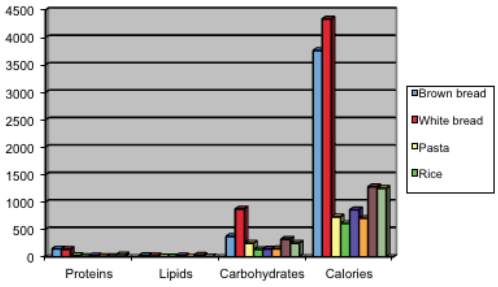

Figure 1. The content of protein, fat, carbohydrate and caloric value of the athlete's ration

C. Advantages and disadvantages

The food categories included in this group may provide almost half the energy needs of the body as well as those of thiamine (Petrescu, 2002). The black bread has a lower caloric value than the white bread, (210 cal % compared to 240 cal %) but it is richer in vitamins and minerals. Due to the fact that it contains more bran it takes longer to digest and therefore it is recommended that, during the first stages and during the recovery phases, the athletes to be administrated intermediary bread, while during competition they should be administrated white bread because it is more easily to be digested (Petrescu, 2002).

CONCLUSIONS

Cereal products are the most important source of energetic material for athlets. Grains and dried legumes can be a source of vegetable proteins. Cereal products and dried legumes are an important source of B vitamins and E vitamin. Dried legumes and grain products are a source of minerals.

REFERENCES

Alexandrescu C., 1994. Raţia alimentară a sportivului, Editura Didactică şi Pedagogică, Bucureşti.

Craciun M., 1996. Alimentaţia sportivului, Editura Didactică şi Pedagogică, Bucureşti.

Hodoşan C., 2014. Chimie anorganică, Editura Pim, Iaşi.

Nistor L., 2015. Agricultura generală, Editura Pim, Iaşi.

Petrescu M., 2002. Regimul şi alimentaţia sportivului în perioada de antrenament şi concursuri, Editura Didactică şi Pedagogică, Bucureşti.

Taras R., Dragatoiu M., Nistor L., Hodosan C., 2012. Study on the mixes of herbicides and fertilizers in the com crops, Scientific Papers, Animal science, Series D, vol.LV, ISSN-L 2285-5750.

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PHENOLIC CONTENT OF SOME MEAL TO RATIONS MIX

O Baris CITIL[1*], Oguz YIGIT[2], Cem KONUK[2], M Selcuk ALATAS[2], Abdullah OZBILGIN[2], Oguzhan KAHRAMAN[2], Mehmet AKOZ[1]

[1]Department of Laborant and Veterinary Health, Karapinar Aydoganlar Vocational School of Higher Education, University of Selcuk, Karapinar, Konya, Turkey, Phone: +90 332 755 6896; Fax: +90 332 755 6300, ozcancitil@gmail.com
[2]Department of Animal Nutrition and Nutritional Disorders, Faculty of Veterinary Medicine, University of Selcuk, Selcuklu, Konya, Turkey, Phone: +90 532 569 1847, Fax: +90 332 2410063, Email: selcukalatas@gmail.com

Corresponding author email: ozcancitil@gmail.com

Abstract

In this study, the phenolic content of sunflower seed meal, cotton seed meal and soybean meal which are added in rations, has been identified. The meals drogs that gathered from 7 different feed factories in Konya, were purified from their fats by extraction with petrolium ether. The extracts prepared from the fat extracted drogs by using 70% aqueous methanol in the agitated water bath at 40 ° C, were used for determination of phenolic compound.
Total phenolic compound of obtained extracts were appointed with spectrophotometer in 750 nm terms of gallic acid as per Folin-Ciocalteu's phenol reactions. The identified phenolic compound of meals was compared with each other. As a result, the sunflower seed meal was identified as the most phenolic compound contained meal.

Key words*: phenolic content, meal.*

INTRODUCTION

Phenolic compounds impart the distinctive acrid flavor and color of the food. As for that, some phenolic compounds play a role in forming the bitter taste. As a component of nutrients, phenolic compounds are important for their functions on human health, effects on generating flavor and odor and contribution to color formation and changes. They also have antimicrobial and antioxidative effects, function on enzyme inhibition and they are determinants of purity control for various foods.

Phenolic compounds cause some unfavorable color changes in foods. The most important of them is enzymatic browning. The enzymes which catalyze reactions that induce phenolic compounds' oxidation are named as poliphenol oxidase enzymes (PPO).

The lipid oxidation that occurs during pulp storage gives animal feeds a bitter taste. Oxidation mechanism develops subsequent to spoilage due to layover. Lipid oxidation products may affect the absorption of some other food substances like proteins (Shahidi et al., 1995). Since oxidized lipids have unfavorable effects on the organism, the importance of inhibiting the lipid oxidation products in the foods has been rising. Consumers usually prefer natural antioxidants rather than synthetic ones (Namiki, 1990). Phenolic substances constitute the most important groups of natural antioxidants (Shahidi et al., 1992). Those are the poliphenolic components that exist in all parts of the plants and the most common herbal phenolic antioxidants are flavonoids, cinnamic acid derivatives, coumarines, tocopherols and phenolic acids. The products that contain phenolic substances are sources of catechin, epicatechin 3-O-gallate, trimeric, tetrameric procyanidins, antimutagenic and antiviral agents (Saito et al., 1998). Phenols also inhibit LDL oxidation (Frankel et al., 1995).

The raw materials of animal feed are the products that can be salvaged by various methods. Fats are one of the important components of animal feed. The residual fat-free part subsequent to fat extraction is important for determining the antioxidant agents from the point of determining the utility of the animal feed. Until today, phenolic substance quantities of some of the fat-free

animal feed raw materials have been calculated via various methods. However, phenolic substance quantities of the pulps added to the rations haven't been compared with each other.

MATERIALS AND METHODS

Sunflower seed meal mixed with rations, cotton seed meal and soybean meal were collected around Konya from seven different feed companies. Feed raw materials around Konya from, Kuzucu, Balci, Tarpas, Seltav and Ozbey company were obtained. The collected samples were placed in one kilogram bags.

In this study we used diethyl ether, methanol, ethanol, chloroform, sulphuric acid, acetone, benzene, folin reagent and gallic acid. Reflux, 250 ml flask, and 100 ml. separatory funnel. Also, beakers, pipettes, flasks, the extractor, Milivial, feed mill, spectrophotometer, Soxhlet, deep-freeze, vortex is used.

Provided meals, with a feed mill, of Selcuk University Faculty of Veterinary Medicine 'in the located 1 mm. Sieve like milled.

Crude analysis of the pulp used AOAC (1990) according to the method, Selcuk University, Faculty of Veterinary Medicine Department of Animal Nutrition and Nutritional Diseases ' s belonging to the Feed Analysis Laboratory, was built in Soxhlet device. Diethyl ether solvent is used in the extraction.

Folin-Ciocalteu method was used for determining the total phenolic substance quantity. Pulp samples and the Gallic acid that would be used as a standard are prepared in 70% methanol. 40 µl from each of the pulp samples were abstracted and 2400 µl of water, 200 µl of undiluted Folin reactive and 600 µl of 20% sodium carbonate were added to the samples. After 2 hours of incubation in room temperature and dark, the absorbance of the reaction admixture was measured versus methanol at 765 nm. Total phenolic content was detected by graphing Gallic acid (0-1 mg/ml) standard curve. The results were indicated as mg of Gallic acid in one gram of microalgae (Singleton et al., 1965).

First of all, mean values of the results of the samples and arithmetic means of seven samples were calculated. Then, standard deviations and standard errors were calculated for each of the mean values. Minimum and maximum values of the samples were determined (Duzgunes, 1987).

RESULTS AND DISCUSSIONS

Phenolic compounds are used to determine the quality factors of animal feed such as flavor, odor and color. They are also helpful in taxonomic studies that aim to differentiate the strains and types and studies on growth, development, rooting and graft incompatibility mechanisms. They are responsible for color and flavor perversion that occurs during animal feed storage. The phenolic substances that are present in herbal structure are very important for plant growth and productivity. In addition, it is known that they play role in many physiological mechanisms of the plants such as cold tolerance and disease resistance mechanisms. For this reason, it is very important to analyze the quantity of phenolic compounds in animal feed raw materials.

Phenolic substances prevent oxidation of LDL-lipoproteins, platelet coagulation and red cell damage by their antioxidant properties. In addition, they are also effective as metal binders, antimutagenic and anticarcinogenic agents (Minussi et al., 2003). They also play a role in cardiac health (Ahn et al., 2002).

Phenolic compounds have an important role in plants' growth and development processes with their complicated chemical structures and various derivatives and they form the second most common constituent of the plant structure after carbohydrates.

Phenolic compounds include many compounds that have an aromatic ring which contains at least one hydroxyl group and are important for their features regarding flavor, aroma, color, quality, nutritive value, storage characteristics, pharmacological and toxic effects. In addition, these compounds are also used in taxonomic studies to identify species and strains. Many plant studies showed that phenolic compounds are mostly present in plastids of cells, while they are found in endoplasmic reticulum during the period after fruit set and dispersed intracellularly during the following stages (Kalalb et al., 1993). Many studies that have been conducted until today suggested that many technical and cultural processes such as plant species and strain, plant and shoot age, hormone

and carbohydrate (saccharose and nitrate) contents of tissues, pruning with ecological factors, ring budding, irrigation, fertilization, use of substances regulating external growth and agricultural struggle modify the synthesis of phenolic compounds (Artik et al., 1997).
In this study, fat contents were 1.62% for sunflower seed pulp, 2.76% for cotton seed pulp and 2.12% for soybean pulp. In addition, productivities of each sample were calculated. These productivity values were 98.39% for sunflower seed pulp, 97.25% for cotton seed pulp and 97.88% for soybean pulp.

CONCLUSIONS

Our analysis showed that calculated phenolic substance content of sunflower seed pulp indicated as equivalent gallic acid was 140.08 g/mg. It was 14.61 g/mg for cotton seed pulp and 2.83 g/mg for soybean pulp. According to these results, the most significant antioxidative effect was in sunflower seed pulp, which means that the sample with the most significant antioxidant capacity was the one which was richest in phenolic substance.

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REFERENCES

Ahn M., Kumazawa S., Usui Y., Nakamura J., Matsuka M., Zhu F., Nakayama T., 2007. Antioxidant activity and Constituents Of Propolis Collected in Various Areas of China, Food Chemistry, 101, 1400-1409.

AOAC, 2003. International. Official Methods of Analysis of AOAC International, 17th Ed. 2nd Revision. Gaithersburg, MD, USA, Association of Analytical Communities.

Artik N., Murakami H., 1997. Türk Elma Suyu Konsantrelerinin Fenolik Madde ve Prosiyanidin Bileşiminin HPLC ile Belirlenmesi, Gıda, 22(5), 327-335.

Duzgunes O, Kesici T, Kavuncu O., Gürbüz F., 1987. Araştırma ve Deneme Metotları, Ankara Üniversitesi Basımevi, Ankara.

Frankel E.N., Waterhouse A.L., Teissedre P.L., 1995. Principal Phenolic Phytochemicals in Selected California Wines and Their Antioxidant Activity in İnhibiting Oxidation of Human Low-Density Lipoproteins, Journal Agricultural and Food Chemistry, 43, 890–894.

Kalalb T.I., Bantash V.G., Matienko B.T., 1993. Ultrastructural and Biochemical Characteristics of Phenolic Inclusions Developing in Pericarp of Apple Trees on Different Parts of a Slope, Hort. Abs., 63(10), 941.

Minussi R.C., Rossi M., Bologna L., Cordi L., Rotilio D., Pastore G.M., Durán N. 2003. Phenolic Compounds and Total Antioxidant Potential of Commercial Wines, Food Chemistry, 82: 409-416.

Namiki M., 1990. Food Science and Nutrition. 29, 273.

Saito M., Hosoyama H., Ariga T., Kataoka S., Yamaji N., 1998. Antiulcer Activity of Grape Seed Extract and Procyanidins, Journal Agricultural and Food Chemistry, 46, 1460–1464.

Shahidi F., Wanasundara K.J., 1992. Critical Revievs in Food Science, Nutrition, 32(1), 67.

Shahidi F., Naczk M., 1995. Food Phenolics Sources Chemistry Effects Applications, Technomic Publication, 235-277.

Singleton V.L., Rossi J.R., 1965. Colorimetry of Total Phenolics With Phosphomolybdic–Phosphotungstic Acid, American Journal of Enology and Viticulture, 16, 144–158.

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VOLATILE FATTY ACIDS AND FATTY ACID COMPOSITION OF SILAGE SPECIES

O Baris CITIL[1*], M Selcuk ALATAS[2], Vahdettin SARIYEL[1], Abdullah OZBILGIN[2], Oguzhan KAHRAMAN[2]

[1,*] Department of Veterinary, Karapinar Aydoganlar Vocational School of Higher Education, University of Selcuk, Konya, Tel: +90 332 755 6896; Fax: +90 332 755 6300, email: ozcancitil@gmail.com
[2]Department of Animal Nutrition and Nutritional Disorders, Faculty of Veterinary Medicine, University of Selcuk, 42075 Konya, Turkey

Corresponding author email: ozcancitil@gmail.com

Abstract

In this study, total fatty acid composition and volatile fatty acid of silage species in Turkey was determined by using Gas Chromatography (Shimadzu 15-A). Total lipids were extracted from the corn silage, alfalfa silage and figüre silage samples by the method of Folch et al. Silage species were a total of 15 different fatty acids. These fatty acids between C12 to C24 have changed. Unsaturated fatty acids were the most corn silage. Linoleic acid levels were at the highest levels in alfalfa silage. In this study, unsaturated fatty acid is higher saturated fatty acid. ω6/ω3 ratio is 6. This value is high. In the report of HMSO, it was suggested that the minimum ratio of PUFA/SFA should be 0.45. In this study this value is 2. According to this result, oil of seed of corn silage is healthy.

Key words*: Fatty acid composition, Silage species, Volatile fatty acid.*

INTRODUCTION

Silage, forage rich in terms of water, concrete, stone, wood or plastic material, leaving a vacuum in the activity of lactic acid bacteria in the prepared feed silo container is obtained by fermenting. So there is a kind of pickles made from rich fodder for the animals in terms of water.

In recent years, our country has increased rapidly create silage and corn silage produced more than about 80% of the total silage (Alçiçek and Karaayvaz, 2003). An important part of the silo feed produced in our country is used for feeding dairy cows, while a small portion is used in beef cattle (Yaylak and Alçiçek, 2003). Corn silage, which should include both energy and lovingly consumed by animals because of silage fodder plants 'best of' bears the distinction of being (DLG, 1997). The feed value of corn silage should be used in both the production costs of intensive feeding animals considered to be spread throughout the country and is a must.

Corn silage is an important dietary source of fatty acids for ruminant breeding animals in Turkey. The fermentation quality of silages has a major effect on feed intake, nutrient utilization and milk production of ruminants (Huhtanen et al., 2002, 2003). Fresh whole corn silage dry matter which generally contains 30-40% grains is rich in linoleic acid and oleic acid and poor in α-linolenic acid, <2% (Chilliard et al., 2007). Concentrations of ALA vary with plant and environmental factors such as stage of maturity, genetic differences, as well as season and light intensity (Elgersma et al., 2006).

MATERIALS AND METHODS

Sample collection

Corn silage, alfalfa silage and figure silage samples, used in this study, were obtained from Konya, Turkey. In the present study, the laboratory chosen for analysis were in 2014. The samples were collected of each season during 2013. The samples were frozen at -26ºC until analyzed. At the beginning of analysis, the samples were allowed to equilibrate to room temperature.

Fatty acid analysis

A water extract of silage was prepared by adding deionised water to 20 g of silage to

achieve a total of 200 g. The values of pH, organic acids lactic acid and volatile fatty acids (VFA: acetic, propionic, n-butyric acids) - were analysed by the method of Naumann and Bassler (1997). The fatty acids in corn silages were determined in lyophilized samples. Lipids from freeze-dried corn and corn silages were extracted using an extraction-transesterification procedure described by Sukhija and Palmquist (1988). A mixture of chloroform and methanol (2:1) was chosen as the extraction solvent. In the extraction of fatty acids from the silages studied, the basic method of Folch, Lees, & Stanley, (1957). For this, samples were homogenized in a chloroform/methanol (2/1, v/v) mixture. The method of AOCS (1972) was employed in order to obtain the methyl esters of fatty acids by using BF3 (14%). The extracted lipids were dissolved in 1mL hexane with internal standard C13:0 and the esterification of lipids was carried out with 2 ml N sodium metoxide (30 min, 50°C) and 3 ml 3 N methanolic HCl (60 min, 50°C). After centrifugation (5 min, 2,500 rpm), samples of the upper hexane layers were used for gas chromatographic analyses. GC analysis of the methyl esters was performed using a GC Shimadzu 15-A model gas chromatograph (GC), equipped with a flame ionization detector (FID) and and a 1.8 m × 3 mm internal diameter packed glass column containing 100/120 Chromosorb WAW coated with 10% SP 2330. Injector and detector temperatures were 225 and 245 ºC, respectively. Column temperature program was 190 oC for 45 min then increasing at 30^{o}C/min up to 220^{o}C where it was maintained for 5 min. Total run time was 51 min. Carrier gas used was nitrogen (1 ml/min).

Identification of fatty acids was carried out by comparing sample FAME peak relative retention times with those obtained for Alltech (Carolean Industrial Drive, Satate Collage, PA) standards. Results were expressed as FID response area relative percentages. Each reported result is the average value of three GC analyses. The results are offered as mean ± SD in Table 1.

Statistical analysis

Each reported result is the average value of three GC analyses. The results were given as means and standard deviations (±SD). Statistical analyses were performed by using SPSS 16.0 software, and multiple comparison tests were carried out. The results were submitted to analysis of variance (ANOVA), at 0.05 significance level, using SPSS 16.0. The mean values were compared by Duncan test.

RESULTS AND DISCUSSIONS

Fatty acid composition of silage are presented in Table 1.

Table 1. Fatty acid composition of corn silage in Turkey

Fatty acids	**%**
C 10:0	0,04
C 12:0	0,3
C 13:0	0,06
C 14:0	1,29
C 15:0	0,33
C 16:0	17,12
C 17:0	0,02
C 18:0	7,67
C 20:0	0,21
C 21:0	0,01
C 24:0	0,03
∑ **Doymuş**	**27,08**
C 14:1	0,28
C 16:1	1,08
C 16:1-T	0,01
C 18:1	27,64
C 20:1	0,29
C 22:1	0,06
∑ **MUFA**	**29,36**
C 16:2	0,01
C 18:2	37,55
C 18:3	5,39
C 20:3	0,07
C 22:2	0,23
C 22:3	0,07
C 22:4	0,23
C 22:6	0,01
∑ **PUFA**	**43,56**
∑ Doymamış	72,92
∑ PUFA / MUFA	1,48
∑ Omega 3	5,54
∑ Omega 6	38,01
∑ Omega 3/6	0,15
∑ Doymuş/Pufa	0,62

We found 25 fatty acids in corn silage. The highest fatty acids in corn silage were found to be 18:2, 18:1, 16:0, 18:0, 18:3, 14:0, 16:1. C8:0 was not found in corn silage. C10:0, C11:0, C12:0, C13:0 were found to be low in the SFA fractions of the silage investigated. Palmitic acid (C16:0) was the primary major SFA (about 17.12). Stearic acid (C18:0) was the seconder major SFA (about 7.67)

Oleic acid (C18:1 n9) was identified as a primary monounsaturated fatty acid (MUFA) in the corn silage for all samples. This fatty acid in corn silage was found to be at levels of about 27.64% in all samples. The highest level of MUFA was oleic acid. According to Cherfaoui et al. (2013), oleic acid is the major monounsaturated fatty acid in other silage species.

Saturated fatty acids were lower than total monounsaturated fatty acids. The ratio of total SFAs was 27.08%. Linoleic acid was the primary polyunsaturated fatty acid, 37.55% for corn silage in all samples. In corn silage, a high amount of linoleic acid and linolenic acid increased the PUFA content in all samples. In our study, total SFA were affected by palmitic acid and stearic acid amount in corn silage. Linoleic acid was major fatty acid in corn silage. Other predominant fatty acids were oleic acid (27.64%) and palmitic acid (17.12%). In this study, DHA, which was recorded in silage to be 0.01%, was observed to be the minör polyunsaturated fatty acid.

In this study, corn silage was rich in PUFA, especially linoleic acid, and other predominant PUFA were linolenic acid in all samples. These results agree with Lauková et al. (2009), who have reported that linoleic acid, linolenic acid and DHA the most abundant PUFA in corn silage lipids in Konya, Turkey. In a previous study, linoleic acid, linolenic acid, DHA, were most abundant PUFA in corn silage in all samples (Han et al., 2013). In our study, total PUFA was higher than total MUFA in corn silage. DHA were low level. These results agree with Arvidsson et al., 2009 who has reported that PUFA is higher than total SFA and total MUFA in corn silage.

The results in the present work indicate that the n-3/n-6 ratio of corn silage is lower in all samples 0.15%. The present study indicates that corn silage are good in terms of n-6/n-3. The n-6/n-3 ratio is a good index for comparing relative nutritional value in corn silage. An increase in the human dietary n-6/n-3 fatty acid ratio is essential to help prevent coronary heart disease by reducing plasma lipids (Gokce et al., 2004).

CONCLUSIONS

This study has revealed that corn silage in the Konya of Turkey is a desirable item in the diet when the levels of linoleic acid and n-6/n-3 ratio are considered. The corn silage identified in this study was found to be good source of n-6 fatty acids.

REFERENCES

Alçiçek A., Karaavaz K., 2003. Sığır besisinde mısır silajı kullanımı. Animalia 203: 68-76.

AOCS, 1972. Official Methods and Recommended Practices of the American Oil Chemists Society, 2nd Ed.; American Oil Chemists Society: Champaign, Illinois.

Arvidsson K., A.M. Gustavsson, K. Martinsson, 2009. Effects of conservation method on fatty acid composition of silage. Animal Feed Science and Technology 148, 241-252.

Cherfaoui M., Durand D., Bonnet M., Bernard L., Bauchart D., Ortigues-Marty I., Gruffat, D., 2013. D., A grass-based diet favours muscle n-3 long-chain PUFA deposition without modifying gene expression of proteins involved in their synthesis or uptake in Charolais steers, The Animal Consortium, 7:11, 1833–1840

Chilliard Y., F. Glasser, A. Ferlay, L. Bernard, J. Rouel, M. Doreau, 2007. Diet, rumen biohydrogenation and nutritional quality of cow and goat milk fat. Eur. J. Lipid Sci. Technol. 109:828-855.

DLG, 1997. Futterwerttabellen für Wiederkäuer. DLG Verlag, Frankfurt/M.

Elgersma, A., S. Tamminga and G. Ellen. 2006. Modifying milk composition through forage. Anim. Feed Sci. Technol. 131: 207-225.

Folch J., Lees M., Stanley A., 1957. Simple method for the isolation and purification of total lipids from animal tissues. Journal of Biological Chemistry, 226, 497–509.

Gokce M. A., Tasbozan O., Tabakoglu S., Celik M., 2004. Seasonal variations in proximate and fatty acid compositions of female common sole (Solea solea). Food Chemistry, 88, 419–423.

HMSO, UK., 1994. Nutritional aspects of cardiovascular disease (report on health and social subjects No. 46), London.

Huhtanen P., Nousiainen J., Khalili I., Jaakkola H., Haikkilä T., 2003. Relationship betwen silage

fermentation characteristics and milk production parameters: analyses of literature data. Livest. Prod. Sci. 81:57-73.
Naumann C., R. Bassler, 1997. Methodenbuch. Band III. Die chemische Untersuchung von Futtermittelm. VDLUFA-Verlag, Darmstadt, Germany.
Sukhija P. S., D.L. Palmquist, 1988. Rapid method for the determination of total fatty acid content and composition of feedstuffs and faeces. J. Agric. Food Chem. 36:1202-1206.
Yaylak E., Alçiçek A., 2003. Sığır besiciliğinde ucuz bir kaba yem kaynağı: Mısır Silajı. Hayvansal Üretim Dergisi 44 (2): 29-36.

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COMPARISON OF THE EFFECT OF PEPPERMINT, ALOE VERA AND VITAMIN E SUPPLEMENTATION ON BROILER IMMUNE RESPONSE

Babak DARABIGHANE[1], Ali MAHDAVI[2], Eleni KASAPIDOU[3]

[1]Young Researchers and Elite Club, Karaj Branch, Islamic Azad University, Karaj, Iran
[2]Faculty of Veterinary Medicine, Semnan University, Iran
[3]Department of Agricultural Technology, Division of Agricultural Products Quality Control, School of Agriculture Technology, Food Technology and Nutrition, Technological Education Institute of Western Macedonia, Florina, Greece

Corresponding author email: b.darabighane@gmail.com

Abstract

Given the currently increased focus of researchers on applications of medicinal herbs as a natural additives to animal and poultry feed for enhanced performance of the immune system, the present study aims to examine effects of peppermint, Aloe vera, and Vitamin E on immune response in broilers. In this experiment, three hundred one-day old male broilers (Ross 308) were used on a completely randomized design in 4 groups with 5 replicates, each consisting of 15 broilers. The experimental groups included the control group (basal diet with no additive), a group that received 10 g/kg dry peppermint leaves (added to basal diet), a group given 10 g/kg Aloe vera gel (added to basal diet), and a group treated with 100 mg/kg Vitamin E. Evaluation of antibody titer against Newcastle disease virus indicated that on day 27, the highest level of antibody titer was observed in the Vitamin E group, showing a significant difference from other groups ($P<0.05$) except for the group that received the dry peppermint leaves. On day 37, the highest level of antibody titer against Newcastle disease virus was found in the Vitamin E group with a significant difference compared to the herb groups (the groups that received dry peppermint leaves and Aloe vera gel) as well as the control group ($P<0.05$). On the other hand, on days 27 and 37, the groups that received dry peppermint leaves and Aloe vera gel showed significant increase in their antibody titer against Newcastle disease virus compared to the control group ($P<0.05$). The results after injection of phytohemagglutinin-P on day 40 demonstrated that broilers in the Aloe vera gel group and those belonging to the dry peppermint leaves group significantly enhanced their response to phytohemagglutinin-P solution compared to the control group and to the broilers that were given Vitamin E ($P<0.05$). On day 42, a significant increase was observed in total white blood cell counts of broilers in the Aloe vera gel group and the dry peppermint leaves group compared to the control broilers, although the largest number of white blood cell was found in the broilers that received Vitamin E ($p<0.05$). In general, our results showed that supplementation of broiler feed with either dry peppermint leaves or Aloe vera gel enhances immune response.

Key words*: Peppermint, Aloe vera, Vitamin E, Immune system, Broiler.*

INTRODUCTION

In the recent years many researchers have focused on improving broiler immune system and enhancing resistance against pathogens by using feed additives. Among these additives, medicinal herbs have received greater attention due to the ban on antibiotic growth promoters and research conducted in this area has shown that addition of herbs to feed can enhance responses of the immune system. In addition, herb supplements have been shown to improve growth performance or alter the intestinal microflora of broilers. Peppermint (*Mentha piperita* L.) has antioxidant, antitumor, anti-allergy, antiviral, antibacterial and fungicidal properties (MacKay and Blumberg, 2006). Peppermint has been applied in veterinary practice for treatment of internal and external parasites as well as digestive diseases (Laudato and Capasso, 2013), and recently it has been used to supplement poultry feed to enhance growth performance and reinforce the immune system. Aloe vera (*Aloe barbadensis* Miller) has also antioxidant, antibacterial, antiviral, antifungal, anti-inflammatory, antitumor, immunomodulatory, wound healing, and anti-diabetic properties (Christaki and Florou-Paneri, 2010). Furthermore, in the past few years, Aloe vera has been used as poultry feed additive to improve growth performance and the immune system and to treat coccidiosis. Vitamin E can also enhance humoral and

cellular immune response (Gore and Qureshi, 1997). Although previous studies have shown positive effects of Peppermint and Aloe vera on the immune system, further studies are needed to determine the extent to which these herbs can induce positive impacts. Therefore, the aim of this study was to evaluate the immunostimulatory effect of dry Peppermint leaves and Aloe vera gel supplementation on broilers. A secondary aim was to study whether using dry Peppermint leaves and Aloe vera gel as natural feed additives for broilers can positively affect the immune system in a similar mode to that of Vitamin E supplementation.

MATERIALS AND METHODS

Three hundred male Ross 308 broilers were assigned randomly to four treatment groups with five replication of fifteen birds each. The treatment groups consisted of the control group (basal diet with no additive), a group receiving dried Peppermint leaves at 10 g/kg (mixed with broiler feed), a group receiving Aloe vera gel at 10 g/kg (mixed with broiler feed), and a group that received Vitamin E at 100 mg/kg (mixed with broiler feed in the form of alpha-tocopheryl acetate). Feed requirements for the broilers at the different growth stages, namely starter (day 0-10), grower (day 11-24), and finisher (day 25-42) were in accordance to the Ross 308 guidelines and the basal experimental diet based on corn-soybean meal was formulated using the UFFDA software. The amount of energy for starter, grower, and finisher periods was 3334, 3150, and 3200 kcal ME/kg, respectively with crude protein content of 25.12%, 22.10%, and 19.76%. During the experimental period, all chickens were given complete access to water and feed. In addition, management factors such as temperature, humidity, light, ventilation, and vaccination were the same for all groups.

Newcastle disease vaccine (LaSota) was administrated with drinking water at 16 days of age. Blood samples from the right wing vein were collected 11 and 21 days after vaccination from three chickens from each replication. Antibody response was determined by using the hemagglutination inhibition (HI) test and the titer obtained through this method was reported as $\log_2$.

On day 40 of the experiment period, three birds were randomly selected from each replication. First, 0.1 ml of phytohemagglutinin-P (PHA-P) solution was intradermally injected to the web of the third and the fourth digit of the right foot of each bird, and 0.1 ml of a phosphate buffer saline (PBS) as the control solution was injected to the web connecting the third and the fourth digit on the left foot. The web thickness was measured by a micrometer before and 24 hours after the injection. Broiler immune response to PHA-P was calculated with the following equation:

(response to PBS solution injected to the left foot) – (response to PHA-P solution injected to the right foot)

where:

- Response to PHA-P solution injected to the right foot = post-injection thickness of skin – pre-injection thickness of skin;
- Response to PBS solution injected to the left foot = post-injection thickness of skin – pre-injection thickness of skin.

White blood cell count was determined on blood samples from the right wing vein from three chickens from each replication that were randomly selected. Natt-Herrick method was used to count white blood cells.

Experimental data was analysed with one way ANOVA and post-hoc analysis was conducted the Duncan's multiple range test at $P < 0.050$. Statistical software package SAS version 9.2 (2009) for Windows (SAS Institute Inc., Cary, NC, USA) was used.

RESULTS AND DISCUSSIONS

Antibody titer against Newcastle disease virus (NDV), response to PHA-P-injection, and total white blood cell count are presented to Table 1. Regarding antibody titer against NDV there were significant differences ($P<0.05$) between treatments and the highest values of antibody titer were observed in the Vitamin E group broilers in both examined periods (days 27 and 37). Furthermore, on day 27, antibody titer was higher ($P<0.05$) in the dry Peppermint leaves group in comparison to the Aloe vera gel group

whereas there was no difference between the two treatments on day 37.

There was a significant increase ($P<0.05$) in the response to PHA-P injection for the dry Peppermint leaves and the Aloe vera gel groups when compared to the Vitamin E group whereas the smallest response to PHA-P was observed in the control group.

With regard to blood cell count, there was a significant increase ($P<0.05$) in the Vitamin E group in comparison to the other groups. Furthermore, a significant increase was also observed in the dry Peppermint leaves and the Aloe vera gel groups compared to the control group.

Table 1. Effect of Peppermint, Aloe vera and Vitamin E supplementation on broiler immune system

Treatment	Antibody response to NDV (log_2)		PHA-P (mm)	White blood cells count ($\times 10^3/\mu l$)
	27 days	37 days	40 days	42 days
Control	1.57^c	2.31^c	0.387^c	21.80^c
Peppermint	2.37^a	3.36^b	0.545^a	22.42^b
Aloe vera	2.21^b	3.32^b	0.547^a	22.47^b
Vitamin E	2.45^a	3.77^a	0.502^b	22.67^a
SEM	0.145	0.139	0.016	0. 76

a, b, c Means with different superscripts within the same column are significantly different ($P<0.05$).

In this experiment we used the medicinal herbs dry Peppermint leaves and Aloe vera gel as well as Vitamin E as immunostimulants, and as seen in the results, these additives (Peppermint, Aloe vera, and Vitamin E) improved antibody titer against NDV, response to PHA-P injection, and total white blood cell count of broilers compared to the control group. A limited number of studies have been conducted on the effect of Peppermint on broiler immune system. Emami et al. (2012) reported that essential oil of Peppermint (200 and 400 mg/kg) failed to enhance antibody titer against sheep red blood cell (SRBC) in male chickens. Moreover, Toghyani et al. (2010) did not observe a significant difference on day 42 in terms of total white blood cells in chickens that received dried Peppermint leaves (4 and 8 g/kg). On the other hand, Sabaghi-Darmiyan et al. (2014) examined the effect of Peppermint powder (1% and 2% of feed) on Japanese quail and reported that Peppermint powder can enhance antibody titer against SRBC compared to the control group, with the highest level of antibody titer observed in a group that received 2% Peppermint powder. Dosti et al. (2012) reported improvements on the immune system capabilities in chickens that received Peppermint powder (10, 15, and 25 g/kg) which resulted in higher antibody titer against Gumboro and Bronchitis as well as larger total white blood cell count compared to the control group. The discrepancies in the results seem to have been stemmed from either the administration form or the dose level.

As shown in Table 1, dry Peppermint leaves increased antibody titer against NDV and total white blood cell count in broilers, particularly on day 40 when a significant increase was observed in the response to PHA-P injected compared to the Vitamin E group. The exact mechanism through which Peppermint affects the immune system is not well understood; however, this may be the result of strong antioxidant and antibacterial properties which indirectly affect this system. In fact, the menthol contained in Peppermint leaves can effectively stimulate appetite and help digestion while also act as an antiseptic (Kamel, 2000). Another study identified menthol as an effective agent that produces antimicrobial properties of Peppermint (Iscan et al., 2002).

Studies examining Aloe vera have revealed that chickens which received Aloe vera gel with their drinking water (Valle-Paraso et al., 2005), Aloe vera gel mixed with their feed (Darabighane et al., 2012), Aloe vera powder mixed with their feed (Alemi el al., 2012) presented increased antibody titer against NDV compared to the control groups (with no additive). Furthermore, broilers that received aquatic and ethanol extract of Aloe vera (Akhtar et al., 2012) and Aloe vera gel mixed with their feed (Darabighane et al., 2012) had enhanced cellular immune response to PHA-P injection in comparison to the control groups. Other studies reported increased number of white blood cell as a result of supplementing feed with Aloe vera gel powder (1%, 0.75%, and 0.5%) (Mahdavi et al., 2012) or addition of Aloe vera gel to drinking water (2%) (Valle-Paraso et al., 2005). Zhang and Tizard

(1996) attributed these positive impacts of Aloe vera on the immune system to acemannan (a polysaccharide contained in Aloe vera gel) that activates macrophages, produces cytokines, and releases nitric oxide. However, the enhancement of intestinal microflora and the subsequent improvement of the immune system response may be attributed to the antibacterial properties of Aloe vera similarly to the overall reported antibacterial properties of medicinal herbs.

CONCLUSIONS

The results of our experiment indicated that supplementation of broiler diet with either dried Peppermint leaves or Aloe vera gel improved broiler immune system response. However, additional studies are required for the determination of optimum supplementation level as well as administration form.

REFERENCES

Akhtar M., Hai A., Awais M.M., Iqbal Z., Muhammad F., ul Haq A., Anwar M.I., 2012. Immunostimulatory and protective effects of Aloe vera against coccidiosis in industrial broiler chickens. Veterinary parasitology, 186(3): 170-177.

Alemi F., Mahdavi A., Ghazvinian K., Ghaderi M., Darabighane B., 2012. The effects of different levels of Aloe vera gel powder on antibody titer against Newcastle disease virus and performance in broilers. Proc. International Poultry Scientific Forum. Georgia World Congress Center, Atlanta, Georgia, 47.

Christaki E.V., Florou-Paneri P.C., 2010. Aloe vera: a plant for many uses. Journal of Food, Agriculture and Environment, 8(2): 245-249.

Darabighane B., Zarei A., Shahneh A.Z., 2012. The effects of different levels of Aloe vera gel on ileum microflora population and immune response in broilers: a comparison to antibiotic effects. Journal of Applied Animal Research, 40(1): 31-36.

Dosti A., Taherpour K., Nasr J., 2012. The effect of dietary Peppermint (Mentha piperita) supplementations on immune system of broiler chickens. Proc. 5th Iranian Congress on Animal Science, Isfahan, Iran.

Emami N.K., Samie A., Rahmani H.R., Ruiz-Feria C.A., 2012. The effect of peppermint essential oil and fructooligosaccharides, as alternatives to virginiamycin, on growth performance, digestibility, gut morphology and immune response of male broilers. Animal Feed Science and Technology, 175(1): 57-64.

Gore A.B., Qureshi M.A., 1997. Enhancement of humoral and cellular immunity by vitamin E after embryonic exposure. Poultry Science, 76(7): 984-991.

Iscan G., KIrimer N.E.S.E., Kürkcüoglu M., Baser H.C., DEMIrci F., 2002. Antimicrobial screening of Mentha piperita essential oils. Journal of Agricultural and Food Chemistry, 50(14): 3943-3946.

Kamel C., 2000. Natural plant extracts: Classical remedies bring modern animal production solutions. In 3rd Conference on Sow feed Manufacturing in the Mediterranean Region 31-8.

Laudato M., Capasso R., 2013. Useful plants for animal therapy. OA Alternative Medicine Italy, 1(1): 1-6.

Mahdavi A., Alemi F., Ghazvinian K., Ghaderi M., Darabighane B., 2012. Study of effects of different levels of Aloe vera gel powder on antibody titre against sheep red blood cells and other blood parameters in broilers. Abstracts, British Poultry Abstracts 8(1): 49-50.

McKay D.L., Blumberg J.B., 2006. A review of the bioactivity and potential health benefits of peppermint tea (*Mentha piperita* L.). Phytotherapy Research, 20(8) : 619-633.

Sabaghi-Darmiyan V., Mehri M., Bagherzadeh-Kasmani F., Ghazaghi M., Vorassi-Ardakani H., Poortaheri M., 2014. The effect of different levels of Peppermint powder on humeral immune response of Japanese quail. Proc. 6th Iranian Congress on Animal Science, Tabriz, Iran.

Toghyani M., Toghyani M., Gheisari A., Ghalamkari G., Mohammadrezaei M., 2010. Growth performance, serum biochemistry and blood hematology of broiler chicks fed different levels of black seed (Nigellasativa) and peppermint (Mentha piperita). Livestock Science, 129(1) : 173-178.

Valle-Paraso M., Vidamo P., Anunciado R., Lapitan A., 2005. Effects of Aloe vera (Aloe barbadensis) on the white blood cell count and antibody titre of broiler chickens vaccinated against Newcastle disease. Philippine Journal of Veterinary Medicine, 42(1) : 49–52.

Zhang L., Tizard I.R., 1996. Activation of a mouse macrophage cell line by acemannan: the major carbohydrate fraction from Aloe vera gel. Immunopharmacology, 35(2) : 119-128.

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NUTRIENT UTILIZATION AND GROWTH PERFORMANCE OF *JALAUNI LAMBS* FED GRASS PEA (*Lathyrus sativus*) HAY BASED DIET

M. M. Das, V. K. Yadav, Archana Singh, P. Sharma, P. K. Ghosh
Indian Grassland and Fodder Research Institute, Jhansi-284003, (Uttar Pradesh), India

Corresponding author email: mmdas1964@gmail.com

Abstract
Grass pea is a very popular crop in many Asian and African countries where it is grown either for livestock feed or human consumption. The most important trait of grass pea consists of its drought tolerance and adaptability to diverse climatic conditions. In spite of the importance of grass pea for human and livestock, the crop has limited uses due to the presence of neurotoxic compound β-ODAP in seeds and plant parts, which causes Lathyrism in human beings and animals. The purpose of this study was to determine the nutrient utilization and growth performance of sheep fed grass pea hay based diet and to compare its feeding value to Berseem hay (Trifolium alexandrinum), a conventional legume fed to livestock in India. Eighteen growing Jalauni lambs of live weight (15.46 ±0.57 kg) were divided in to three groups of six animals in each. Animals of G1 (control) was fed berseem hay ad libitum as basal roughage whereas in the diet of G2 and G3, berseem hay was replaced with grass pea hay 50% and 100%, respectively. All the groups received 200 g crushed maize grain daily for 90 days. At the middle of the experimental feeding, a digestion cum metabolism trial was conducted for 7 days. DM intake (kg per 100 kg live weight and g per kg $W^{0.75}$) was comparable among the groups. Digestibility of nutrients viz., DM, OM, CP, NDF, ADF were none significantly different among the groups. Digestible crude protein (DCP) intake (g/d) ranged from 64.68±4.22 in G2 to 68.00±3.01 in G3. Total digestible nutrients (TDN) intake (g/d) was also comparable among the groups. Nutrient content (%) in terms of DCP and TDN were (8.64±0.56 and 62.89±1.73), (8.42±0.73 and 62.89±0.64), (9.03±0.24 and 64.63±0.63), respectively in different diets. Daily live weight gain (g/d) was (84.10±3.59) in G1, (83.53±4.30) in G2 and (86.05±3.77) in G3, respectively. No adverse effect on feeding grass pea hay on body condition was observed in experimental lambs. It was concluded that nutrient intake and utilization and growth performance were comparable in Jalauni lambs fed either berseem hay or grass pea hay based diet and grass pea hay could safely be incorporated to ruminant's diet without any adverse affection body condition.

Key words: *Berseem hay, Grass pea hay, Growth performance, Jalauni lambs, Nutrient utilization.*

INTRODUCTION

Grass pea (*Lathyrus sativus*) is a dual purpose annual legume grown for its seed for human consumption and for fodder for livestock feeding. The main feature of this legume crop consists of its sturdiness, drought tolerance, and adaptability to a wide range of soil types, including marginal ones (Yan et al., 2006). Grass pea because of its high protein content has made it possible to be a popular crop in subsistence farming in certain developing countries that suffer from adverse weather conditions. In spite of the importance of grass pea for human and livestock, the crop has limited uses due to the presence of neurotoxic compound β-ODAP in seeds and plant parts, which causes Lathyrism in human beings and animals (Hanbury et al., 2000).

The experiment was carried out to determine the nutrient utilization and growth performance of sheep fed grass pea hay based diet and to compare its feeding value to berseem hay (*Trifolium alexandrinum*), a conventional legume fed to livestock in India.

MATERIALS AND METHODS

Grass pea, low ODAP containing variety was harvested at full flowering stage and berseem during the end of February from Experimental farm of Indian Grassland and Fodder Research Institute at Jhansi. Eighteen growing *Jalauni* lambs of live weight (15.46±0.57kg) were used to investigate the effect of different level of replacement of berseem hay with grass pea hay on feed intake, nutrient digestibility coefficients, nitrogen utilization, rumen fermentation and growth performance. The animals were randomly assigned to three experimental groups (six animals in each treatment). The three experimental groups were considered as G1: berseem hay (100%)

+ 200 g crushed maize grain; G2: berseem hay: grass pea hay (50:50) + 200 g crushed maize grain and G3: grass pea hay (100%) + 200 g crushed maize grain. Rations were offered in two portions, crushed maize grain at 8.30 a.m. followed by different roughage sources at 9.30 a.m. for a period of 90 days. Water was offered twice daily at 11.00 a.m. and 4.00 p.m. Fortnightly body weight were recorded. At the middle of the experimental feeding, animals were placed in metabolic cages for quantitative collection of faeces and urine separately and a digestion cum metabolism trial of 7 days collection period was conducted to evaluate the nutritive value, balance of N from various diets.

Dry matter in feed and faeces was determined by oven drying at 100^{o}C overnight. For chemical analysis, pooled samples of feed offered, refusals and faeces were dried at 60^{o}C and ground to pass through a 2 mm sieve. Wet faeces and urine samples, preserved in diluted and concentrated sulfuric acid, respectively were analysed for N by the standard Micro Kjeldahl method. Feed and faecal samples were analysed for CP, EE and total ash contents (AOAC, 2000) and fiber fractions were analysed as per Van Soest et al. (1991).

Before the onset of digestibility trial, rumen liquor was collected at 2 h of post feeding through an oesophageal tube. Ruminal pH was immediately determined using digital pH meter. Rumen liquor samples were analysed for total N (Micro-kjeldahl), ammonia N concentrations were determined applying NH_3 diffusion technique using Kjeldahl distillation method according to A.O.A.C (2000), total VFA (Barnett and Reid, 1957).

Statistical analysis of data was performed using SPSS (13.0) statistical package. Data on nutrient intake, digestibility coefficients, rumen metabolites etc. were analysed by one way analysis of variance (ANOVA). Significance was declared at $P<0.05$; differences between means were tested using least significant difference. All statistical procedures were carried out as per Snedecor and Cochran (1994).

RESULTS AND DISCUSSIONS

Chemical analysis and cell wall constituents of feed ingredients are presented in Table 1. Crude protein (15.69%), crude fiber (28.59%) and organic matter (88.10%) contents of berseem hay were similar to earlier report (Hamed et al., 2012) where as NDF content (53.98%) was higher which might be due to late harvesting of the berseem crop in the present study. The CP content of grass pea hay in the present study was lower than the report of Vahdani et al., 2014, however, similar with the values reported by Tuna et al. (2004). Similarly, NDF and ADF values were also higher in the ongoing study than those reported earlier (Poland et al., 2003). Differences in growing conditions, cultivars used and different vegetative stages at harvest may explain part or more of these differences.

Table 1. Chemical analysis and cell wall constituents of feed ingredients

Item	Maize grain	Berseem hay	Grass pea hay
Dry matter	94.64	92.13	93.47
Chemical analysis on DM basis			
Organic matter	97.6	88.1	93.77
Crude protein	10.28	15.69	14.99
Crude fiber	2.39	28.59	31.85
Ether extract	2.14	2.26	2.18
Nitrogen free extract	78.84	41.56	44.75
Ash	2.39	11.89	6.22
Cell wall constituents			
NDF	15.54	53.98	58.08
ADF	5.46	37.9	41.51
ADL	1.56	7.01	9.3
Hemi-cellulose*	10.08	16.08	16.57
Cellulose**	3.9	30.89	32.21

NDF: Neural detergent fiber. ADF: Acid detergent fiber.
ADL: Acid detergent lignin.
* Hemicellulose= NDF – ADF. * *Cellulose= ADF – ADL.

Dry matter, TDN, DCP intakes by the experimental groups fed different experimental diets are presented in Table 2. The results showed that inclusion of grass pea hay as replacer of berseem hay in lamb diet did not affect feed consumption as DM, TDN and DCP intakes in comparison with the berseem hay containing diet.

Table 2. Nutrient intake and utilization in *Jalauni* lambs fed grass pea hay based diet

Parameters	G_1	G_2	G_3	SEM	P value
Body weight (kg)	20.13	20.25	19.45	-	-
DMI(g/d)	779	775	752	28.61	0.787
DMI% BW	3.87	3.84	3.86	0.18	0.993
CPI (g/d)	114.21	111.76	109.91	3.95	0.749
TDNI (g/d)	488	487	486	17.09	0.993
DCPI(g/d)	67.1	64.68	68	3.79	0.819
Digestibility coefficients (%)					
DM	66.29	65.27	64.28	1.3	0.568
OM	67.75	66.33	65.96	1.24	0.582
CP	58.88	59.32	60.78	3.84	0.935
NDF	53.95	54.32	50.76	1.87	0.377
ADF	54.83	54.1	56.2	1.68	0.68
EE	64.6	63.44	62.11	1.58	0.561
NFE	72.72	72.02	70.45	0.83	0.06
N intake (g/d)	17.9	17.9	17.99	0.79	0.995
Fecal N (g/d)	7.07	7.17	6.77	1.06	0.963
Urinary N (g/d)	5.92	6.19	6.26	0.43	0.835
N balance (g/d)	4.9	4.53	4.95	0.54	0.834
N retention as % NI	27.4	25.77	27.38	3.25	0.921
Nutrient density (%)					
DCP	8.64	8.42	9.03	0.55	0.731
TDN	62.89	62.89	64.63	1.12	0.48

Similar nutrient intake was recorded in sheep fed berseem hay based diet (Hamed et al., 2012). The intake of DCP was comparable among the groups and was within the suggested range (ICAR,1998) whereas TDN intake was 16% higher in all the groups than the requirement for achieving a daily gain of 100 g/d. Digestibility of DM, OM, CP and NDF in the present study was comparable among the groups, however, higher than the values reported in Ossimi sheep fed diets containing different sources of roughages (Hamed et al., 2012) and in Varamini rams fed grass pea hay diet (Vahdani et al., 2014). On the contrary, Abdel-Magid et al. (2008) found that pea forage containing diet and berseem hay diet had similar values of digestibility of OM, CP and CF. N intake as well as N excretion through faeces and urine was similar in lambs fed either sole berseem hay based diet or grass pea hay supplemented diet. The pattern of excretion of N through faeces and urine corroborated with the findings of Das et al. (2013) in lambs fed berseem hay based feed block supplemented with different level of maize grain. N balance was comparable among the groups and similarly, Forster et al. (1988) mentioned that N retention was not affected by diet when lambs were fed 30% ground maize and 70% chopped forage of 0, 25, 50, 75 or 100% pea hay with Lucerne. No significant differences in ruminal pH were observed after 2 h post feeding (Table 3). TVFA's concentration in the rumen is governed by several factors such as dry matter digestibility, rate of absorption, rumen pH, transportation of the digesta from the rumen to the other parts of the digestive tract and the microbial population in the rumen and their activities. Similar pH and digestibility values with different experimental diets in the present experiment indicated comparable TVFA concentration among the groups (128.3-130.3 meq/l). Ruminal ammonia concentration values were also comparable with earlier report (Das et al., 2013).

Table 3. Rumen metabolites in *jalauni* lambs fed different experimental diets

Parameters	G_1	G_2	G_3	SEM	P value
pH	6.41	6.48	6.54	0.14	0.82
TVFA (meq/L)	129.7	130.3	128.3	2.98	0.78
NH3-N (mg/dl)	28.93	29.77	30.24	2.11	0.908
Total N (mg/dl)	79.33	78.86	81.22	3.48	0.884

Average daily gain (g/d) of lambs fed different experimental did not differ significantly (Table 4). Similarly, grass pea hay compared to alfalfa hay as sole forage fed *ad libitum* to pregnant ewes did not change body weight and body condition score (Poland et al., 2003).

Table 4. Growth performance of *Jalauni* lambs fed different experimental diets

Parameters	G_1	G_2	G_3	SEM	P value
Initial body wt (kg)	15.52	15.52	15.3	0.76	0.986
Final body wt (kg)	23.08	23.13	23.04	0.84	0.996
Gain (kg)	7.56	7.51	7.75	0.43	0.926
Daily gain(g/d)	84.1	83.53	86.05	4.75	0.926
Feed intake /kg gain (kg)	9.2	9.05	8.92	0.59	0.982

CONCLUSIONS

It could be concluded that grass pea hay can be used as an alternative sources of legume

roughage successfully in sheep diets instead of berseem hay for similar feed intake, digestion coefficient, nitrogen utilization, ruminal fermentation and growth performance without any adverse affect on body condition.

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REFERENCES

Abdel-Magid Soha S., Abd El-Rahman H.H., Mohamed M.I., Awadalla I.M., 2008. Utilization of chick pea straw and pea straw in feeding growing Rahmani lambs. American-Eurasian Journal of Agricultural & Environmental Sciences, 4 (2): 214-217.

AOAC., 2000. Official Methods of Analysis of the AOAC International. 17th Edition, Gaitherburg, MD: AOAC International.

Barnett A.J.G., Reid R.L., 1957. Studies on the production of volatile fatty acids from grass in artificial rumen.1. Volatile fatty acids production from fresh grasses. Journal of Agriculture Science, 48: 315-321.

Das M.M., Singh K.K., Pathak P.K., 2013. Effect of energy supplementation to berseem (*Trifolium alexandrinum)* hay based feed block on growth performance in *Jalauni* lambs. Range Management and Agroforestry, 34(1): 108-111.

Forster L.A., Perry H.P., Fontento I.P., 1988. Nutritional value of flat pea hay for ruminants. Nut. Abs & Rev. Series, B: 58 No. 3:172.

Hamed A.A. Omer, Mohamed A. Tawila, Sawsan M.G., 2012. Feed and water consumptions, digestion coefficients, nitrogen balance and some rumen fluid parameters of Ossimi sheep fed diets containing different sources of roughages**.** Life Science Journal 9(3): 805-816.

Hanbury C. D., White C.L., Mullan B.P., Siddique K.H.M., 2000. A Review of the potential of *Lathyrus sativus* L. and *L. cicera* L. grain for use as animal feed. Animal Feed Science and Technology, 87:1–27.

ICAR., 1998. Nutrient requirements of livestock and poultry. Indian Council of Agricultural Research, New Delhi.

Poland C., Faller T., Tisor L., 2003. Effect of chickling vetch (*Lathyrus sativus* L.) or alfalfa (*Medicago sativa*) hay in gestating ewe diets. Originally published in the 2003 Sheep Day Report, North Dakota State University.

Tuna C., Coskuntuna L., Koc F., 2004. Determination of nutritional value of some legume and grasses. Pakistan Journal of Biological Sciences*,* 7(10): 1750-1753.

Snedecor G.W., Cochran W.G., 1994. Statistical Methods, 8th ed. Iowa State University press, Iowa, USA.

Vahdani N., Moravej H., Rezayazdi K., Dehghan-Banadaki M., 2014. Evaluation of nutritive value of grass Pea hay in sheep nutrition and its palatability as compared with alfalfa. Journal of Agricultural Science and Technology, 16: 537-550.

Van Soest P.J., Robinson J.B., Lewis B.A., 1991. Methods for dietary fiber, neutral detergent fiber and non-starch polysaccharides in relation to animal nutrition. Journal of Dairy Science*,* 74: 3583–3597.

Yan Z.Y., Spencer P.S., Li Z.X., Liang Y.M., Wang Y.F., Wang C.Y., Li F.M., 2006. *Lathyrus sativus* (Grass pea) and its neurotoxin ODAP: Review. Phytochemistry*,* 67:107–121.

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INFLUENCE OF USING DIFFERENT OIL SOURCES IN QUAIL NUTRITION ON MEAT COMPOSITION AND QUALITY PARAMETERS

Reyhan GUVEN[1], Birol KILIÇ[1], Cem Okan OZER[1,2]

[1]Suleyman Demirel University, Faculty of Engineering, Department of Food Engineering, Isparta, Turkey
[2]Nevsehir Hacı Bektaş Veli University, Faculty of Engineering and Architecture, Department of Food Engineering, Nevsehir, Turkey

Corresponding author email: birolkilic@sdu.edu.tr

Abstract

In this study, effects of using different oil sources (sunflower oil, olive oil, fish oil, flax seed oil and nettle oil) in Japanese quail feeding on pH, colour, moisture, protein, fat, myoglobin, total iron, thiobarbituric acid reactive substances (TBARS) and fatty acid composition of quail meat were investigated. After two weeks of prefeeding period with standard diet, quails were fed with diet containing five different oil sources for a period of five weeks and slaughtered at the end of the seven weeks. After that, breast meat was removed from carcass and physico-chemical properties of quail meat were evaluated. The results indicated that the meat samples obtained from quails fed with diet containing olive oil and nettle oil had the highest pH levels compared to other groups ($p<0.05$). No significant differences for fat, protein and moisture levels, and colour values among treatment groups were determined. The lowest myoglobin and total iron levels were determined in meat samples obtained from quails received diet containing flax seed oil and nettle oil ($p<0.05$). As far as lipid oxidation is concerned, the highest TBARS values were found to be in meat samples obtained from quails received nettle oil ($p<0.05$). It was determined that the ratio of polyunsaturated (PUFA) and saturated (SFA) fatty acids were higher than 0.4 and the highest PUFA/SFA ratio was found in the meat samples obtained from quails received flax seed oil in diet ($p<0.05$). Furthermore, quail meat obtained from quails fed with fish oil and flax seed oil had higher eicosapentaenoic acid and docosahexaenoic acid compared to other groups ($p<0.05$). into quail diet.

Key words*: Quail, nutrition, oils, meat quality.*

INTRODUCTION

Animal products, in particular poultry meat, represent an important part of our diet. Poultry meat is distinguished for its low energy concentration and it has high nutrient density compared with other food substitutes(Hargis and Vanelswyk, 1993). Poultry meat is a good source of high biological value protein. Furthermore, it provides iron and zinc of high bioavailability in lower quantities than red meats, but important amounts compared with food of vegetable origin. Poultry meat has significant content of vitamins from group B such as thiamine, riboflavin, niacin and vitamin B_6, although vitamin B_{12} content is less than in other meats.

The demand of consumers for quail meat has increased in recent years due to the pursuit of economic and healthy meat. Quail meat which can be produced quickly and easily has been one of the most important alternatives for economic meat source.

The quails are the smallest species of game bird which are farmed as well. These are found in wild environment in Europe, Asia, America and Australia but commercial strains are farmed for meat and eggs worldwide. The most common specie of quail is Japanese quail (*Coturnix coturnix japonica*) which is used in commercial enterprises (Minvielle, 2004).

The quail meat may be considered as a competitive source against the broiler meat. According to some studies, it is believed that quail meat is nearly a chicken and even better than it. Quail meat includes high protein, polyunsaturated fatty acids and essential trace minerals and fat. Because of high metabolic activity in this bird, the amount of glycogen stored in muscles increased, resulting in high quality (Boni et al., 2010).

Researchers have focused on studies related with modification of poultry meat composition

using different strategies and thus to produce the useful food products for human health. One of the most effective methods is the use of dietary strategies to improve the quality of the poultry carcass and meat (Barroeta, 2007). This modification is to increase the amount of unsaturated FA, especially the omega 3 (n3) family which has beneficial effects on human health (Salainatcloustnobar et al., 2008). However, some studies indicated that increasing the unsaturation degree of the meat leads to organoleptic and nutritional problems and requires assessment of the oxidation processes of the lipid fraction (Lopez-Ferrer et al., 1999).
In the present study, the objective was to determine the effect of using different oil sources (sunflower oil, olive oil, fish oil, flax seed oil and nettle oil) in Japanese quail feed on pH, colour, moisture, protein, fat, ash, myoglobin, total iron, thiobarbituric acid reactive substances (TBARS) and fatty acid composition of quail meat.

MATERIALS AND METHODS

Animals and diet

250, 14 day-old Japanese quail (*Coturnix coturnix japonica*) were used in the experiment. The quails were obtained from Suleyman Demirel University, Faculty of Agriculture, Isparta, Turkey.
Quails were fed by launching feed containing 20% crude protein and 2900 kcal ME/kg energy for the first 2 weeks (prefeeding). The later 5 weeks, quails were fed by growing feed containing 20% crude protein, 2900 kcal ME/kg energy and 2% sunflower oil (S), olive oil (O), fish oil (F), linseed oil (L) and nettle oil (N). Treatments were applied under *ad libitum* feeding conditions.
The quails were slaughtered at the end of the seven weeks and carcasses were trimmed for breast meat (*Pectoralis major)* by removing skin, bones, and connective tissue. Breast meat samples within each group were vacuum packaged, and stored at -80°C pending further experimentation and analysis.

Analysis

pH measurements were carried out using Orion Model 420 digital pH meter (Orion, Boston, USA). The pH was determined after mixing a 10 g sample with 90 ml distilled water and equilibrating for 10 min. Moisture, protein, fat and ash of samples were determined according to procedures of AOAC (1995)and (AOAC, 2000). Colour measurement was taken with a Hunterlab model Precise Colour Reader TCR 200 (BAMR Ltd, Claremont, South Africa) colorimeter. Evaluation of oxidative stability was performed by measuring the formation of thiobarbituric acid reactive substances (TBARS). TBARS values of samples were determined according to Kilic and Richards (2003). Myoglobin, total iron content and fatty acid composition of samples were determined according to Topel (1949), EPA (2000) and Özer and Kiliç (2014).
The statistical evaluation of the results was performed using the SPSS 18.0.0 (SPSS Inc., Chicago, USA). The generated data were analysed by analysis of variance (ANOVA). Differences among mean values were established using the Duncan test and were considered significant when $P<0.05$.

RESULTS AND DISCUSSIONS

The proximate compositions of quail meat from are shown in Table 1. No significant differences for fat, protein and moisture levels, and colour values among treatment groups were determined.

Table 1. Proximate compositions of quail meats

Groups	Fat (%)	Protein (%)	Moisture (%)
S	5,03[a]	17,72[a]	72,16[a]
O	5,03[a]	18,05[a]	73,35[a]
F	5,02[a]	17,69[a]	73,54[a]
L	5,04[a]	18,10[a]	73,36[a]
N	5,04[a]	17,62[a]	72,41[a]
SEM	0,01	0,02	0,01

All values are the mean of three replicates, SEM: standard error of the mean
a, b (↓) Different letters within a column are significantly different.

TBARS and pH levels of quail meat are presented Figure 1. The results indicated that the meat samples obtained from quails fed with diet containing olive oil and nettle oil had the highest pH levels compared to other groups (P<0.05).

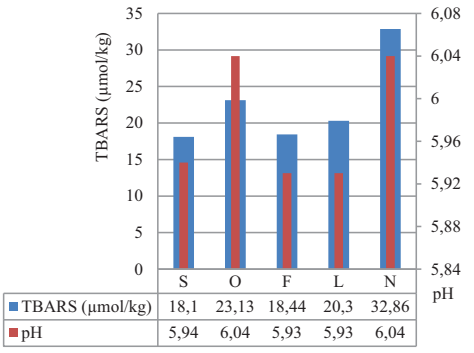

Figure 1. TBARS and pH levels of quail meats, All values are the mean of three replicates

As far as lipid oxidation is concerned, the highest TBARS values were found to be in meat samples obtained from quails received nettle oil (P<0.05). This observation could be related to PUFA and long fatty acid contents of meat. In a similar study, Barroeta (2007) indicated that chicken meat containing high PUFA is more the susceptibility of meat to oxidation.

Table 2. Myoglobin and total iron levels of quail meats

Groups	Total iron (mg/kg)	Myoglobin (mg/g)
S	128,2^{b}	1,70^{b}
O	131,3^{ab}	1,80^{ab}
F	129,3^{b}	1,93^{b}
L	117,4^{c}	1,19^{c}
N	116,4^{c}	1,21^{c}
SEM	0,11	0,06

All values are the mean of three replicates, SEM: standard error of the mean
a, b, c (↓) Different letters within a column are significantly different.

The lowest myoglobin and total iron levels were determined in meat samples obtained from quails received diet containing flax seed oil and nettle oil (P<0.05)
The fatty acid composition of quail meats is presented in Table 3. The results indicated that linoleic acid contents in the S and N group, oleic acid content of O group and linolenic acid content in the L group was higher compared with other groups.

Table 3. Fatty acid composition of quail meats

Fatty Acids	S	O	F	L	N
C10:0	0,47	0,29	0,68	0,27	0,37
C12:0	1,50	1,17	1,27	1,36	0,79
C16:0	19,89	17,25	22,26	16,50	17,70
C16:1	3,04	6,45	5,78	5,44	3,43
C18:0	20,04	19,27	18,01	19,09	21,68
C18:1	19,62	24,02	22,91	19,96	19,23
C18:2	22,60	19,89	17,90	20,20	25,16
C18:3	0,04	0,11	0,17	4,29	0,06
C20:0	11,53	10,22	5,85	9,21	11,52
C20:5(n-3)	0,10	0,01	1,26	0,98	0,04
C22:6 (n-3)	1,16	1,31	3,91	2,70	0,02
∑SFA	41,90	37,98	42,22	37,22	40,54
∑UFA	58,10	62,02	57,78	62,78	59,47
∑PUFA	35,43	31,55	29,09	37,39	36,81
PUFA/SFA	0,85	0,83	0,69	1,00	0,91

It was determined that the ratio of polyunsaturated fatty acids (PUFA) and saturated fatty acids (SFA) were higher than 0.4 and the highest PUFA/SFA ratio was found in the meat samples obtained from quails received flax seed oil in diet (P<0.05). Furthermore, quail meat obtained from quails fed with fish oil and flax seed oil had higher eicosapentaenoic acid (C20:5, n-3) and docosahexaenoic acid (C22:6, n-3) compared to other groups (p<0.05).

CONCLUSIONS

This study concluded that the use of sunflower oil, olive oil, fish oil, flax seed oil and nettle oil can be an effective strategy for modifying fatty acid composition, total iron and myoglobin content and susceptibility to oxidation of quail meat when incorporated in quail diet at the level of 2%.

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REFERENCES

AOAC 1995. Official Methods of Analysis (16th ed) Association of Official Analytical Chemists, Washington, DC.

AOAC. 2000. Official Methods of Analysis (16th ed) Association of Official Analytical Chemists, Washington, DC. In.

Barroeta A.C., 2007. Nutritive value of poultry meat: relationship between vitamin E and PUFA. World Poultry Sci J 63, 277-284.

Boni I., Nurul H., Noryati I. 2010. Comparison of meat quality characteristics between young and spent quails. International Food Research Journal 17, 661-666.

EPA, 2000. Inductively Coupled Plasma-Atomic Emission Spectrometry. Method 6010C. In.

Hargis P.S., Vanelswyk M.E., 1993. Manipulating the Fatty-Acid Composition of Poultry Meat and Eggs for the Health Conscious Consumer. World Poultry Sci J 49, 251-264.

Kilic B., Richards M.P., 2003. Lipid oxidation in poultry doner kebab: Pro-oxidative and anti-oxidative factors. J Food Sci 68, 686-689.

Lopez-Ferrer S., Baucells M.D., Barroeta A.C., Grashorn M.A., 1999. n-3 enrichment of chicken meat using fish oil: Alternative substitution with rapeseed and linseed oils. Poultry Sci. 78, 356-365.

Minvielle F., 2004. The future of Japanese quail for research and production. World's Poultry Science Journal 60, 500-507.

Özer C., Kiliç B., 2014. Effect of conjugated linoleic acid enrichment on the quality characteristics of Turkish dry fermented sausage. J Food Sci Technol, 1-10.

Salainatcloustnobar R., Aghdamshahriar H., Gorbani A., 2008. Enrichment of Broiler Meat with n-3 Polyunsatrurated Fatty Acids. Asian J Anim Vet Adv 3, 70-77.

Topel D.G., 1949. Determination of myoglobin in pork muscle. Annals of Physics 156, 44-51.

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EFFECT OF SUPPLEMENTING DIFFERENT LEVELS AND SOURCES OF PHYTASE ENZYME TO THE LAYING HENS DIETS ON PRODUCTIVE PERFORMANCE

Sunbul Jassim Hamodi[1], Firas Muzahem Hussein[2], Huda Qassim Al-Hamdany[1]

[1]College of Agriculture, University of Baghdad, Animal Resources Department, Iraq
[2]State Board of Agricultural Research, Ministry of Agriculture, Iraq

Corresponding author email: sunbulj.hamodi@yahoo.com

Abstract

This study was conducted in the Poultry Research Station – State Board of Agricultural Research - Ministry of Agricultural in Abu Ghraib, to study the effect of supplementing different levels and sources of Phytase enzyme to laying hens diets on productive performance and quality of eggs produced .

Nine hundred sixty egg-brown hens (Lohmann Brown-Classic) 24 weeks-old distributed randomly to six treatments with various sources of phytase, each source contain three levels which include 16 treatments with 2 replicates (30 hen per replicate). Chickens fed on diets equal protein and metabolic energy according to the age periods in Lohmann Index as in follow:

Treatment 1 (T1): Control group (without any supplement and without reduction of calcium and phosphorus).

Treatment 2 (T2): Supplement phytase from fungal source (Aspergillus oryza).

Treatment 3 (T3): Supplement phytase from bacterial source (E. coli).

Treatment 4 (T4): Supplement phytase from phytase mixture

For every three source of phytase added 3 levels as follows:

A: a diet reduced phosphorus (0.09) and calcium (0.07) which included Phytase adding by 250 FTY / kg feed

B: a diet reduced phosphorus (0.12) and calcium (0.10) which included Phytase adding by 350 FTY / kg feed

C: a diet reduced phosphorus (0.15) and calcium (0.13) which included Phytase adding by 450 FTY / kg feed

Treatment 5 (T5): Supplement Phytase from yeast (Saccharomyces cerevisia).

Treatment 6 (T6): Supplement Phytase from Alfalfa plant.

For every two source of Phytase (T5 and T6) added 3 levels 2.5, 3.5 and 4.5 FTY for each source. All three levels diet reduced phosphorus and calcium as in T2, T3 and T4.

The results of experiment showed:

Significant differences ($P<0.05$) between treatments in accumulative egg production (HD%), egg weight, egg mass and feed conversion ratio, while the adding of T2 treatment at level (A,C) and T4 treatment at level(C) were highly significant than other treatments.

All treatments with adding Phytase enzyme showed significant superiority ($P<0.05$) for qualitative characteristics of eggs produced from 24-55 weeks as follows.

From this study we can concluded that supplementing fungal phytase at levels 250 and 450 FTY/kg feed in layer diets with decrease phosphore 0.09 and 0.15 % and calcium 0.07 and 0.13% respectively gave the best results also when supplementing mixture enzyme at level 450 FTY/kg feed.

Key words*: phytase, laying hens, productive performances, eggs quality.*

INTRODUCTION

The poultry industry at the present time faced many environmental problem, like poultry waste that accumulation in very large quantities (Toth et. al., 2006). China produced nearly 2.21 billion tons of poultry waste in 2003 (Huang et. al., 2006) and its content 40% organic material.

Environmental pollution with phosphorus increased attention by the associations of environmental protection, especially if environmental pollution sources of this element from poultry manure over and above rainwater and sewage that have a role in this pollution (Howarth et al., 2002; Sim and Sharpley, 2005).

All of this is linked to the operations of poultry feeding, where the form of cereal crops and their by-products and oil seeds main ingredients in diets because of nutritional value, these components contain

phosphorus element which has importance in metabolism as a source of energy through the entry in the synthesis of ATP and creatine phosphate also being primarily created in nucleic acids DNA and RNA and responsible for phosphorylation of some nutrients during metabolism as well as its entry in the synthesis of many compounds such as co-carboxylase, phosphokinase and Vit.B1 (Thiamine pyrophosphate) (Hatten et al., 2001).

Two-thirds of phosphorus in poultry diets be a complex compound unavailable form known as phytic acid (organic phosphorus) (Lott et al., 2000; Gibson et al., 2010).

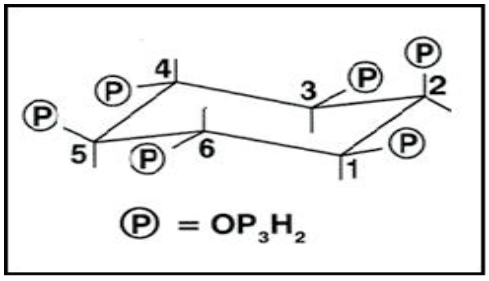

Fig.1 The chemical composition of phytic acid (Kumar et al., 2010)

Due to the inability of poultry to use phytate phosphorus to the lack of phytase enzyme that responsible for smashing phosphorus bond from inositol ring and make it available for mono gastric animals (Singh et al., 2007).

This has led the developed countries to adding phytase enzyme to poultry diets in order to reduce phosphorus in feces (Francesch et al, 2005; Ciftci et al., 2005). Studies have shown the importance of phytase enzyme to reduce the ratio of phosphorus in poultry feces to more than 30% (Pulmstead, 2007).

Musapuor et al. (2005) explained that the addition of phytase enzyme to the laying diets have a positive impact in improving the performance and the production of eggs ,also added to diets poor with phosphorus improved the level of calcium and phosphorus and egg weight and production (Scott et al., 1999).

Snow et al. (2003) pointed that laying hens fed on phytase enzyme by 300FTY/kg for 21 days did not have any significant effect on egg production. While the addition of Silversides and Hurby (2009) from phytase enzyme at levels 300 and 600FTY/kg to Lohmann hens with presence of phosphorus at perfect level doesn't appear any effect in phosphorus, calcium, energy and protein so from this results the researchers concluded that the addition of phytase enzyme to poor diets can ensuring necessity body from phosphorus and reduce phosphorus excreted with feces and thus reduce environmental pollution. Haitham (2010) found that using phytase enzyme Natophus and Phyzyme at level 300 and 450 respectively (two different microbial sources of phytase enzyme) to corn-soybean diets have a positive impact on the productive performance, feed efficiency and egg weight. Also Mohebbifar and Torki (2011) revealed that using 0.3 gm from phytase enzyme /kg at levels 0.33 and 0.29 gm from phytate phosphorus /gm led to significant increase in egg production due to increased feed consumption and feed conversion ratio compared with control treatment.

The aim of this study added different sources of phytase enzyme and different levels to laying hen diets and their effects on productive performance.

MATERIALS AND METHODS

Using 960 Lohmann Brown-Classic laying hens at 24 week old distributed randomly on 16 treatment (2 replicate / treatment)30 hen per replicate .Treatments were as follows:

T1: Treatment 1 as Control (without adding enzyme and non-reduced phosphorus and calcium).

T2: Treatment 2 adding phytase enzyme from fungal source.

T3: Treatment 3 adding phytase enzyme from bacterial source.

T4: Treatment 4 adding phytase enzyme from enzyme mixture.

Treatments 2,3,4 adding by three levels as in follow:

Level A: Diet reduced phosphorus (0.09%) and calcium (0.07%) with adding 250 FTY phytase enzyme /kg feed.

Level B: Diet reduced phosphorus (0.12%) and calcium (0.10%) with adding 350 FTY phytase enzyme /kg feed.

Level C: Diet reduced phosphorus (0.15%) and calcium (0.13%) with adding 450 FTY phytase enzyme/kg feed.

T5: Treatment 5 adding phytase enzyme from yeast source.

T6: Treatment 6 adding phytase enzyme from plant source.

Treatments 5 and 6 adding by three levels as in follow:

Level A: Diet reduced phosphorus (0.09%) and calcium (0.07%) with adding 2.5 FTY phytase enzyme /kg feed.

Level B: Diet reduced phosphorus (0.12%) and calcium (0.10%) with adding 3.5 FTY phytase enzyme /kg feed.

Level C: Diet reduced phosphorus (0.15%) and calcium (0.13%) with adding 4.5 FTY phytase enzyme/kg feed.

Tables 1, 2, 3 indicate the diets used in the experiment for the periods from 18-28, 29-45, 46-63 weeks of age.

Table 1. Components and chemical composition (%) of the diet used in laying hens from age 18 to 28 week

Treatment / Components	T1 control	T2,T3, T4(1)			(S.C)T5			T6(ALFALFA)		
		A FTY 250	B FTV 350	C FTY 450	A FTY 2.5	B FTY 3.5	C FTY 4.5	A FTY 2.5	B FTY 3.5	C FTY 4.5
Corn	53	53	50.89	53.02	53	52.081	52	52.047	50.49	50
wheat	7.34	7.71	10	8.21	8.466	9.37	9.11	7.59	9.1	9
SBM (44% cp)	24	24.02	24	24	23.208	23.32	23.659	23.437	22.862	22.91
Protein Centre (2)	5	5	5	5	5	5	5	5	5	5
fat	2	2	2	1.8	1.9	1.9	2	2.143	2.32	2.407
Di-calcium phosphate (18.5 P)	1.75	1.25	1.07	0.91	1.25	107	0.91	1.25	1.07	0.91
Calcium carbonate (limestone)	6.82	6.93	6.95	6.97	6.93	6.95	6.95	6.88	6.88	6.87
NaCI	0.09	0.09	0.09	0.09	0.09	0.09	0.09	0.09	0.09	0.09
Yeast S.C	0	0	0	0	0.156	0.219	0.281	0	0	0
Dried leaves alfalfa	0	0	0	0	0	0	0	1.563	2.188	2.813
Total	100	100	100	100	100	100	100	100	100	100
Calculated Chemical analysis(3)										
Energy (kcal I kg)	2804	2825	2811	2812	2813	2813	2820	2798	2800	2797
% Crude protein	18.05	18.1	18.2	18.1	17.92	18.04	18.1	18.0	17.94	18.0
Lysine%	0.99	0.99	1.0	1.0	0.98	0.99	1.0	0.98	0.97	0.97
Methionine%	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40
Methionine+ Sistine%	0.69	0.69	0.70	0.69	0.69	0.69	0.69	0.69	0.69	0.69
Calcium%	3.46	3.39	3.36	3.33	3.36	3.33	3.46	3.33	3.46	3.39
Available Phosphorus%	0.50	0.41	0.38	0.35	0.38	0.41	0.35	0.41	0.38	0.35
Arginine	0.85	0.85	0.86	0.86	0.85	0.85	0.85	0.85	0.83	0.83
Linoleic acid%	1.29	1.29	1.29	1.29	1.28	1.28	1.28	1.30	1.30	1.30

(1) T2 and T3 and T4 represent sources of phytase enzyme treatments fungal ' bacterial and mixture respectively

(2)Protease Center for poultry feed Breedcom-5 special product by Dutch WAFI company, (3) Metabolic Energy (kilocalories = 2100 crude protein 40%, fat 5%, crude fiber 2%, Calcium 8%, phosphorus 2%, lysine 3.75%, methionine 2.85%, of the date, and according to the (NRC, 1994)

Table 2. Components and chemical composition (%) of the diet used in laying hens from age 29 to 45 week

Treatment / Components	T1 control	T2,T3, T4(1)			(S.C)T5			T6(ALFALFA)		
		A FTY 250	B FTV 350	C FTY 450	A FTY 2.5	B FTY 3.5	C FTY 4.5	A FTY 2.5	B FTY 3.5	C FTY 4.5
Corn	50.74	50.43	50.72	50.517	50.334	50	50.009	51.087	51.85	52.777
wheat	18.34	19	18.15	18.983	19	19.49	19.8	17	16.3	15
SBM (44% cp)	17.3	17.35	18.06	17.57	17.3	17.221	17	17.2	16.662	16.59
Protein Centre (2)		5	5	5	5	5	5	5	5	5
fat	0	0	0	0	0	0	0	0	0	0
Di-calcium phosphate (18.5 P)	1.6	1.05	0.88	0.72	1.05	0.88	0.72	1.05	0.88	0.72
Calcium carbonate (limestone)	6.93	7.07	7.1	7.12	7.07	7.1	7.1	7.01	7.03	7.01
NaCl	0.09	0.09	0.09	0.09	0.09	0.09	0.09	0.09	0.09	0.09
Yeast S.0	0	0	0	0	0.156	0.219	0.281	0	0	0
Dried leaves alfalfa	0	0	0	0	0	0	0	1363	2.188	2.813
Total	100	100	100	100	100	100	100	100	100	100
Calculated Chemical analysis(3)										
Energy (kcal I kg)	2722	2732	2733	2739	2731	2733	2739	2712	2712	2712
% Crude protein	16.4	16.5	16.7	16.5	16.5	16.5	16.5	16.4	16.3	16.3
Lysine%	0.85	0.86	0.87	0.86	0.86	0.86	0.86	0.85	0.84	0.84
Methionine%	0.38	0.38	0.38	0.38	0.38	0.38	0.38	0.38	0.38	0.38
Methionine+ Sistine%	0.66	0.66	0.66	0.66	0.66	0.66	0.66	0.65	0.65	0.64
Calcium%	3.46	3.39	3.36	3.33	3.39	3.36	3.33	3.39	3.36	3.33
Available Phosphorus%	0.50	0.41	0.38	0.35	0.41	0.38	0.35	0.41	0.38	0.35
Arginine	0.85	0.85	0.86	0.86	0.85	0.85	0.85	0.85	0.83	0.83
Linoleic acid%	1.29	1.29	1.29	1.29	2731	2733	2739	1.30	1.30	1.30

Table 3. Components and chemical composition (%) of the diet used in laying hens from age 46 to 63 week

Treatment / components	T1 control	T2,T3, T4(1)			(S.C)T5			T6(ALFALFA)		
		A FTY 250	B FTV 350	C FTY 450	A FTY 2.5	B FTY 3.5	C FTY 4.5	A FTY 2.5	B FTY 3.5	C FTY 4.5
Corn	45.04	45.17	45.1	44.95	45.1	45.786	45.55	45.13	45.3	43.3
wheat	26.86	27.06	27.45	28.212	27.841	27	26.854	25.8	25	26.76
SBM (44% cp)	14	14.1	14	13.58	13.55	13.6	13.9	13.667	13.8	13.399
Protein Centre (2)	5	5	5	5	5	5	5	5	5	5
fat	0.2	0.18	0.1	0.07	0.05	0.05	- 0.05	0.4	0.442	0.65
Di-calcium phosphate (18.5P)	1.4	0.89	0.72	0.528	0.528	0.72	0.89	0.89	0.72	0.528
Calcium carbonate (limestone)	7.45	7.55	7.58	7.61	7.6	7.575	7.55	7.5	7.5	7.5
NaCI	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Yeast S.0	0	0	0	0	0.281	0.219	0.156	0	0	0
Dried leaves alfalfa	0	0	0	0	0	0	0	1.563	2.188	2.813
Total	100	100	100	100	100	100	100	100	100	100
Calculated Chemical analysis(3)										
Energy (kcal 1 kg)	2722	2733	2732	2737	2727	2733	2735	2723	2720	2127
% Crude protein	15.6	15.6	15.6	15.6	15.6	15.6	15.6	15.6	15.6	15.6
Lysine%	0.78	0.78	0.78	0.77	0.78	0.78	0.78	0.77	0.78	0.77
Methionine%	0.37	0.36	0.37	0.37	0.37	0.37	0.37	0.37	0.37	0.37
Methionine+ Sistine%	0.64	0.64	0.64	0.64	0.64	0.64	0.64	0.63	0.63	0.63
Calcium%	3.60	3.53	3.5	3.47	3.53	3.5	3.47	3.53	3.5	3.47
Available Phosphorus%	046	0.37	0.34	0.31	0.37	0.34	0.31	0.37	0.34	0.31
Arginine	0.77	0.77	0.78	0.78	0.77	0.77	0.77	0.77	0.77	0.77
Linoleic acid%	1.28	1.20	1.23	1.25	1.20	1.20	1.20	1.3	1.3	1.4

(1) T2 and T3 and T4 represent sources of phytase enzyme treatments fungal ' bacterial and mixture respectively (2)Protease Center for poultry feed Breedcom-5 special product by Dutch WAFI company, (3) Metabolic Energy (kilocalories = 2100 crude protein 40%, fat 5%, crude fiber 2%, Calcium 8%, phosphorus 2%, lysine 3.75%, methionine 2.85%, of the date, and according to the (NRC, 1994

For bacterial phytase we used *E. coli* sources of enzyme for fungal phytase *Aspergillus oryzae*, enzymes mixture contain (Xylanases, B-glucanase, Protease, Alfa-amylase, phytase, for yeast (*Saccharomyces cerevisia*), and for plant (dried alfalfa leaves powder).

Table 4. Estimation phytase enzyme in alfalfa leaves powder and *Saccharomyces cerevisia*

Material	Phytase enzyme activity (FTY/kg material)
Saccharomyces cerevisia	1600
Alfalfa plant leafs	160

The analysis was conducted in the Food Testing Center in Iowa in America.

It measured productive performance such as Hen day production (H.D.%) during 32 week for eight periods ,each period 28 days; cumulative egg production (egg/hen) during 224 day, egg weight, egg mass, feed conversions ratio, also qualitative characteristics of eggs such as yolk index, yolk weight, yolk and white relative weight and shell thickness.

Statistical analysis of this study using General, Linear Model of SAS statistical program was conducted (SAS, 2001).

To determine the effect of treatments studied it was using Complete Randomized Design.

RESULTS AND DISCUSSIONS

Table 5 shows no significant differences between treatments during the periods 1, 2, 3, 4, 5, 7 and 8 and the rate of egg production for the period 24-55wk recorded significant superior for treatments adding phytase enzyme at levels 250 and 450 FTY/kg feed and enzyme mixture at level 450 U/kg feed, so this was reflected positively to significant for the same treatments to produce cumulative eggs, followed by other treatments addition phytase enzyme than control group, while the lowest rate for this character recorded for the treatment added S.c.yeast at level (3.5/kg feed).

Table 6 revealed significant difference between treatments during all periods in egg weight, so egg weight from age 24-55 week has recorded higher rates for enzyme mixture at level 250 U/kg feed and alfalfa leaves powder at level 2.5 FTY/kg feed treatments by 65.45 and 65.30 gm respectively compared with enzyme treatments at various sources and levels (reduced with Ca, p) and control treatment which did not differ while adding yeast treatment at level 4.5 FTY/kg feed recorded the lowest rate of egg weight 62.65 gm.

Egg mass represents the number of eggs produced multiplied by the average egg weight and from the Table 7 there were no significant differences between experimental treatments during the first five periods and for the period 24-55 week treatment added fungal phytase enzyme at level(250 FTY/kg feed) recorded significant superior at rate 59.83 g/ bird/day and a cumulative egg mass 13401 g/bird/224 day, while S.C. yeast treatment (3.5 FTY/kg feed) recorded minimum egg mass 54.32 gm/bird/day, which reflected negatively on the a cumulative egg mass 12167 g / bird / 224 day .

Feed consumption known indicator of the nutritional content that provided to birds and the limiting factor for the efficiency of production and profitability, especially that experimental diet adding phytase enzyme at different sources and levels low in Ca and P, and according to Lohman Guide provided 120 g feed / hen / day to meet the production need and did not recorded any residual amount of feed because all feed consumed from treatments birds .

Feed consumption is an important economic indicator and what determine the values of this index were feed conversion ratio and increase egg production, egg weight and other qualitative characteristics of eggs are only the result of inputs conversion efficiency to economic value outputs. Table 8 included the average feed conversion ratio for experimental treatments, there were significant differences ($P < 0.05$) for all periods. When calculated rate of feed conversion ratio (g feed / g eggs) for a period of 24-55 weeks we showed significant improvement ($P < 0.05$) for treatment of adding fungal phytase enzyme (250 FTY / kg feed) and treatment adding mixture enzymes with 2.01 and 2. 04 g feed / g egg respectively while S.C. yeast treatment (3.5 FTY / kg feed) recorded the worst feed conversion ratio (2.22 g feed/g eggs).

The relative weight of the shell, is an important indicator of shell thickness Table 9 revealed significant differences for all production periods between the various treatments in the experiment. The total rate of relative shell weight for period (24-55) leaves weeks, treatment of adding dried alfalfa leaf

powder at level (3.5 FTY / kg feed) showed significantly superior (P<0.05) recorded 10.39%, then fungal phytase enzyme treatment at level 250 FYT/ kg feed recorded 10.16% compared to other treatments.

These results showed a positive effect because the diets differed from the conventional, in terms of the fact that rations provided complete requirements of calcium and phosphorus for the bird while diets adding phytase enzyme various sources and levels (fungal, bacterial, a mixture of enzymes, S.c. yeast, and dried alfalfa leaves) reduced with Ca and P depend on phytase enzyme levels, and that hence the non-significant increase in these treatments compared with control group. The positive result in the search indicating the benefit for bird from unavailable phosphorus in the plant sources and its relationship with many nutrient and mineral linked with and reflection on the production, this may be due to the lytic action of phytase enzyme on phytic acid then release linked nutrients and mineral like proteins or carbohydrates or minerals and vitamins (Oatway et al., 2001; Gatlin et al., 2007; Hardy, 2010) ,the enzyme works to raise the level of phosphorus and availability and thus increase the performance of vital functions because of its role in metabolites of carbohydrates, amino acids, fat and entry in the synthesis of nucleic acids and storage of energy in the body (Cao et al., 2007). As well as the action of enzyme on the liberalization of calcium that enters in many processes and pathways of metabolic and increase the permeability of cell membranes, which helps the occurrence of absorption of nutrients in the intestines and facilitate fluid passage and some ions into and out of cells process and thus maintain the balance of the contents of (Fleet et al., 2008; Schoch et al., 2012), where each of Ca and P share together in many biological processes within the body and which affect directly to increase activity of thyroid gland as a result of secreted hormone responsible for regulating their levels in the blood, thereby increasing the metabolism which will reflect positively on egg production (Elsayed et al., 2010) in addition to release manganese by enzyme that important in activation many internal enzymes in the metabolism of protein, fat and carbohydrate to prowrement generated energy therefore is an important element in production of eggs (Yildiz et al., 2011). So release nutrient and mineral elements means increased availability and thus achieve maximum benefit from digested and absorbed (Peter and Baker, 2001; Aksakal and Bilal, 2002), in addition to release inositol to be benefit from body (Wu et al., 2006). This is another side to increase in egg production for treatments adding phytase enzyme at various sources and levels, particularly for the sixth period (44-47 weeks) of the production (Table 5) which coincided in the eighth month (August) of the year, which have very high temperatures (33-34.5ºC), that means exposure of birds to heat stress led to negative impact on the productive performance, but experimental treatments have proven that add phytase enzyme at various sources showed significantly superior (P<0.05) in comparison with control group (T1). This is due to the improvement in utilization of myo-Inositol which turns in the secondary path to vitamin C in animal body (Panda et al., 2007). On the other hand entry inositol in another path for the synthesis of fat in the body after decomposition glycerol several paths to get phosphotedil Inositol (Maurie et al., 2003) and therefore, both benefit of inositol whether to get vitamin C or release energy are important sources to reduce heat stress during this period, which reflected positively in improving productivity traits especially production eggs (Panda et al., 2008).

The superiority significant for the treatment of adding a mixture of enzymes at levels (350 and 450 u/kg feed) may be due to the enzyme mixture, which included Xylanases, ß-glucanase, α-Amylase, phytase, the Xylanases enzyme works on Arabinoxylase, and ß-glucanase, works on beta-glucane which connect many nutrients and thus reduce viscosity of intestine, also α-Amylase enzyme is working on analysis of starch and that some of it is linked to phytic acid after its release by phytase enzyme (Cowieson et al., 2008) that the mixture enzymes reflected positively on egg production.

The superiority shown for the treatments of adding dried alfalfa leaves powder (3.5 and 4.5 FTY/kg feed), which was similar to fungal phytase enzyme treatment at level (350 FTy/kg feed)in cumulative egg production during 224 days may be due to the contains of

alfalfa with many vitamins such as vitamin A, vitamin E and carotenoids (Mohammed and Al-Janabi, 1989) and because of its effect in increasing egg production in birds as a result of their impact on increased secretion of sex hormones that works as vitamin E to stimulate the secretion Luteinizing Hormone Releasing Factor (LHRH) from hypothalamus (Karanth et al., 2004), and thus enhance the secretion of both hormones FSH and LH and progesterone and estrogen, which leads to enhance egg production to the presence of a positive and significant between the concentration of these hormones in blood plasma and egg production.

I believe that the findings from the results when we added phytase enzyme with reduction in Ca and P lead to get maximum benefit from the nutrients release from feed and thus appeared clearly in many traits.

We find that the significant increase for treatments adding phytase enzyme at various sources and levels with decrease Ca and P in rations compared with control treatment (T1) led a positive reflection in the production process and this can be explained by several reasons, including that the most important factor in the impact on production is nutrition and especially the productive performance of laying hens is influenced by the level of the source of diets and in addition to other factors related to management of birds, this side reflects the superiority of treatments adding phytase enzyme at various sources and levels on egg weight to the ability of enzyme to analysis phytic acid and release nutrients either carbohydrates, and fats, and proteins, and elements to be more availability , thus affecting to increase the size of product egg (Liu et al., 2007), also release phytase enzyme for magnesium, which is an important element in metabolism of protein and protein synthesis (Maguire and Cowan, 2002), as is magnesium responsible for activated alkaline phosphatase enzyme (ALP) that being necessary for deposition of calcium and phosphorus in bones and increases metabolism of calcium and phosphorus, which encourage the transition of vital components of blood to liver, thus increasing liver function of vital components manufacturing (proteins, carbohydrates, fats) to go overage the need for management and production, transmission the necessary nutrients for the manufacture of egg yolk components (Weiser et al., 1990). Also the role of enzyme in release inositol ring which is part of secondary course be vitamin C (antioxidant), where he pointed by Panda et al., 2008, that have positive effect of the reaction of vitamin C on poultry production of eggs, weight and egg mass associated with increased protein concentration in egg yolk. And perhaps improve unsaturated fatty acids (Omega-3 and Omega-6). When we added phytase enzyme thus release associated fat and may have a role in improving chicken requirements from the essential fatty acid, in addition to advantage of vitamins soluble in fat (A, E, D, K), which is positively reflected a significant improvement to egg weight (Zyla et al., 2012).

Adding dried alfalfa leaves powder at level (2.5 FTy/kg feed) may be due to their contain with many enzymes such as phytase, amylase, beta-glucanase (Edimister et al., 2001), amylase works on analysis of starch into its basic components, and beta-glucanase which works to break down the compound complex beta- glucane and release nutrients associated with it, which has a high affinity for link with water, causing increase viscosity (Cowieson et al., 2006) and thus led to benefit from feed, which reflects positively on the production and egg weight. This is in addition to containing alfalfa a good quality protein and element such as calcium, phosphorus, and source of vitamin A.

The reason for increase relative shell weight in dried alfalfa leaves treatment may be due to contain alfalfa 9-13 gm/kg calcium is an important element in the formation of egg shell, as well as 2-3 gm/kg phosphorus, (Mohammed and Al-Janabi, 1989).Thus led to increase calcium by absorption from intestine and then move into blood and increase concentration of calcium in blood plasma (Al-Hassani, 2000), in addition to the containment. Flavonoids known as Isoflavones and glycoside saponine compounds for its role in influencing stimulation of sex hormones as estrogen that works to transform calcium from medullary bone to blood.

This reflected positively on increased shell thickness and shell weight, and this enhance the strength and hardness for egg produced (Fayad and Nagy, 1989).

Table 5. Effect of adding different levels and sources of phytase enzyme to the diets of laying hens (Lohmann brown) in the perecentage of egg production (HD%) (±standard error) during production periods (24-55 weeks) of age

Treatments		Average percentage of egg production (HD%) of production periods (weeks)								Overall average 24 -55 weeks	Cumulative production of eggs (egg/chicken/224 day)
		1 27 −24	2 31 −28	3 35 −32	4 39−36	5 43 −40	6 47−44	7 51 −48	8 55−52		
T1		5.70±87.5	2.20±96.3	2.50±94.2	4.51±87.9	3.3±85.0	2.20±65.1 b	0.61±84.7	0.6±88.7	0.77± 86.26 cde	0.57± 193.21 f
T2	A	0.29±88.5	0.17±98.0	1.11±95.3	4.21±90.3	1.1±90.1	3.72±85.4 a	4.7±94.2	2.8±94.0	0.57±92.06 a	0.57±206.21 a
	B	0.89±78.5	1.92±88.7	2.01±88.5	7.52±92.7	8.8±91.4	6.92±86.4 a	2.9±88.0	3.9±90.4	0.50±88.13 bc	0.57±197.42 c
	C	1.42±88.2	0.12±94.3	0.31±95.0	5.62±91.9	4.4±88.1	4.40±86.6 a	4.2±90.2	2.4±92.3	2.10±90.87 a	0.57± 203.55 b
T3	A	7.81±82.7	2.20±92.2	3.50±90.7	3.50±85.9	4.2±87.2	5.32±84.6 a	6.0±90.2	4.1±87.7	0.75±87.72 bc	0.57±196.48 cd
	B	0.05±83.5	0.05±94.1	0.11±92.5	4.60±86.1	0.9±84.8	3.22±82.8 a	5.2±86.4	4.1±87.2	0.73±87.22 bc	0.57±195.36 de
	C	10.6±88.8	8.92±93.7	9.40±90.4	5.42±91.3	1.1±90.2	5.14±83.4 a	7.2±86.0	7.8±84.8	0.50±88.24 b	0.57±197.65 c
T4	A	3.80±83.5	2.42±94.3	1.50±95.0	0.11±88.3	1.2±84.3	0.95±77.2 ab	0.1±86.5	2.8±87.5	0.69±87.11 bc	0.57±195.13 de
	B	0.53±87.0	1.43±93.2	0.11±92.9	0.82±86.7	0.5±86.2	0.67±78.8 ab	2.6±85.1	0.2±85.4	0.48±86.98 bc	0.57 ±194.84 def
	C	6.01±86.3	1.32±96.7	1.2±96.41	5.50±90.6	1.6±92.9	6.10±84.3 a	2.1±93.6	1.5±90.3	0.59±91.85 a	0.57±205.75 a
T5	A	0.29±84.1	0.23±90.4	1.1±91.13	0.07±88.7	1.4±87.9	0.97±82.3 a	2.1±82.4	0.7±85.1	0.72±86.53 bcd	0.57± 193.83 ef
	B	2.92±81.6	0.35±91.5	1.9±91.10	0.71±85.7	1.1±83.5	1.67±73.3 ab	1.3±86.2	2.6±83.1	0.66±84.55 e	0.57± 189.40 g
	C	8.03±83.8	6.09±95.3	1.3±95.31	3.60±83.6	2.3±85.2	2.30±85.6 a	1.9±87.2	1.9±83.8	0.50±87.54 bc	0.57±196.08 cd
T6	A	4.92±78.9	7.40±89.7	8.3±90.32	10.0±88.0	6.0±86.6	6.01±82.4 a	1.3±83.2	9.3±81.7	0.55± 85.06 de	0.57±190.53 fg
	B	4.34±83.6	0.32±92.1	2.1±95.20	1.56±89.7	4.8±83.7	3.91±84.8 a	1.9±89.2	2.3±87.6	0.63± 88.29 b	0.57±197.77 c
	C	1.96±84.7	1.63±96.7	1.7±93.90	6.10±94.6	0.8±85.9	0.14±81.1 ab	1.9±83.6	3.8±81.3	1.01±87.77 bc	0.57±196.61 cd
significant		N.S	N.S	N.S	N.S	N.S	*	N.S	N.S	*	*

* Different letters within the same column indicated the presence of significant differences ($p \leq 0.05$),. T1= control. T2 and T3 and T4 enzyme phytase treatments fungal *AspergilIus oryzae* , bacterial E.coli and a mixture of enzymes, respectively ,and the letters A, B, C (250, 350, 450 FTY 1kg feed T5 and T6 treatment me yeast SC and alfalfa plant respectively, letters C, B, A (2.5 and 3.5 and 4.5 FTY /kg feed).

Table 6. Effect of adding different levels and sources of phytase enzyme to the diets of laying hens (Lohmann brown) average of egg weight (g) (±standard error) during production periods (24-55 weeks) of age

Treatments		Average egg weight (g) of production periods (weeks)								Average egg weight (g) 24.55 weeks
		1 27 -24	2 31 -28	3 35 -32	4 39-36	5 43 -40	6 47-44	7 51 -48	8 55-52	
T1		0.05±64.01 ab	0.05±66.51 ab	0.01±65.06 ab	0.02±63.51 abc	0.03±61.07 ab	0.08±62.20 c	0.02±65.22 ab	0.08±65.92 b	0.07±64.30 abc
T2	A	0.02±64.65 ab	0.01±64.53 cde	0.63±64.80 ab	0.08±63.91 abc	0.44±61.83 ab	0.06±63.14 abc	0.09±65.55 ab	9.01±68.39 a	0.00±65.00 ab
	B	0.03±63.75 abc	0.06±65.36 abcde	0.01±64.38 abc	0.04±64.78 ab	0.55±62.85 ab	0.06±64.22 ab	b0.53±65.32 a	0.03±67.38 ab	0.05±64.75 abc
	C	0.08±63.85 abc	0.09±63.70 e	0.50±63.6 bc	0.05±63.10 c	0.04±60.23 b	0.02±62.91 abc	0.41±63.23 b	0.04±65.33 b	0.01±63.25 c
T3	A	0.05±63.65 abc	0.09±65.30 abcde	0.09±64.32 abc	0.60±65.24 ab	0.90±62.70 ab	0.96±63.52 abc	0.00±65.08 ab	0.21±66.85 ab	0.04±64.60 abc
	B	0.03±64.33 ab	0.06±66.63 a	0.08±65.64 a	0.07±64.50 ab	0.08±61.71 ab	080±63.21 abc	0.21±65.48 ab	2.01±65.72 b	0.10±64.70 abc
	C	0.05±64.25 ab	0.01±65.91 abc	9.01±64.89 ab	0.10±65.82 a	0.08±63.70 ab	0.45±64.17 ab	0.03±64.72 ab	0.03±66.86 ab	0.02±65.05 ab
T4	A	0.05±64.45 ab	0.00±66.72 a	0.09±65.59 ab	0.08±64.80 ab	0.06±62.80 ab	0.01±64.80 ab	0.02±67.20 a	0.43±67.39 ab	0.45±65.45 a
	B	0.03±63.32 abc	0.03±65.33 abcde	0.02±63.82 abc	0.99±63.92 abc	0.33±61.16 ab	0.02±65.62 a	0.54±65.50 ab	0.21±67.41 ab	0.40±64.53 abc
	C	0.02±63.75 abc	0.03±65.73 abcd	0.08±63.72 abc	0.66±63.92 abc	0.11±60.91 ab	0.04±63.24 abc	0.43±65.63 ab	0.05±66.21 b	0.55±64.15 abc
T5	A	0.02±63.76 abc	0.07±63.72 e	0.08±64.63 ab	0.06±63.31 abc	0.07±61.61 ab	0.21±63.34 abc	0.03±64.91 ab	0.01±63.94 b	0.05±63.65 b
	B	0.01±64.52 ab	0.05±64.82 bcde	0.03±65.13 ab	0.08±64.40 abc	0.07±62.40 ab	0.03±62.21 c	0.60±65.39 ab	1.02±64.81 b	0.06±64.20 abc
	C	0.04±62.20 c	0.04±63.68 e	0.04±62.53 c	0.87±63.75 ab	0.08±59.80 b	0.40±62.23 c	0.03±64.09 b	0.32±64.44 b	0.05±62.65 c
T6	A	0.06±65.05 a	0.08±66.80 a	0.08±65.38 ab	0.90±65.14 ab	0.04±63.34 ab	0.05±64.50 ab	0.03±65.19 ab	0.21±66.68 ab	0.20±65.30 a
	B	0.06±63.00 b	0.06±64.07 de	0.00±64.14 abc	0.09±64.51 ab	0.01±65.92 a	0.03±63.90 abc	0.02±64.80 ab	0.33±65.73 b	0.45±64.51 abc
	C	0.05±63.85 abc	0.01±66.43 ab	0.01±65.24 ab	0.60±65.17 ab	0.05±63.19 ab	0.20±64.20 ab	0.04±64.73 ab	0.11±65.92 b	0.03±64.85 ab
significant		*	*	*	*	*	*	*	*	*

* Different letters within the same column indicated the presence of significant differences ($p \leq 0.05$),. T1= control. T2 and T3 and T4 enzyme phytase treatments fungal *Aspergillus oryzae* , bacterial E.coli and a mixture of enzymes, respectively, and the letters A, B, C (250, 350, 450 FTY 1kg feed T5 and T6 treatment me yeast SC and alfalfa plant respectively, letters C, B, A (2.5 and 3.5 and 4.5 FTY /kg feed).

Table 7. Effect of adding different levels and sources of phytase enzyme to the diets of laying hens (Lohmann brown) in egg mass (g /bird / day) (± standard error) during production periods (24-55 weeks) of age

Treatment		day) for production periods (weeks)/ bird/Average egg mass (g								Average from 24-55 weeks	Cumulative egg mass (g/ bird / day 224)
		1 27 -24	2 31 -28	3 35 -32	4 39-36	5 43 -40	6 47-44	7 51 -48	8 55-52		
T1		0.03±56.50	0.67±64.03	0.04±61.28	0.08±55.81	0.07±51.90	0.35±40.67 b	0.01±55.22	0.02±58.45 ab	±55.461.23 efgh	±124235.72 j
T2	A	0.02±57.21	0.01±63.25	0.23±61.75	0.34±57.70	0.05±55.68	0.01±53.92 a	0.03±61.70	0.04±64.28 a	±59.831.76 a	±134016.90 a
	B	0.03±50.04	0.05±57.97	0.00±55.97	0.09±60.05	0.30±57.39	0.03±55.46 a	0.06±57.46	0.02±60.91 ab	±57.071.09 cde	±127833.67 e
	C	0.01±56.31	0.03±60.06	0.03±60.42	0.87±57.98	0.34±53.03	0.05±54.47 a	0.03±57.00	0.05±60.29 ab	±57.502.13 bc	±128795.09 c
T3	A	0.70±52.63	0.12±60.20	0.09±58.32	0.12±56.04	0.05±54.67	0.06±53.72 a	0.07±58.70	0.04±58.62 ab	±56.671.26 cdefg	±126936.72 g
	B	0.05±53.69	0.02±62.67	0.11±60.68	0.34±55.53	0.09±52.33	0.02±52.32 ab	0.04±56.50	0.03±57.29 ab	±56.401.98 cdefg	±126339.02 h
	C	0.10±55.77	0.09±61.75	0.21±58.66	0.45±60.07	0.45±57.45	0.51±53.51 a	2.07±55.64	0.08±56.69 ab	±57.411.34 bcd	±128595.34 d
T4	A	0.38±53.81	0.03±62.89	0.34±62.31	0.45±57.21	0.04±52.94	0.06±50.02 ab	0.04±58.12	0.02±58.96 ab	±57.051.98 cde	12778±5.47 e
	B	0.50±55.12	0.13±60.85	0.40±59.28	0.09±55.40	0.06±52.71	0.02±51.69 ab	1.03±55.74	0.04±57.56 ab	±56.122.21 cdefgh	±125715.88 i
	C	0.06±55.01	0.22±63.53	0.06±61.40	0.72±57.89	0.05±56.57	0.04±53.27 a	0.12±61.40	0.03±61.56 ab	±58.922.01 ab	±131972.34 b
T5	A	0.29±53.57	0.11±57.58	0.13±58.89	0.43±56.14	0.06±54.14	0.05±52.12 ab	0.03±53.47	0.01±54.37 b	±55.091.40 fgh	±123396.87 k
	B	0.29±52.63	0.23±59.31	0.01±59.33	0.05±55.19	0.09±52.10	0.02±45.59 ab	0.04±56.36	0.03±53.84 b	±54.321.39 h	±121675.67 m
	C	0.08±52.12	0.10±60.68	0.15±59.59	0.32±51.74	0.04±50.94	0.04±53.24 a	0.06±55.88	0.02±53.96 b	±54.821.58 gh	±122807.83 l
T6	A	0.49±50.86	0.02±59.91	0.88±59.03	0.47±57.32	0.02±54.81	0.03±53.14 a	0.43±54.23	0.06±54.47 b	±55.531.03 defgh	±124397.71 j
	B	0.03±52.66	0.21±59.00	0.09±61.06	0.34±57.85	0.03±55.15	0.06±54.18 a	0.12±57.80	0.03±57.50 ab	±56.961.89 cdef	±127606.85 f
	C	0.19±54.08	0.42±64.23	0.12±61.26	0.04±61.65	0.05±54.28	0.05±52.06 ab	0.34±53.95	0.06±53.57 b	±56.922.01 cdef	±127495.50 f
significant		N.S	N.S	N.S	N.S	N.S	*	N.S	*	*	*

* Different letters within the same column indicated the presence of significant differences ($p \leq 0.05$),. T1= control. T2 and T3 and T4 enzyme phytase treatments fungal *Aspergillus oryzae* , bacterial E.coli and a mixture of enzymes, respectively ,and the letters A, B, C (250, 350, 450 FTY 1kg feed T5 and T6 treatment me yeast SC and alfalfa plant respectively, letters C, B, A (2.5 and 3.5 and 4.5 FTY /kg feed).

Table 8. Effect of adding different levels and sources of phytase enzyme to the diets of laying hens (Lohmann brown) In feed conversion ratio (g feed / g egg) (± standard error) during production periods (24-55 weeks) of age

Treatment		Feed conversion ratio (g feed /g egg) for production periods (weeks)								Total feed conversion ratio (24-55) week
		1 27 -24	2 31 -28	3 35 -32	4 39-36	5 43 -40	6 47-44	7 51 -48	8 55-52	
T1		0.15±2.12 f	0.15±1.87 h	0.06±1.96 fg	0.09±2.15 b	0.07±2.31 b	0.13±2.94 a	0.25±2.17 b	0.01±2.05 de	0.00±2.20 ab
T2	A	0.05±2.10 f	0.02±1.90 gh	0.04±1.94 g	0.05±2.08 c	0.00±2.15 gh	0.03±2.22 efg	0.08±1.94 i	b0.06±1.79 h	0.03±2.01 f
	B	0.01±2.40 a	0.03±2.07 a	0.04±2.10 a	0.08±2.00 d	0.17±2.09 i	0.23±2.16 h	0.10±2.09 efg	0.14±1.97 f	0.17±2.11 de
	C	0.02±2.13 f	0.00±1.99 cd	0.04±1.98 ef	0.02±2.07 c	0.03±2.26 d	0.10±2.20 g	0.07±2.10 def	0.15±1.99 f	0.01±2.09 e
T3	A	0.15±2.28 c	0.13±1.99 cd	0.10±2.06 b	0.09±2.14 b	0.02±2.19 ef	0.06±2.23 efg	0.15±2.04 h	1.10±2.05 de	0.09±2.12 de
	B	0.01±2.23 d	0.03±1.91 fg	0.03±1.98 ef	0.13±2.16 b	0.07±2.29 bcd	0.06±2.29 d	0.08±2.12 cde	0.09±2.09 c	0.02±2.13 d
	C	0.15±2.17 e	0.00±1.94 ef	0.10±2.05 bc	0.02±2.00 d	0.00±2.09 i	0.09±2.24 ef	0.12±2.15 cb	0.07±2.11 c	0.05±2.09 e
T4	A	0.08±2.23 d	0.02±1.91 fg	0.05±1.93 g	0.09±2.10 c	0.56±2.26 d	0.02±2.39 c	0.05±2.06 gh	0.03±2.03 e	0.02±2.11 de
	B	0.12±2.18 e	0.01±1.97 de	0.05±2.02 cd	0.03±2.16 b	0.26±2.27 cd	0.11±2.32 d	0.08±2.15 bc	0.03±2.08 cd	0.06±2.14 cd
	C	0.01±2.18 e	0.04±1.89 gh	0.01±1.95 fg	0.01±2.08 c	0.13±2.12 hi	0.09±2.25 e	0.34±1.95 i	0.28±1.93 g	0.11±2.04 f
T5	A	0.09±2.24 d	0.00±2.08 a	0.02±2.04 bcd	0.09±2.14 b	0.00±2.21 e	0.16±2.30 d	0.66±2.24 a	0.08±2.21 ab	0.05±2.18 b
	B	0.06±2.28 c	0.01±2.02 bc	b0.01±2.02 cd	0.00±2.17 b	0. 04±2.30 bc	0.13±2.63 b	0.04±2.13 cd	0.06±2.22 ab	0.01±2.22 a
	C	0.19±2.30 c	0.09±1.98 d	0.12±2.01 de	0.03±2.32 a	0.09±2.35 a	0.03±2.25 e	0.00±2.15 bc	0.07±2.22 ab	0.06±2.20 ab
T6	A	0.02±2.36 b	0.08±2.00 bcd	0.20±2.03 bcd	0.05±2.09 c	0.03±2.19 e	0.10±2.25 e	0.11±2.21 a	0.07±2.20 b	0.09±2.17 bc
	B	0.06±2.28 c	0.06±2.03 b	0.03±1.96 fg	0.01±2.07 c	0.05±2.17 fg	0.05±2.21 fg	0.04±2.07 fgh	0.04±2.08 cd	0.08±2.11 de
	C	0.01±2.22 d	0.07±1.87 h	0.03±1.96 fg	0.05±1.95 e	0.10±2.21 e	0.01±2.30 d	0.05±2.22 a	0.11±2.24 a	0.06±2.12 de
significant		*	*	*	*	*	*	*	*	*

Table 9. Effect of addina different levels and sources of phytase enzyme to the diets of laying hens (Lohmann brown) In relative shell weight(%) (± standard error) during production periods (24-55 weeks) of age

Treatment		relativesheliweight for production Periods (week)								Rate the relative weight of the shell 24-56 weeks
		1 24-27	2 28-31	3 32-35	4 36-39	5 40-43	6 44-47	7 48-51	8 52-55	
T1		035±9.39 c	0.35±9.76 abc	0.37±9.59 abc	0.35±9.45 abcd	0.0.37±8.73 ef	0.37±9.20 c	0.38±10.5 ab	0.32±10.00 cde	0.1419.58 bc
T2	A	0.37±10.27 bc	0.35±10.08 ab	0.3419.88 abc	0.35±9.48 abcd	0.32±9.99 abcd	0.34±10.14 abc	0.39±9.83 bc	0.37±11.61 a	0.16±10.16 ab
	B	0.37±9.36 bc	0.371,9.97 ab	0.36±9.60 abc	0.371-9.58 abcd	0.34±10.00 abcd	0.33±9.14 c	0.37±8.09 d	0.35±10.30 dc	0.17±9.54 bc
	C	0.33±9.51 c	0.361:10.29 ab	0.35±9.52 abc	0.37±9.59 abcd	0.34±8.98 cdef	0.32±9.45 bc	0.37±10.64 ab	0.38±9.18 de	0. 15±9.65 bc
T3	A	0.36±10.20 bc	0.34±8.74 c	0.34±9.51 abc	0.32±9.51 abcd	0.31±8.98 cdef	0.37±10.02 abc	0.38±9.04 cd	0.34±9.43 f	0.20±9.44 C
	B	0.37±10.27 bc	0.35±10.07 ab	0.33±7.34 e	0.33±10.57 a	0.34±8.11 f	0.361±10.00 abc	0.381±10.16 bc	0.37±9.28 f	0.27±9.48 c
	C	0.34±9.26 c	0.36±10.27 ab	0.36±10.36 a	0.34±9.22 bcd	0.34±9.41 bcde	0.34±10.59 ab	0.35±9.69 bc	0.35±9.14 f	0.20±9.74 bc
T4	A	0.37±9.41 c	0.34±8.72 c	0.33±8.78 cd	0.35±10.02 abc	0.31±10.77 a	0.31±9.54 bc	0.35±10.77 ab	0.36±9.07 e	010±9.63 bc
	B	0.361±9.80 bc	0.30±9.89 abc	0.32±10.40 a	0.33±10.05 ab	0.32±10.28 ab	0.33±10.28 abc	0.39±10.67 ab	0.39±9.39 f	0.19±10.10 abc
	C	0.35±10.73 ab	0.33±9.78 abc	0.3619.33 abc	0.32±9.80 abcd	0.35±8.89 def	0.36±9.69 abc	0.33±7.43 e	0.331±10.05 cde	0.19±9.45 c
T5	A	0.36±10.26 bc	0.32±9.76 abc	0.36±7.91 de	0.34±9.29 bcd	0.30±9.84 abcd	0.37±10.80 a	0.31±10.65 ab	0.36±10.16 cde	0.31±9.84 abc
	B	0.34±9.76 bc	0.36±9.23 bc	0.33±9.51 abc	0.35±9.53 abcd	0.36±9.42 bcde	0.31±9.46 bc	0.33±10.32 ab	0.34±9.56 cde	0.2029.60 bc
	C	0.36±9.74 bc	0.35±7.59 c	0.33±10.36 a	0.33±9.45 abcd	0.34±10.13 abc	0.33±9.57 bc	0.36±9.84 bc	0.34±10.63 abc	0.12±9.66 bc
T6	A	0.36±9.36 c	0.32±10.57 a	0.38±8.99 bc	0.27±8.69 d	0.33±10.46 ab	0.30±10.00 abc	0.34±9.85 bc	0.33±10.00 cde	0.23±9.74 bc
	B	0.34±10.47 abc	0.38±10.13 ab	0.32±9.99 ab	0.33±8.84 cd	0.32±9.87 abcd	0.30±9.87 abc	0.36±11.47 a	0.38±11.47 ab	0.16±10.39 a
	C	0.34±11.50 a	0.33±10.09 ab	0.34±10.31 a	0.33±9.05 bcd	0.35±7.92 f	0.31±6.92 d	0.34±9.69 bc	0.34±10.47 bc	0.21±9.49 c
significant		*	*	*	*	*	*	*	*	*

* Different letters within the same column indicated the presence of significant differences ($p \leq 0.05$),. T1= control. T2 and T3 and T4 enzyme phytase treatments fungal *AspergilIus oryzae* , bacterial E.coli and a mixture of enzymes, respectively ,and the letters A, B, C (250, 350, 450 FTY 1kg feed T5 and T6 treatment me yeast SC and alfalfa plant respectively, letters C, B, A (2.5 and 3.5 and 4.5 FTY /kg feed).

REFERENCES

Aksakal, D.H., T. Bilal., 2002. Effects of microbial phytase and 1, 25 – dihyroxycholecacifrol on the absorption of minerals from broiler chicken diets containing different levels of calcium. Ind. Vet. J. 79 : 446 – 450 .

Al-hussani, D.H., 2000. Physiology poultry. The Ministry of Higher Education and Scientific Research - University of Baghdad - National Library for printing and publishing – Baghdad.

Cao, L, W. Wang, C. Yang, Y. Yang, J. Diana, A .Yakupitiyage, Z. Luo, D. Li., 2007. Application of microbial phytase in fish feed. Enz.Microb.echnol. 40: 497-507.

Cheng, Z., M. Elmes, S. E. Kirkup, E. C. Chin, D. R. E. Abayasekara, D. C. Wathes, 2005. The effect of a diet supplemented with the n-6 polyunsaturated fatty acid linoleic acid on prostaglandin production in early- and late-pregnant ewes. J. Endocrinal., 184: 165- 178.

Ciftci, M., B. Dalkilic, M. Ali-Azman., 2005. Effects of microbial phytase supplementation on feed consumption and egg production of laying hens. Int. J. Poult. Sci., 4: 758-760.

Cowieson, A. J., V. Ravindran, P. H. Selle, 2008. Influence of dietary phytic acid and source of microbial phytase on ileal endogenous amino acid flows in broiler chickens. Poult.Sci. 2008 87: 2287-2299.

Cowieson, A., T. Acamovic, and M. Bedford, 2006. Supplem-entation of corn-soy-based diets with an *Eschericia coli*-derived phytase: Effects on broiler chick performance and the digestibility of amino acids and metabolizability of minerals and energy. Poult. Sci. 85: 1389-1397.

Edminster, C., D. Miller, J.Moutray, 2001. Alfalfa industry in the United State of America. China Grassland Society, Beijing Agriculture committee.

Elsayed, M.A., M.M. Wakwak, K.H.M. Mahrose, 2010. Effect of pyridoxine injection in Japanese Quail eggs on hatchability, performance and some of physiological parameters. Isotope and Rad. Res., 472(1), 109-123.

Fayad, H. A., S.A., Nagy, 1989. Technology poultry products. The first edition, the Directorate of Higher Education Press, Baghdad – Iraq.

Ferguson, E. M., H.J. Leese, 2006. A potential role for triglyceride as an energy source during bovine oocyte maturation and early embryo develop-ment. Mol. Reprod. Dev., 73: 1195-1201.

Fleet,JC, C.Gliniak, Z. Zhang, Y.Xue, K.B Smith, R.Mcreedy, and S.A.Adedokon .,2008. Serum metabolite profiles and target tissue gene expression define the effect of cholecalciferol intake on calcium metabolism in rats and mice. Journal of Nutrition.;138(6):1114–20.

Fletcher R.A., 1971. Effect of vitamin A deficiency on pituitary - gonad axis of hecalifirnia quail (Lophorty x califirnicus). J. Exp. Zool .176 : 25-29.

Francesch, M., J. Broz, J. Brufau, 2005. Effects of an experimental phytase on performance, egg quality, tibia ash content, and phosphorus bioavailability in laying hens fed on maize- or barley-based diets. Br. Poult. Sci. 46:340-348.

Gatlin, D.M, F.T. Barrows, P. Brown, K. Dabrowski, G.T. Gaylord, R.W Hardy, E. Herman, G.Hu, Å.Krogdahl, R. Nelson, K. Overturf, M. Rust, W. Sealey, D. Skonberg, E.J Souza, D. Stone, R. Wilson, E. Wurtele, 2007. Expanding the utilization of sustainable plant products in aqua feeds: a review. Aquaculture Research 38:551-579.

Gibson, R.S., K.B. Bailey, M. Gibbs, E.L. Ferguson, 2010. A review of phytate, iron, zinc, and calcium concentrations in plant-based complementary foods used in low-income countries and implications for bioavailability'. Food Nutrition Bulletin, 31:S134-46

Haitham M.Y., 2010.Compared to exporters of feed Phytase for laying hens feed of corn and soybean meal. Egypt. Poult.Sci., 501-516.

Hardy, R.W., 2010. Utilization of plant proteins in fish diets: effects of global demand and supplies of fishmeal. Aquaculture Research. 41 (5): 770-776.

Hatten, L.F., D.R. Ingram, S.T. Pittman, 2001. Effect of phytase on production parameters and nutrient availability in broilers and laying hens: A review. J. Appl. Poult. Res.10:274-278.

Howarth, R.W., A.N. Sharpley, D. Walker, 2002. Sources of nitrogen pollution to coastal waters of the United States. Estuaries 25:656-676.

Huang, H.X., S.T.Li, X.L.Li, J.Yao, W.D.Cao, M.Wang, Liu, 2006. Analysis on the status of organic fertilizer and its development strategies in China. Soil Fertilizer, 1: 3–8.

Karanth, S., W.H. Yu, C.A. Mastronardi, S.M. McCann, 2004. Inhibition of stimulated ascorbic acid and luteinizing hormone-releasing hormone release by nitric oxide synthase or guanyl cyclase inhibitors. Exp. Biol. Med., 229:72–79.

Knuckles B.E., E.M., Biekoff, G.O. Kohler, 1972. Pro-Xan process: Methods for increasing protein recovery form alfalfa. J Agr Food chem., 20:1055-1057.

Kumar V., K. Amit, P.S. Harinder, K. Becker, 2010.ietary roles of phytate and phytase in human nutrition: A review. Food Chemistry 120 : 945-959.

Liu, G., H. Liu, F. D. Li, J. S. Sands, S. Zhang, A.J. Zheng, Y.J. Ru, 2007. Efficacy of Phytases on Egg Production and Nutrient Digestibilityin Layers Fed Reduced Phosphorus Diets. Poult.Sci., 86:2337–2342.

Lott J.N.A., I. Ockenden, V. Raboy, G.D. Batten, 2000. Phytic acid and phosphorus in crop seeds and fruits: a global estimate. Seed Sci Res., 10:11–33.

Maguire, M. E., J. A. Cowan, 2002.Magnesium Chemistry and Biochemistry. Biometals 15, 203-210,

Mauriès M., 2003. Luzerne : culture, récolte, conservation, utilisation. France Agricole editions. http://books.google.fr/books?id=phLup1wSc9UC.

Mohammed, A., M.J. Gibney, T.G. Taylor, 1991. The effect of dietary levels of inorganic phosphorus, calcium and cholecalciferol on the digestibility of phytate phosphorus by the chick. British Journal of Nutrition 66: 251 -259.

Mohammed, A.S., A.N. Al-Janabi, 1989. The scientific basis for chicken feed. The Ministry of Higher Education and Scientific Research. Faculty of Agriculture, University of Baghdad.

Mohebbifar, A. M. Torki, 2011. Phytase supplementation of low phosphorous diets included graded levels of ricebran on productive performance of laying hens ,International Conference on Biology Environment and Chemistry. IPCBEE, vol.1 .

Musapuor, A., J. Pourreza, A. Samie, H.M. Shahrbabak, 2005. Effects of cholecalciferol and phytase on phytate phosphorus utilization in laying hens. Int. J. Agric. Biol., 7: 643-645.

Nahm, K.H., 2007. Efficient phosphorus utilization in poultry feeding to lessen the environmental impact of excreta.World's Poult. Sci. J., 63: 625-654.

Oatway L., T. Vasanthan, J.H. Helm, 2001. Phytic acid. Food reviews International, 17 (4): 419-431.

Panda, A.K., S.V. Ramarao, M.V. Raju, R.N. Chatterjee, 2008.Effect of dietary supplementation with vitamins E and C on production performance, immune responses and antioxidant status of White Leghorn layers under tropical summer conditions.British Poult. Sci., 49 (5): 592- 599

Panda, A.K., S.V.Rao, , M.V. Raju, S.S.Gauja and S.K.Bhanja.,2007. Performance of broiler chickens fed low non phytate phosphorus diets supplemented with microbial phytase. Journal of Poult. Sci. 44(3): 258-264.

Peter, C.M., D.H. Baker, 2001. Microbial phytase does not improve protein-amino acid utilization in soybean meal fed to young chicks. J. Nutr., 131:1792-1797.

Plumstead, P.W., 2007. Strategies to reduce fecal phosphorus excretion in the broiler industry without affecting performance. Ph.D. Thesis, North Carolina State University.

SAS.,2001. SAS / STAT Users Guide for personal computer ; Release 6-12 . SAS Institute Inc. Cary, NC. USA.

Schoch, C.L., K.A. Seifert, S. Huhndorf , V. Robert, J.L. Spouge, C.A. Levesque, W. Chen, F.B. Consortium, 2012. Nuclear ribosomal internal transcribed spacer (ITS) region as a universal DNA barcode marker for Fungi. Proceedings of the National Academy of Sciences, USA, 109: 6241–6246.

Scott, M.L., M.C. Nesheim, R.J. Young, 1982. Proteins and amino acids. Nutrition of Chicken. M. L. Scott and Association Publisher, Ithaca, NY, 58-118.

Scott, T.A., R. Kampen, F.G. Silversides, 1999. The effects of phosphorus, phytase enzyme and calcium on the performance of layers fed corn-based diets. Poult. Sci., 78: 1742-1749.

Silversides, F.G., M. Hruby, 2009. Feed formulation using phytase in laying hen diets1. J. Appl. Poult. Res. 18 :15–22doi: 10.3382/japr.2008-00035.

Silversides, F.G., M. Hruby, 2009. Feed formulation using phytase in laying hen diets1. J. Appl. Poult. Res. 18 :15–22doi: 10.3382/japr.2008-00035

Sims, J.T., A.N. Sharpley, 2005. Phosphorus; Agriculture and the Environment. Am. Soc. Agron. Monograph. Agronomy 46, American Society of Agronomy, Madison, WI, 1121.

Singh A., J.D. Hamme, O.P. Ward, 2007. Surfactants in microbiology and biotechnology. Part 2. Application aspects. Biotechnol. Adv., 25: 99-121.

Snow J.L., M.W. Douglas, C.M. Parsons, 2003. Phytase Effects on Amino Acid Digestibility in Molted Laying Hens. Poult.Sci., 82:474–477.

Toth, J.D., Z. Dou, J.D. Ferguson, D.T. Galligan, C.F. Ramberg, 2006. Nitrogen- vs. phosphorus-based dairy manure applications to field crops: nitrate and phosphorus leaching and soil phosphorus accumulation. J. Environ. Qual., 35:2303-2312.

Walter, P.S., A.A. Carlos, K. Carruthers, S. Kulkarni, F.L. Goggin, A. Lorence, 2010. Exploring the impact of wounding and jasmonates on ascorbate metabolism. Plant Physiology and Biochemistry, Volume 48, Issue 5, May, 337–350.

Wathes, D.C., D. Robert, E. Abayasekara, R.J. Aitken, 2007. Poulyunsaturated fatty acids in Male and female reproduction. Biol. Reprod., 77 (2): 190-201.

Weiser, H., H. Schlacheter, H.P. Probst, 1990. The effectiveness of vit.D3 and its metabolites in relation vit. C. Internat. J. Vitamin Nutr. Res., 60: 205 .

Wu X., T. Zhang, J. Bossuyt, X. Li, T.A. McKinsey, J.R. Dedman, E.N. Olson, J. Chen, J.H. Brown, D.M. Bers, 2006. Local InsP3-dependent perinuclear Ca2+ signaling in cardiac myocyte excitation-transcription coupling. J. Clin. Invest., 116:675–682.

Yildiz, A.Ö., Y. Cufadar, O. Olgun, 2011. Effects of dietary organic and inorganic manganese supplementation on performance, egg quality and bone mineralization in laying hens. Revue Méd.Vét., 162, 10, 482-488.

Żyla K., M. Grabacka, M. Pierzchalska, R. Duliński, A. Starzyńska, 2012. Effect of inositol and Phytase on hematological indices and α-1 acid glycoprotein levels in laying hens fed phosphorus-deficient corn-soybean meal-based diets. Poult. Sci.,vol. 92, no. 1, 199-204.

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UTILIZATION OF COCONUT WATER-BASED ELECTROLYTE SOLUTION AND ROSELLE EXTRACT ON BODY WEIGHT AND PHYSIOLOGICAL RESPONSES ON PADJADJARAN RAM

Elvia HERNAWAN[1], An-An YULIANTI[1], ElinNURLINA[2]

[1]Faculty of Animal Husbandry of Padjadjaran University, Bandung, Indonesia
[2]Faculty of Engineering of JenderalAchmadYani University, Cimahi, Indonesia

Corresponding author email: hernawan.elvia@yahoo.com

Abstract
Animal transportation usually causes a weight loss. This surely becomes an economical loss. Animal suspected to be under stress circumstances during the transportation which caused physiological change. Twenty Padjadjaran rams which have average weight between 17 – 26 kg were used in this research. Research was done experimentally using Completely Randomize Design with four levels of electrolyte solution treatment which are P1 (0 mL), P2 (75 mL), P3 (12.5 mL), and P4 (150 mL). Every treatment was added by 25 mg of Roselle extract and replicated five times. Rams were transported for 8 hour using pick-up car with capacity of 20 rams. Observed variables were body weight, and physiological responses which consist of rectal temperature, respiration rate, heart rate, glucose level and blood's urea. The observation showed that body weight was not significantly different (P>0.05) between treatments. The respiratory rate and heart rate also resulted not significant (P>0.05), although it can be tolerated because rectal temperature was in a normal range. Values of glucose and blood's urea decreased although it were still not significantly different (P>0.5). In conclusion, the utilization of coconut water-based electrolyte solution with roselle extracts addition can suppress body weight loss without gives any major effect physiologically to the Padjadjaran ram.

Key words*: electrolyte, coconut water, roselle, transportation, Padjadjaran ram.*

INTRODUCTION

Transportation plays an important part in Farm animal to ensure the distribution of both livestock and its production. Transportation can improve mobility of the livestock but it also can be a potential source of physiological disruption. Livestock is not fed and watered during transportation, meanwhile there are stressors that arise that need to be taken care immediately (Knowles and Warriss, 2000). To overcome this condition, additional energy is needed to perform homeostasis process (Campbell, 2010) that will result in body weight decrease around 8-10% after transportation (Cockram, 2007).

Stressors during transportation will cause physiological stress such as thirst, hunger or psychological stress such as fear, restlessness. It can be happen because of separation from the bigger herd, bump, physical bump and environmental stress such as climate, weather, feed, noise and human presence (Minka and Ayo, 2012). Physiological reactions during transportation are very dynamic, especially in heat release in form of evaporation such as sweat excretion, excessive salivation, heart rate increase, respiration and panting (Bernardini, 2012). It will cause body fluid and electrolyte become unbalance if it occurs during long period of time because half of fluid either from extracellular or intracellular fluid and important electrolyte will be excreted from body (Knowles and Warriss, 2000). Body fluid and electrolyte that are excreted from body will immediately overcome by administration of isotonic fluid instead of water (Koswara, 2009).

Coconut water (*Cocos nucifera*) is one of natural liquid that isotonic with body fluid and equipped with energy resources, vitamins and minerals. Meanwhile roselle is herbs that contain high ascorbic acid and active compounds such as flavonoids and polyphenols that act as secondary anti-oxidant (Ramirez-Rodrigues et al., 2011). These two materials can be utilize as ready to use electrolyte source to supress oxidant or free radical to prevent reactive characteristic of oxidant (Pokorný, 2007).

The objective of this research is to understand effect of utilization of coconut water based electrolyte and roselle extract on Padjadjaran rams body weight and physiological condition.

MATERIALS AND METHODS

Twenty Padjadjaran rams with body weight between 17 – 26 kg and age between 1 – 1.5 years old were transported for 8 hours withoutt a rest. Research was done experimentally with Completely Randomize Design (CRD) that consist of 4 treatments that each repeated 5 times. Mineral contents of coconut water and roselle extract were used to design the treatment formula can be seen in Table 1.

Table 1. Coconut Water (*Cocosnucifera*) and Roselle Extract (*Hibiscus sabdarifa*) Contents in Treatment

Material	Concentration
Coconut Water[a]	
Sodium (mg/L)	105
Potassium (mg/L)	312
Chlorine (mg/L)	183
Roselle Extract	
Vitamin C (mg/100g)[b]	250.75
Flavonoid (mg/L)[c]	29.74
Phenol Total (mg/L)[c]	292.01

Source :[a] Young (2009) ; [b]Lab. Penelitian dan Pelayanan UNPAD (2014) ; [c] Ramirez-Rodrigues (2011)

Rams were given treatment formula before transportation and not allowed to be fed and watered during it. Content of the treatment formula can be seen in Table 2. A pick-up truck with capacity of 20 rams was used during transportation. Rams were weighed, measured its physiological status and taken its blood sample before and after the transportation. Each rams was given a mark to make observation easier.

Measurement methods:

1. Body weight was measured using scale with capacity of 100 kg with deviation of 0.1 kg.
2. Physiological status such as heart rate, respiration rate, and rectal temperature were measured using stethoscope and counter. Heart rate measured by counting heart rate for 1 minute. Respiration rate measured by counting inspiratory and expiratory for 1 minute. Meanwhile rectal temperature measured by inserting clinical thermometer to rectum for 3 minutes.
3. Blood samples were taken as much as 3 mL using syringe. Then it was stored in vacuum tube EDTA and cold temperature to prevent coagulation before it was analyzed. Blood samples were used to determined glucose level and blood urea. Measurement was done by Haematology analyzer in a commercial laboratory named Multitest in Bandung.

Table 2. Content of Coconut Water and Roselle Extract Based Electrolyte Solution for Each Treatment

Electrolyte Solution Formulation	Treatment			
	P0	P1	P2	P3
Coconut Water (mL)	0	50	75	100
Sucrose (g)	0	4,5	6,75	9
Sodium Chloride (g)	0	0,5	0,75	1
Sodium Benzoate (g)	0	0,1	0,15	0,2
Potash (g)	0	3,1	4,65	6,2
Citric Acid (g)	0	0,1	0,15	0,2
Vit.C in Roselle Extract(mg)	0	25	25	25
Distilled Water	75	75	112,5	150

RESULTS AND DISCUSSIONS

Live Body Weight

Administration of coconut water-based electrolyte and roselle extract showed change in body weight with range from 0 – 5.47%. Statistical analysis showed that the change in body weight between treatments was not significantly different. It has to be noted that control treatment (P0) had the highest body weight loss. Meanwhile there were not any body weight loss for treatment P1 and P2, and 2.38% for treatment P3 (Figure 1).

Dehydration often happen in transported livestock and it is usually considered as cause of weight loss (Benardini, 2012).In dehydration state there will be a exchange of water and electrolyte from intracellular and interstitial to intravascular (Cockram, 2007). That fluid exchange depends on tonicity and hidrostatic pressure of remaining extracellular fluid. It will also affect dehydration degree of organs (Schaefer et al.,1997). Administration of

coconut water-based electrolyte and roselle extract before transportation is a form of prevention toward dehydration. Coconut water-based electrolyte and roselle extract formulated to replace lost electrolyte. Coconut water is a fluid that istonic with blood fluid and Its contain compunds such as sodium bicarbonate, sodium sitrat, potash (Koswara, 2009).

This research showed that treatment P0 experienced highest weight loss compare to other treatments. This allegedly occurred because rams in treatment P0 were only given distilled water (aquades) before transportation so that the rams did not have electrolyte supply to replace what was lost. Unlike treatment P0, other treatments were given coconut water before transportation. Coconut water that

Table 3. Effect Of Coconut Water-Based Electrolyte Solution and Rosella Extract on Body Weight And Physiological Responses on Padjadjaran Ram

Parameter	Treatment							
	P0		P1		P2		P3	
	1	2	1	2	1	2	1	2
Body Weight (kg)	20.48 ± 2.65	19.36 ± 2.74	21 ± 0.62	21,08 ± 0,73	22.16 ± 3.26	22.16 ± 3.54	21.88 ± 3.75	21.36 ± 3.84
Heart Rate (Freq/minute)	81 ± 16.16	73 ± 33.38	65 ± 8.82	62 ± 5	62 ± 3.88	63 ± 4.47	79 ± 22.75	63 ± 7.29
Respiration rate (Freq/minute)	42 ± 4.17	45 ± 8.06	37 ± 3.42	45 ± 6.95	39 ± 6.20	42 ± 4.35	45 ± 17.05	40 ± 9.05
Rektal temperature (°C)	39.8 ± 0.1	39.7 ± 0.28	39.7 ± 0.19	39.34 ± 0.15	39.78 ± 0.08	39.68 ± 0.24	39.98 ± 0.24	39.7 ± 0.19
Blood glucose (mg/dL)	49.2 ± 10.43	37.46 ± 22.82	71.60 ± 36.80	28.60 ± 7.44	54.33 ± 11.78	27.40 ± 6.58	73.60 ± 28	52.2 ± 29.72
Blood urea (mg/dL)	40.94 ± 14.81	33.50 ± 2.93	42.08 ± 13.93	34.08 ± 2.61	41. 68 ± 14.19	33.42 ± 1.41	45.42 ± 13.06	35.30 ± 3.33

1:before transportation; 2: after transportation

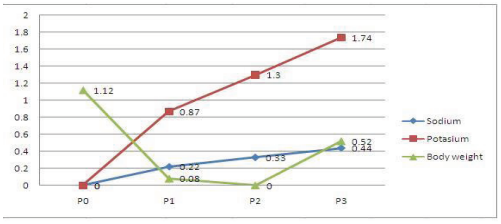

Figure 1. Body Weight Change after transportation

Observation indicate that addition of coconut water-based electrolyte and roselle extract tend to suppress the body weight loss in 8 hours of transportation process. The given treatment alledgedly replace lost electrolyte immediatly along with urine, feces or sweat (Bernandini, 2012; Gortel et al., 1992, cited by Schaefer et al., 1997). This will make pool size water space in interstitial undisturbed, (Gortel et al., 1992, cited by Schaefer et al., 1997). On the other hand, livestock will experience stressor along transportation which can affect physiological and biological process in their body. Heat levelling and metabolism increase caused by increase of cortisol concentration in fight and flight have to be provided by additional energy, (Campbell, 2010).

contains electrolyte (Na and K) and sucrose that can be used as energy source.It also contains vitamin C as anti-oxidant.

The non significant result of weight loss parameter is in line with Hobson (1997) statement, especially in rams which prioritize sufficient of water better than electrolyte condition. Administration of electrolyte intended to improve livestock's performance (Schaefer, 1997), meanwhile roselle extract contains vitamin C that can suppress free radical that often happen during transportation (Minka and Ayo, 2012). Another reason of non significant result is that treatment P0 were given distilled water equivalent to treatment P1 so that these two treatments have different amount of nutrient and electrolyte in water coconut-based electrolyte and roselle extract. Because of that reasons, it is strongly possible that result of treatment P1 and P2 are contributed from coconut water-based electrolyte and roselle extract although the weight loss still not significant.

In this research, body weight loss percentage range after 8 hours of transportation is lower than observation result of Purnomoadi et al 2003 in Endang Purbowati et al. (2005) that range about 1.0 – 1.2 kg or equal to 7.1 – 8.2%.

Heart Rate, Respiration Rate and Rectal Temperature

Data about heart rate, respiration rate, and rectal temperature can be seen at Table 3. Heart rate after transportation was decreased in treatment P0 (9,88%), P1 (3,08%) and P3 (20,25%), meanwhile it was increased in treatment P2 (1.61%). Respiration rate was increased for treatment P0 (6%), P1 (21.9%), P2 (10.4%), meanwhile it was decreasing in treatment P3 (6.9%). For rectal temperature, all of treatment were decreased as in 10.9% for P0, 3.6% for P1, 0.7% for P2, and 16.4% for P3. Statictical analysis showed that addition of coconut water-based electrolyte and roselle extract was not significantly different ($P > 0.05$) for those 3 parameters. This can be interpreted that eventhough a change occured between after and before the transportation, it did not cause significant difference physiologically.

The result of observation from this research were opposite from others previous research where almost every transportation could increase heart rate, respiration rate and body temperature (Lefcourt et al., 1986, cited by Kassab, 2014). This contradiction was alledgedly happen in this research because those parameters were higher before transportation condition than after transportation condition as result of handling. Every stressor from internal or external that experience by livestock is a stimulus that will be responded immediatly by system neuroendocrin through sympatho-adrenal-medullary (SAM) axis. SAM axis is a short term respond system that very effective to solve problem (Griffin, 1989, cited by Parker, 2004). It will be secreted by catecholamine from adrenal medulla and functioned to improve vigilance (fight and flight). An increase in heart rate and dilation of blood vessel are parts of homeostasis process. The objective of heart rate increase is to accelerate blood pumping to cells throughout body. Meanwhile blood vessel dilation has purpose to release heat out of body through sweat, however rams have a more effective system to do it by evaporation or an increase in respiration rate (Knowles and Warriss, 2000).

Respiration is a form of respond from livestock to release or replace heat with surrounding heat, (Yani, 2006). An increase of blood temperature by 1^0C will activate heat receptor on peripheral and hypothalamic (Bouchma and Knochel, 2002, cited by Sugito, 2009). In the end, all of this process inside body will be manifested in body temperature. Body temperature in homeotherm animal have to remain constant, therefore it has to be arrange by balancing heat loss and heat gain inside body (Yousef, 1985). If livestock cannot lose heat form inside the body, it will cause organs temperature to increase. If this situation happen in long term periode, it will be handled by glucocorticoid, a hormone secreted by the adrenal cortex on stimulation from corticotropic realising hormone (CRH) and adenocorticotropic hormone (ACTH). Analogically, the more higher concentration of glucocorticoid especially cortisol in blood the more higher stress experience by livestock (Bernardini, 2014), Because of that reason a decrease in cortisol level in blood can be interpretated as a sign that homeostatis process have been completed.

In this research, decrease in heart rate and respiration rate happened in mid and end of transportation, meanwhile body temperature would back to normal. The heart rate recorded in a range of 62-83 freq/minute which is considered normal at 60–80 frequency/minute (Smithand Mangkoewidjoio, 1988). Respiration rate recorded in a range of 37-45 frq/minute which higher than normal average by Frandson (1996) at 19 frequency/minute. The high respiration rate is a homeostatis mechanism in order to maintain body temperature. This condition can be seen from average body temperature about 39.7-39.9^{o}C which is still within a normal average about 39.2–40^{0}C (Smith and Mangkoewidjojo, 1988). Rectal temperature for rams as homoioterm animals are about 0.6–1.0^{0}C. Capability of endoterm animals to maintain its body temperature is an autonomic process from heat production. Those temperature will protect enzymates process inside the body from interruption (Roberto and Michael, 1992).

Based electrolyte solution is believed to be part of omoreseptor (Soeharsono, 2010), who helped oversee and control systems of the body fluids, electrolytes such as sodium and potassium and other ions play a role in regulating traffic nutrients into the cell. Similarly, the role of roselle extracts as

antioxidants, ie vitamin C, flavonoids inhibit free radicals formed when phosphorylation in the mitochondria (Ramirez-Rodriguez, 2011).

The mean rectal temperature of sheep after treatment showed lower transport before the transport. This is allegedly closely related to system heat setting, the heat for the animals homeoterm sourced from heat metabolism, but also influenced by the external environment, including temperature, humidity, radiant sun and wind movement (Cockram, 2007; Yani, 2006). During the 8 hour trip, sheep in the fasting state of eating and drinking while the first 4 hours ambient temperature of transportation reached the peak is 32^{o}C. The condition affects the physiological functions of the body, but the four-hour journey end relative ambient temperature dropped to 22.7^{o}C (cool). Heat exchange occurs between the body of livestock and the environment is relatively easy so it does not require additional energy for the process of homeostasis.

Body temperature in the range - therefore in his body control system equipped with a highly sensitive and receptor systems (osmoreceptors and baroreceptors) (Raharja, 2010). Both systems actively work related to the changes that occur during the transport takes place. Barriers process heat release in sheep often occurs because almost the entire body covered in fur, so that evaporation is considered more effective way. Sheep known as the panting animal, while releasing heat from its body can be done in several ways, one of which is the mechanism of evaporation. Evaporation is an effective way to eliminate body heat load, every gram of moisture evaporation will eliminate body heat calorie 0.582 (Yousef, 1985). Air humidity can be used to control evaporation heat loss livestock from the skin and respiratory system. High humidity can cause evaporative heat loss of livestock hampered.

Blood Glucose and Blood Urea

Energy needed during transportation depends on stressor level that experienced by livestock so that glucose level in blood as precursor have to be maintained in a relatively satble condition. Glucose always have to be available in body because of its function as primary precursor energy for metabolism. Glicogen is first choice followed by fat and protein in reserved energy.

From observation, glucose level of rams before transportation are about 49.2 – 73.6 mg/dL, but after 8 hour of transportation it decreased. Treatment P1 have the highest decrease with 43 mg/dL or 60.06%. Treatment P2 and P3 decreased by 26.30 mg/dL (49.57%) and 21.4 mg/dL (23.86%), meanwhile treatment P0 decreased by 11.74 mg/dL (23.86%). This condition showed that glucose level tend to decrease along with incrase of coconut water-based electrolyte and roselle extract dosage.

Glucose is a nutrient that can be immediately convert to be energy source. Normal ram has glucose level of 35 – 60 mg/dL (Riis et al, 1983). Glucose level of Padjadjaram rams that were given coconut water-based electrolyte and roselle extract before transportation in Padjadjaran rams is not significantly different ($P > 0.05$). It showed that eventhough there is a difference in glucose level between before and after transportation, but it did not give any physiological meaning.

Decreased glucose level in treatment P1 alledgedly happen bacause the dosage of treatment solution given. Treatment P1 was given 75 mL of treatment solution that means almost all of glucose that available used for homeostatic process. Meanwhile treatment P2 and P3 were given 112.5 mL and 150 mL of treatment solution that means there is more glucose available. That condition made the decrease in treatment P2 and P3 lower than P1.

Sucrose in treatment solution is function as energy source. Sucrose is a non-reducing disaccharide that have α-ß-glycosidic bond. To break the bond of sucrose into glucose and fructose takes specific enzymes (Syahrir, 2011) which may lack in rumen's fluid of treatment's rams. Therefore only part of sucrose is used as energy source by rumen's microbe in intestine. Glucose enters blood sirculation ang use as precursor energy in cellular metabolism. It is strongly possible that during 8 hour of transportation, rams in treatment only used glucose from sucrose that contained in treatment solution and were not used glicogen.

Nitrogen in blood urea is an indicator to identified protein metabolism. Low level of blood urea indicate process of protein saving meanwhile high level of blood urea indicate catabolism process, (Peel et al 1981 in Isdoni 1996). Observation showed that there is a

decrease in blood urealevel from before and after transportation respectively 7.44 mg/dL (18.17%) for P0, 8 mg/dL (19.01%) for P1, 8.26 (19.82%) for P2 and 10.12 (22.28%) for P3. It showed that the more higher dosage of treatment solution given the more ureablood level decrease. This condition means that during transportation there were not any mass protein splitting because blood urea was decrease.

Statistical analysis showed that administration of coconut water-based and roselle extract is not significantly different ($P > 0.05$) between treatment. There was not shortage of energy in every treatment so that there was not any mass protein splitting either. Blood urea from every treatment is relatively the same around 33.42-35.30 mg/dL. Those number are considered a normal state which is between 15.0-36.0 mg/dL (Bendryman et al., 2000).

CONCLUSIONS

Administration of coconut water-based electrolyte and roselle extract before transportation do not give negative physiological responses, but there is a tendency of body weight loss supression without significant effect to heart rate, respiration rate, rectal temperature, glucose level and blood urea.

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REFERENCES

Bendryman, S.S., R.S. Wahyunidan H. Puspitawati. 2000. Pengaruh pemberian rimpang temulawak (Curcuma xanthorrhiza) dantemuhitam (Curcuma aeruginosa) dalam urea molasses blok (UMB) pada gambaran darah dan fungsi hati dan ginjal domba yang diinfeksidengancacing Haemonchus contortus. Media Kedokteran Hewan. 16(1): 1-8.

Bernardini, D., Gerardi, G., Peli, A., Costa, L. N., Amadori, M., Segato, S. 2012.The effects of different environmental conditions on thermoregulation and clinical and hematological variables in long-distance road-transported calves. Journal of animal science, 90(4), 1183-1191.

Campbell, N. A., Reece, J. B., Urry, L. A., Cain, M. L., Wasserman, S. A., Minorsky, P. V., Jackson, R. B. 2010. Biologi: EdisikeDelapan, Jilid 3. Penerjemah: Wulandari, D. T. PenerbitErlangga: Jakarta.

Cockram M.S., 2007. Sheep Transport. DalamLivestock handling And Transport Ed. Grandin, T. CAB International: Oxfordshire, Britania Raya. 12, 184-198.

Endang Purbowati, Agung Purnomoadi, 2005. Respon Fisiologis Domba Lokal Jantan Pada Rentang Bobot Hidup Yang Lebar Akibat Pengangkutan Dari Dataran Tinggi Ke Dataran Rendah. Seminar Nasional Teknologi PeternakandanVeteriner .

Frandson, R.D., Wilke, W.L., Fails, A.D., 2009. Anatomy and Physiology of Farm Animals. Seventh Edition. Wiley-Blackwell.USA. 39.

Hobson, P.N., C.S. Stewart, 1997. The Rumen Microbial Ecosystem. Blacide Academic & Professional. Chapman & Hall, London, 585.

Isdoni, Hera M., Aryani Sismin S., 1996. Gambaran Nitrogen Urea DarahKambing Bunting. Media Veteriner, 111(2).

Jung, Eun-Kyung, Young-Jun Kim, Nami-Joo, 2013. Physicochemical properties and antimicrobial activity of Roselle (*Hibiscus sabdariffa* L.)."Journal of the Science of Food and Agriculture 93.15: 3769-3776.

Kassab, A.Y., A.A. Mohammed, 2014. Ascorbic Acid Administration As Anti-Stress Before Transportation Of Sheep. The Egyptian Journal of Animal Production 51:19-25.

Knowles, T.G., P.D. Warriss, 2000. Stress Physiology of Animals During Transport. DalamLivestock handling and Transport Ed. Grandin, T. CAB International: Oxfordshire, Britania Raya.2, 385-407.

Koswara S., 2009. Minuman Isotonik. Ebook Pangan. Bogor.

*** Laboratorium Penelitian, Pelayanan Jurusan Kimia, 2014. Universitas Padjadjaran, Bandung.

Minka, N.S., Ayo, J.O., 2012. Assessment of thermal load on transported goats administered with ascorbic acid during the hot-dry conditions. International journal of biometeorology, 56(2), 333-341.

Parker, A.J., 2004. Water, Electrolyte and Acid-Base Transported Bos Indicus Steers. Doctoral Thesis. James Cook University. Australia. 8.

Pokorný, J., 2007. Are natural antioxidants better–and safer–than synthetic antioxidants?.European Journal of Lipid Science and Technology, 109(6), 629-642.

Rahardja, D.P., 2010. Ilmu Lingkungan Ternak. Penerbit Masagena, Makassar

Ramirez-Rodrigues, M.M., Plaza, M.L., Azeredo, A., Balaban, M.O., Marshall, M.R., 2011. Physicochemical and phytochemical properties of cold and hot water extraction from Hibiscus sabdariffa. Journal of food science, 76(3), 428-435.

Riis P.M., 1983. Dynamic Biochemistry of Animal Production. NY. pp 363

Refinetti R., Menaker M., 1992.The Circadian Rhythm of Body Temperature. Physiology & Behavior.Vol 51, 613-637

Schaefer, A.L., Jones, S.D., Stanley, R.W., 1997. The Use of Electrolyte Solutions for ReducingzTransport Stress. Journal of Animal Science 1997, 75: 258-265.

Smith, J.B., Mangkoewidjojo, S., 1988. Pemeliharaan, Pembiakandan Penggunaan Hewan Percobaan di Daerah Tropis. Penerbit Universitas Indonesia.

Soeharsono, 2010. Fisiologi Ternak. Widya Padjadjaran. Bandung.

Sugito., Manalu, W., Astuti, D.A., Handharyani E., dan Chairul, 2007. Efek Cekaman Panas dan Pemberian Ekstrak Heksan Tanaman Jaloh (Salix Tetrasperma Roxb) Terhadap Kadar Kortisol, Triiodotironin dan Profil Hematologi Ayam Broiler. Jurnal Ilmiah Puslitbangnak Vol. 12 No.3. Bogor.

Syahrir S., F.K. Tangdilintin, K.G. Wiryawan, A. Parakkasi, Winugroho, 2011.Potensi Senyawa 1-*Deoxynojirimycin* Untuk Melambatkan Hidrolisis Beberapa Jenis Karbohidrat Oleh Enzim Rumen. JITP Vol. 1 No. 2.

Yani A., B.P. Purwanto. 2006. Pengaruh Iklim Mikroterhadap Respons Fisiologis Sapi Peranakan Fries Holland and Modifikasi Lingkunganuntuk MeningkatkanProduktivitasnya (ULASAN). Media Peternakan, 35-46 Vol. 29 No. 1

Yousef M.K**.,** 1985. Thermoneutral Zone. In: M.K.Yousef (Ed.). Stress Physiology of Livestock. Vol.II. CRC Press, Inc. Boca Raton, Florida, 68-69.

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FOLIC ACID IN RUMINANT NUTRITION

Oguzhan KAHRAMAN[1]*, O. Baris CITIL[2], M. Selcuk ALATAS[1], Abdullah OZBILGIN[1]

[1]Department of Animal Nutrition and Nutritional Disorders, Faculty of Veterinary Medicine, Selcuk University, Konya 42100, Turkey
[2]Department of Veterinary, Karapinar Aydoganlar Vocational School of Higher Education, Selcuk University, Karapinar / KONYA

*Corresponding author email: vetoguzhan90@gmail.com

Abstract

Folic acid plays an essential role in ruminant nutrition. Microorganisms in the rumen can synthesise folates, but these amounts are not sufficient to achieve the best efficiency of dairy cows. However, the amount of folates synthesised in the rumen could possibly, to some extent, be affected by the forage: concentrate ratio. The supply of folates by the diet and the synthesis by ruminalmicroflora is sufficient to prevent folic acid deficiency in dairy cows and to maintain normal gestation and lactation. Degradation of orally supplemented folic acid in the rumen seems to be very high (about 97 %), as supplementation of folic acid hardly increases folate concentrations in the digesta at the duodenum. However, it must be considered that dietary supplements of folic acid higher than 0·5 mg/kg body weight increased serum folate concentrations in all available studies and milk folate concentrations in most studies. Additionally, milk production tended to be increased in some studies. Therefore, degradation of folic acid in the rumen may be overestimated as folates can be absorbed at the proximal duodenum. For future research it is necessary to consider the whole flow and the metabolic pathways of folates from the rumen to duodenum, blood, tissue, milk and transfer to calf to declare requirement values for cows.

Key words*: Folic acid, folate, dairy cows.*

INTRODUCTION

Folic acid is one of the vitamins in the B complex and it is necessary for the synthesis of nucleic acids. Their biologically active forms are folates. Folates are essential for the transfer of one-carbon units from donor molecules into biosynthetic pathways leading to methionine, purine and pyrimidines. In general, it is assumed that B-vitamin requirements for ruminants can be met by microbial synthesis in the rumen, even when the animals are fed a diet providing very small amounts of those vitamins.

Folic acid has the single, important biochemical function in mammals to accept and release one-carbon units (Choi and Mason, 2000). This role is essential for the synthesis of purine and pyrimidine and the de novo synthesis of methyl groups for formation of the primary methylating agent, S-adenosylmethionin (Bailey and Gregory, 1999). Given this last role, the metabolic demand for folic acid is likely to be high because exogenous supply of methyl groups is low in ruminants (Snoswell and Xue, 1987). Moreover, it seems that the supply in folic acid could be limiting based on estimated ruminal synthesis and dietary supply (Zinn et al., 1987; NRC, 2001).

Folic acid is very important during lactation and for DNA synthesis of fetal and placental tissues during pregnancy (McNulty et al., 1993), therefore a suboptimal supply should be avoided. In agricultural practice in dairy cows, gestation and lactation are concomitant during several months per year, so the avoidance of progressive folate deficiency must be a priority. The objective of this review is to elucidate the relationship between dietary folic acid levels and milk production in dairy cows and emphasize the importance of folic acid and it's duty on the metabolism.

CHEMICAL STRUCTURE

The vitamin folic acid (chemical name pteroylglutamic acid) consists of three parts: a pteridine nucleus, para-aminobenzoic acid and glutamic acid (Girard, 1998). The name folic acid is deduced from folium, the Latin word for leaf, because native forms of folic acid were originally isolated from spinach leaves (Mitchell

et al., 1944). In chemistry the name folic acid is only used for the synthetic form. It is a stable compound and the basal structure of a wide family of vitamin coenzymes (Lucock, 2000). In nature, more than 100 compounds, with the basal structure of folic acid, feature a common vitamin activity. These pteroylglutamate forms of folic acid are generally called folates (Finglas et al., 2003; Girard, 1998; Bender, 1992).

ABSORPTION AND BIOCHEMICAL FUNCTIONS

There are several excellent reviews on absorption and biochemical functions of folates (Scott, 1999; Bassler, 1997). Derived from studies with non-ruminant animals, two mechanisms of folate absorption from the intestinal tract seem to exist: an active saturable process and a non-saturable passive process. In fact, the relative importance of passive absorption changes according to folate supply, increasing with the amounts of folates available (Selhub et al., 1983; Bassler et al., 2002). However, folates are perhaps degraded, converted and synthesised in the four stomachs of ruminants (Zinn et al., 1987), and even absorbed on a small scale (Re´rat et al., 1958). Unfortunately the forms and the availability of the forms present in rumen contents and duodenal digestion are unknown

The folates are involved in two major metabolic pathways, the DNA cycle and the methylation cycle. When the supply in one-carbon units is inadequate, the utilization of folate coenzymes for biological methylation and nucleotide synthesis appears to compete (Choi and Mason, 2000).

A deficit of folates can lead to a decrease in S-adenosylmethioninelevels and to an abnormal DNA precursor metabolism resulting in faulty DNA synthesis and a decrease in NAD (James et al., 1994), as a decrease in NAD levels is consistent with an increase in DNA repair activity (James et al., 1989).

As folates influence DNA synthesis and the methionine cycle, they are involved in the metabolic pathways of reproduction and milk protein synthesis; therefore they are very important especially in gestating and lactating cows. An additional special situation for cows is that they have a very high demand for methyl groups in early lactation. Concurrently some precursors for methylated compounds (for example, serine and glycine) are also needed for gluconeogenesis, as the amounts of glucose reaching the small intestine through the digestive system are generally low. So, coincident demand for precursors of methylated compounds leads to competition between different metabolic pathways, for example, gluconeogenesis, lecithin synthesis, DNA synthesis and remethylation of methionine (Girard and Matte, 2006; Bruesemeister and Suedekum, 2006).

SOURCES AND STABILITY OF FOLATES

Folic acid is widely distributed in nature; green leafy materials, cereals and extracted oilseed meals are good sources of the vitamin. Folic acid is reasonably stable in foods stored under dry conditions but it is readily degraded by moisture, particularly at high temperatures. It is also destroyed by the ultraviolet light (Mcdonald et al., 2002).

INDICATIONS OF A FOLIC ACID REQUIREMENT FOR DAIRY COW

Obviously, in high-producing dairy cows that are in gestation and lactation, frequently both at the same time, for the greatest part of their life, demand for methylneogenesisas well as DNA biosynthesis and cell division are highly solicited pathways, which are likely to rely heavily on folate metabolism. Although recovery of nucleic acids from microbial digestion could decrease the pressure on the DNA cycle competition between gluconeogenesis and methylneogenesis for substrates are likely to be high in lactating high-producing dairy cow, especially in early lactation. During those periods when there is a shortage in precursors for de novo synthesis of methylated compounds, an adequate supply in folates should improve efficiency of transfer of one-carbon units. Thus, an adequate supply of both methyl group precursors and the appropriate co-factors (folates, B12) is likely to be crucial for an optimal metabolic efficiency and milk production (Girard and Matte, 2005).

A few observations on cattle suggest that, in spite of an adequate ruminal function, folate supply could vary in time. In growing steers, folate supply, evaluated from the amounts

reaching the duodenum, was marginal as compared to requirement evaluated from recommendations for a 35 kg growing pig and adapted for steers on a body weight basis (Zinn et al., 1987). Some other observations seem to substantiate folate supply variations in cows. Non-gestating cows have serum concentrations of folates superior to those of gestating cows (Arbeiter and Winding, 1973; Tremblay et al., 1991). Total serum folates of dairy cows decrease by 40% from 2 months after calving to next calving (Girard et al., 1989). Changes in serum concentrations are likely to give an indication that the relationship between folate supply and its tissue utilization differs among the different physiological stages studied substantiating this choice of vitamin for further studies in dairy cows.

MICROBIAL SYNTHESIS, DEGRADATION AND ABSORPTION OF FOLATES IN THE GASTROINTESTINAL TRACT OF RUMINANTS

It is well known that the microbial activity and the ruminal population are influenced by the level of concentrates in the diet and the type of feed (Hungate, 1966). As some bacterial species are able to synthesise folates, and some others need them (Wolin and Miller, 1988), different amounts of folates can be synthesised and used in the rumen depending on the feed composition. For steers, Hayes et al. (1966) and Girard et al. (1994) described a relationship between the proportion of concentrates in the diet and the amount of folates in the rumen. High-concentrate diets resulted in an increase of folates.

EFFECTS OF SUPPLEMENTED FOLIC ACID ON RUMINANTS

M. Duplessis et al. (2014) reported that milk fat concentration was decreased during the first 60 DIM (days in milk) for both primiparous and multiparous cows receiving the vitamin supplement. Also they found that supplementation of folic acid and vitamin B-12 given 21 day before the expected calving date until 60 DIM did not increase milk yield of dairy cows in early lactation and during the 305 day lactation period in commercial dairy herds. Supplementation of folic acid does not influence feed intake (Graulet et al., 2007). For gestating primiparous and multiparous cows, Girard et al. (1995) found a non-significant increase in milk production of 14% in the last part of lactation due to an i.m. injection of 160 mg folic acid once per week.

Girard et al. (1989) observed that total serum folates of dairy cows decreased by 40% from 2 months postpartum (around mating) to parturition. According to that study of dairy cows, which are generally considered to be independent of an exogenous supply of folic acid (Agricultural Research Council. 1980; National Research Council, 1989) the synthesis of folates by ruminal microorganisms was not sufficient to prevent a decline in serum folates during gestation and lactation.

CONCLUSIONS

It is concluded from this review that folic acid plays an important role in the synthesis of milk protein from dietary protein. However; the levels at which folic acid should be supplemented in dairy cattle diets are not determined clearly in many studies. Future research on ruminant diets should be headed towards on determining the folic acid digestion mechanism at rumen and intestinal level and their role in milk protein synthesis. Research efforts should also be focused on finding the balance between supply and demand of folic acid in ruminant diets.

REFERENCES

Agricultural Research Council. 1980. The Nutrient Requirements of Ruminant Livestock. Commonw. Agric. Bur., Slough, England.

Arbeiter, V.K., Winding, W., 1973. Folatbestimmungenim Serum von RindernmitbesonderemBezug auf die Fruchtbarkeit. Wien. Tiera¨rztl.Monatsschr.60, 323-326.

Bailey L.B., Gregory İİİ, J.F., 1999. Folate metabolism and requirements. J. Nutr. 129, 779-782.

Bassler K.H., 1997. Enzymatic effects of folic acid and vitamin B12. İnt J VitamNutr Res 67, 385–388.

Bassler K.H., Golly I., Loew D., Pietrzik K., 2002. Vitamin-Lexikonfur Arzte, Apotheker und Ernahrungswissenschaftler (Vitamin Encyclopedia for Physicians, Pharmacists and Nutrition Scientists), 3rd ed. Munich and Jena: Urban & Fischer.

Bender D.A., 1992. Folic acid and other pterins and vitamin B12. İn Nutritional Biochemistry of the Vitamins, 269-317 [DA Bender, editor]. Cambridge: Cambridge University Press.

Bruesemeister F., Suedekum K.H., 2006. Rumen-protected choline for dairy cows: the in situ evaluation of a commercial source and literature evaluation of effects on performance and interactions between methionine and choline metabolism. Anim Res 55, 93-104.

Choi S.W., Mason, J.B., 2000. Folate and carcinogenesis: an integrated scheme. J. Nutr. 130, 129-132.

Duplessis M. et al., 2014. Milk production and composition, and body measurements of dairy cows receiving intramuscular injections of folic acid and vitamin B-12 in commercial dairy herds Livestock Science 167, 186-194

Finglas P.M., Wright A.J.A., Wolfe C.A., Hart D.J., Wright D.M., Dainty J.R., 2003. İs there more to folates than neural-tube defects? Proc.Nutr.Soc. 62, 591-598.

Girard C.L., 1998. B-complex vitamins for dairy cows: a new approach. Can. J. Anim. Sci., 78, 71–90.

Girard C.L., Chiquette J., Matte J.J., 1994. Concentrations of folates in ruminal content of steers – responses to a dietary-supplement of folic-acid in relation with the nature of the diet. J. Anim.Sci., 72, 1023–1028.

Girard C.L., Matte J.J., 2005. Folic acid and vitamin B12 requirements of dairy cows: A concept to be revised

Girard C.L., Matte J.J., 2006. İmpact of B-vitamin supply on major metabolic pathways of lactating dairy cows. Can. J. Anim.Sci. 86, 213–220.

Girard C.L., Matte J.J., Tremblay G.F., 1989. Serum folates in gestating and lactating dairy cows. J. Dairy Sci. 72, 3240– 3246.

Girard C.L., Matte J.J., Tremblay G.F., 1995. Gestation and lactation of dairy cows – a role for folic acid. J. Dairy Sci. 78, 404–411.

Graulet B., Matte J., Desrochers A., Doepel L., Palin M., Girard C., 2007. Effects of dietary supplements of folic acid and vitamin B12 on metabolism of dairy cows in early lactation. J. Dairy Sci., 90, 3442–3455.

Hayes B.W., Mitchell G.E., Little C.O., Bradley N.W., 1966. Concentrations of B-vitamins in ruminalfluid of steers fed different levels and physical forms of hay and grain. J. Anim.Sci., 25, 539–542.

Hungate R.E., 1966. Variations in the rumen.İn The Rumen and its Microbes, pp. 376–418 [RE Hungate, editor]. New York: Academic Press İnc.

James S.J., Miller B.J., Mcgarrity L.J., Morris S.M. 1994. The effect of folic-acid and/or methionine deficiency on deoxyribonucleotide pools and cell-cycle distribution in mitogen-stimulated rat lymphocytes. Cell Prolif 27, 395–406.

James S.J., Yin L.,Swendseid M.E., 1989. DNA strand break accumulation, thymidylate synthesis and NAD levels in lymphocytes from methyl donor-deficient rats. J. Nutr. 119, 661–664.

Lucock M., 2000. Folic acid: nutritional biochemistry, molecular biology, and role in disease processes. Mol. Genet. Metab. 71, 121–138.

McNulty H., McPartlin J.M., Weir D.G., Scott J.M., 1993. Folate catabolism is increased during pregnancy in rats. J. Nutr. 123, 1089–1093.

Mitchell H.K., Snell E.E., Williams R.J., 1944. Folicacid. İ. Concentration from spinach. J. Am. Chem.Soc. 66, 267–268.

National Research Council, 2001. Nutrient Requirements of Dairy Cattle.7th revised edition. National Academy Press, Washington, DC.

National Research Council. 1989. Nutrient Requirements of Dairy Cattle. 6th rev. ed. Natl. Acad. Sci., Washington, DC.

Rerat A., Molle J., le Bars H., 1958. Mise en e´vidence chez le Mouton de la perme´abilite´ du rumen aux vitamines B et conditions de leur absorption a` ceniveau (Demonstration in the sheep of the permeability of the rumen to the B vitamins and the conditions for their absorption at that level). C R Hebd. Seances Acad. Sci., 246, 2051–2054.

Scott J.M., 1999. Folate and vitamin B12.Proc.Nutr.Soc. 58, 441–448.

Selhub J., Dhar G.J., Rosenberg I.H., 1983. Gastrointestinal absorption of folates and antifolates. Pharmacol.Ther., 20, 397–418.

Snoswell A.M., Xue G.P., 1987. Methyl group metabolism in sheep. Comp. Biochem. Physiol. 88B, 383– 39

Tremblay G.F., Girard, C.L., Bernier-Cardou, M., Matte, J.J., 1991. Nycterohemeral variations of concentration of serum folates in dairy cows. Can. J. Anim. Sci. 71, 919– 923.

Wolin M.J., Miller T.L., 1988. Microbe–microbe interactions.İn The Rumen Microbial Ecosystem, pp. 343–359 [PN Hobson, editor]. London and New York: Elsevier Applied Science.

Zinn R.A., Owens F.N., Stuart R.L., Dunbar J.R., Norman B.B., 1987. B-vitamin supplementation of diets for feedlot calves. J. Anim. Sci. 65, 267–277.

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STRATEGIES TO REDUCE METHANE PRODUCTION IN RUMINANTS

Oguzhan KAHRAMAN[1], Abdullah OZBILGIN[1], M. Selcuk ALATAS[1], O. Baris CİTİL[2]

[1]Department of Animal Nutrition and Nutritional Disorders, Faculty of Veterinary Medicine, Selcuk University, Konya 42100, Turkey
[2]Department of Veterinary, Karapinar Aydoganlar Vocational School of Higher Education, Selcuk University, Karapinar, Konya

Corresponding author email: vetoguzhan90@gmail.com

Abstract

Ruminant animals play an important role in the food chain for evaluate cellulose and non-protein nitrogenous (NPN) compounds absorbed partially or not by other farm animals and humans. However, ruminant animals also bring some disadvantages. Methane, produced as a natural consequence of the ruminal digestion and it is a potential green house gas, is a problem, both ecologically and economically. Methane emissions from ruminant livestock are a contributor to total global anthropogenic emissions of greenhouse gases which have a global warming potential. Also methane produced by ruminants represents a loss of energy for ruminants.
Methane is formed in the fore-stomach (reticulorumen, more commonly known as the rumen) of ruminants by a group of microbes called methanogens, which form a subgroup of the domain Archaea. Their effect on producing methane is mentioned.
In this review, current approaches towards mitigation of methane in pastoral farming are summarised. The strategies to diminish methane output from livestock are required for ecological and economical dairy production. Research strategies based on vaccination, enzyme inhibitors, phage, homoacetogens, feed supplements, and animal selection are reviewed. Numerous studies have been completed on use of plant secondary metabolites (PSM) in substitute for chemical feed additives because some of them modify rumen fermentation and reduce CH4 production. Also this review describes the basic conceptual aspects of ruminal methanogenesis, which is a way of keeping a low H2 pressure in the rumen by reducing CO2, and steps where it may be possible to intervene to reduce CH4 production

Key words*: Methane, Plant secondary metabolits, Ruminant, Greenhouse gas.*

INTRODUCTION

Agriculture was responsible for 10–12% of total global non-CO_2 greenhouse gas (GHG) emissions in 2005, but emissions of CH4 and N_2O increased globally by nearly 17% from 1990 to 2005, with both gases contributing equally to the increase (Smith et al., 2007). Enteric CH_4 fermentation accounted for about 32% of total non-CO_2 emissions from agriculture in 2005 (Smith et al., 2007). If CH4 emissions grow in direct proportion to projected increases in livestock numbers, then global CH_4 emissions from livestock production are expected to increase 60% by 2030 (FAO, 2003). Efforts are being made by governments around the world to develop mitigations to reduce CH_4 emissions from ruminant livestock. However, livestock producers are unlikely to adopt these strategies if they reduce animal production and, hence, profitability.

Lowering global methane emissions is an important part of any effort to reduce anthropogenic GHG emissions. However, reducing the number of ruminants being farmed is not an option as the worldwide demand for meat and milk is predicted to double by 2050 (FAO, 2008).

FATS – EFFECTS ON CH_4 EMISSIONS

One of the energy sources is fat and it can reduce production of methane. İn case of using fat as energy source, the microbial flora in the rumen and energy use efficiency can change and methane production can reduce (McGinnetal, 2004). Thus, in a study with dairy cows did by Giger-Reverdinetal (2003) reported that adding fatty acids with a carbon quantity of medium length (8-16 C) reduces the methane production and this reduction is proportional with fat's degree of unsaturation. Martin et al. (2008) claimed that adding raw linseed, extracted

linseed and line seed oil to dairy cow rations reduce the methane production substantially and they concluded that reduction of feed fermentation with fat addition. This inhibits cellulolytic bacteria and protozoons.

There are five possible mechanisms by which lipid supplementation reduces CH_4: by reducing fibre digestion (mainly long-chain fatty acids); by lowering DMI (if total dietary fat exceeds 6-7%) the suppression of methanogens (mainly medium-chain fatty acids); the suppression of rumen protozoa; and to a limited extent, through biohydrogenation (Johnson and Johnson, 1995; McGinn et al., 2004; Beauchemin et al., 2008).

There is opportunity to add fat supplements to TMR to reduce enteric CH_4 emissions. Use of by product feeds from agricultural/food processing industries, which contain fat, is a useful approach to reducing enteric CH_4 emissions and global GHG emissions, particularly since GHG emissions arising from producing the by-product are accounted for by the primary product, at least in some jurisdictions. Examples of by-products that contain fat and are suitable for adding to ruminant diets are whole cottonseed, brewers grains, cold pressed canola, and hominy (maize) meal.

Using DDGS in cattle diets to supply digestible energy often lowers diet starch content, but generally increases dietary fat content and enteric CH_4 is reduced in a manner commensurate with increased dietary fat concentration. The effect was demonstrated recently by McGinn et al. (2009) in growing beef cattle fed a diet in which barley grain (350 g/kg DM) was replaced by dried maize DDGS. Incorporating DDGS in the diet increased the dietary fat content from 20 to 51 g/kg DM and enteric CH_4 decreased from 23.8 to 19.9 g CH_4/kg DM intake. This reduction in CH4 is equivalent to a 1.26 g/kg DM intake decline/10 g/kg increase in dietary fat, which is consistent with the overall rate of decline we report for other fat sources.

Like fish oil, micro-algae are rich in omega-3 fatty acids, which have been shown to reduce CH_4 production in vitro (Fievez et al., 2007). Micro-algae can be mass produced (Rosenberg et al., 2008). For example, MBD Energy Limited (Melbourne, VIC, Australia) use waste CO_2 gases from coal-fired power plants combined with sunlight and waste water to produce algae meal which can be used as livestock feed. The oil contained in this meal could be useful in reducing CH_4 emissions from ruminants, due primarily to its negative impacts on methanogen growth in the rumen, but testing is required in animals to as certain that enteric CH4 production is reduced without lowering feed intake or digestibility.

FORAGE QUALITY

Improving forage quality, either through feeding forage with lower fibre and higher soluble carbohydrates, changing from C4 to C3 grasses, or even grazing on less-mature pastures, can reduce CH_4 production (Ulyatt et al., 2002; Beauchemin et al., 2008). Methane production per unit cellulose digested has been shown to be three times that of hemicellulose (Moe and Tyrrell, 1979), while cellulose and hemicellulose ferment at slower rates than do non-structural carbohydrates, thus yielding more CH_4 per unit substrate digested (McAllister et al., 1996).

HIGHER STARCH DIETS

It is well known that feeding grain based diets lowers enteric CH4 emissions (g/kg DM intake) compared with feeding forage based diets (Johnson and Johnson, 1995). Starch fermentation promotes propionate production in the rumen creating an alternative hydrogen sink to methanogenesis (Murphy et al., 1982), lowers ruminal pH and inhibits growth of rumen methanogens (Van Kessel and Russell, 1996), and decreases rumen protozoal numbers limiting transfer of hydrogen from protozoa to methanogens (Williams and Coleman, 1988). Whether feeding more grain reduces net farm GHG emissions is less certain, and ultimately depends on the farming system (Beauchemin et al., 2010). Nevertheless, the scope for increasing the amount of grain fed to ruminants is limited and feeding grain ignores the importance of ruminants in converting fibrous feeds, unsuitable for direct human consumption, to the high quality protein sources milk and meat (Garnett, 2009).

RATIO OF FORAGE/CONCENTRATED FEED

It was reported by several researchers that reducing the ratio of roughage/concentrated feed and pelleting of the forage cause an increase in the production of propionic acid and reduction in the formation of methane (Johnson and Johnson, 1995; Reynolds et al., 2001). However, Reynolds et al. (2001) reported that loss of energy reduced substantially in the beef heifers with methane. In another study it was expressed that adding concentrated feed in the rations of beef cattles reduced methane emission (Olivera et al., 2007).

HOMOACETOGENS

Autotrophic H_2-utilising acetogenic bacteria, also known as homoacetogens, are able to employ H_2 as an energy source for growth, using it to reduce CO_2 to acetate. Redirection of the rumen fermentation by the activity of homoacetogens has been postulated as a way of increasing feed-use efficiency (Joblin K., 1999). Instead of feed energy being lost as methane, the energy represented by the H_2 would be diverted to acetate formation and hence enhance animal productivity. In addition, a reduction in methane production would occur.

VACCINATION AGAINST RUMEN METHANOGENS

Vaccination against rumen methanogens has the potential to reduce methane emissions by decreasing the number or activity of methanogens in the rumen. Such a vaccination approach against rumen-dwelling organisms has met with success in vaccinating animals against the rumen dwelling bacterium *Streptococcus bovis* (Gill et al., 2000; Shu et al., 2001).

In an Australian study, immunisation of sheep with a whole-cell preparation from three methanogens reduced methane production (per kg/DMI) by 7.7% (Wright et al., 2004). However, when the study was repeated with a mixture of five methanogens, vaccination failed to demonstrate any methane abatement, although it changed the microbial fauna in the rumen (Williams et al., 2009). These results highlight the difficulty of producing effective vaccines to reduce methane emissions in ruminants based on crude whole-cell preparations, which are more likely to target selected methanogen species.

BACTERIOPHAGES

Bacteriophages are present in all biological ecosystems. Their relative simplicity and modular structure (Brussow et al., 2004) makes them important agents for genetic exchange between various microbial hosts (Stanton, 2007; Chen and Novick, 2009). Furthermore, their ability to penetrate and subsequently lyses their host cells makes phages and their genes potential sources of mitigation strategies.

In contrast to the nearly 300 bacteriophage genomes reported (Ackermann and Kropinski, 2007), only six archaeal phages have been sequenced and described so far, and only two are from methanogens: Methanobacterium phage psi M1 and M2 (a variant of M1) (Pfister et al., 1998), and Methanothermobacter phage psi M100 (Luo et al., 2001).

More methanogen phages need to be identified, sequenced and characterised to identify and employ such phage-based strategies effectively. However, the high specificity of phages may be a limiting factor in their effectiveness in reducing the total methane emissions, since there appears to be a high diversity of methanogens in the rumen (Janssen and Kirs, 2008).

PLANT SECONDARY COMPOUNDS

Condensed tannins (CT) have been shown to reduce CH_4 production by 13–16% (DMI basis) (Waghorn et al., 2002; Woodward et al., 2004) mainly through a direct toxic effect on methanogens. Plant saponins also potentially reduce CH_4, and some saponin sources are clearly more effective than others, with CH4 suppression attributed to their anti-protozoal properties (Beauchemin et al., 2008)

DIETARY SUPPLEMENTS

Dietary supplements can potentially profitably reduce CH_4 emissions from intensive ruminant production systems, with many strategies

already available for on-farm implementation. Yeast cultures of *Saccharomyces cerevisiae* potentially stimulate acetogenic microbes in the rumen, consuming H_2 to form acetate (Chaucheyras et al., 1995), and thus potentially reducing CH_4 production.

Enzymes, in the form of cellulases and hemicellulases added to the diets of ruminants, improved ruminal fibre digestion and productivity (Beauchemin et al., 2003) and reduced CH_4 by 28% in vivo and 9% *in vivo*, respectively, perhaps by reducing the acetate-to-propionate ratio (Beauchemin et al., 2008).

Dicarboxylic acids, like fumarate, malate, and acrylate, are precursors to propionate production in the rumen and can act as an alternative H_2 sink, restricting methanogenesis. McAllister and Newbold (2008) reviewed studies that showed 0%– 75% reductions in CH4 achieved by feeding fumaric acid.

Halogenated analogues, such as bromochloromethane (BCM) and chloroform, are potent inhibitors of CH_4 formation in ruminants, with BCM reducing CH_4 emissions by 57%, 84%, and 91% (DMI basis) in feed-lot steers, at increasing dose rates (Tomkins and Hunter, 2004).

ANIMAL BREEDING

Animal breeding has long been shown to increase productivity and to reduce susceptibility to disease, and has the potential to contribute towards reducing methane emissions from livestock. Breeding for increased productivity reduces methane emission intensity by increasing the proportion of feed energy used for production purposes while diluting the maintenance requirements (Chagunda et al., 2009). However, productivity increases also require the use of increasing amounts of concentrate feeds.

CONCLUSIONS

Reduction of ruminal methane production in ruminants is a difficult issue. The variations in technological and economic infrastructures in the regions where, livestock carried out and in the feeding habits , requires the implementation of different strategies in this area. But it can be useful if some of the precautions taken in part in solving this problem. We can achieve progress towards reducing methane production from biotechnology, reducing the number of animals by increasing the efficiency of animal, producing high quality of forages and pastures, the use of high alternative forage and concentrate feeds which has high content of substances such as tannin and saponin and also using of probiotics which, can compete with methanogens by suppressing them with secondary plant components such as essential oils.

REFERENCES

Ackermann H.W., Kropinski A.M., 2007.Curated list of prokaryote viruses with fully sequenced genomes. Research in Microbiology 158, 555–566.

Beauchemin K.A., Colombatto D., Morgavi D.P., Yang W.Z., 2003. Use of exogenous fibrolytic enzymes to improve feed utilization by ruminants.J. Anim. Sci. 81 (E. Suppl. 2), E37–E47.

Beauchemin K.A., Kreuzer M., O'Mara F., McAllister T.A., 2008. Nutritional management for enteric methane abatement: a review. Aust. J. Ep. Agric. 48, 21–27. doi:10.1071/EA07199..

Beauchemin K.A., Janzen H.H., Little S.M., McAllister T.A., McGinn S.M., 2010. Lifecycle assessment of greenhouse gas emissions from beef production in western Canada: a case study. Agric. Syst. 103, 371–379.

Brussow H., Canchaya C., Hardt W.D., 2004. Phages and the evolution of bacterial pathogens: from genomic rearrangements to lysogenic conversion. Microbiology Molecular Biological Review 68, 560–602.

Chaucheyras F., Fonty G., Bertin G., Gouet P., 1995. In vitro utilization by a ruminalacetogenic bacterium cultivated alone or in association with an archaea methanogen is stimulated by a probiotic strain of Saccharomyces cerevisiae. Appl. Environ. Microbiol. 61, 3466–3467.

Chen J., Novick R.P., 2009. Phage-mediated intergeneric transfer of toxin genes. Science 323, 139–141.

Chagunda M.G.G., Römer D.A.M., Roberts D.J., 2009. Effect of genotype and feding regime on enteric methane, non-milk nitrogen and performance of dairy cows during the winter feeding period. Livestock Science 122, 323–332.

Food and Agriculture Organization of the United Nations (FAO), 2008.The State of Food Insecurity in the World.<http://www.fao.org/docrep/011/i0291e/i0291e00.htm>.

FAO, 2003. World Agriculture: Towards 2015/2030. An FAO Perspective.FAO, Rome, Italy, 97 pp.

Fievez V., Boeckaert C., Vlaeminck B., Mestdagh J., Demeyer D., 2007. In vitro examination of DHA-edible micro-algae 2.Effect on rumen methane production and apparent degradability of hay. Anim. Feed Sci. Technol. 136, 80–95.

Garnett T., 2009. Livestock-related greenhouse gas emissions: impacts and options for policy makers. Environ. Sci. Policy 12, 491–503.

Giger-Reverdin S., Morand-Fehr P., Tran G., 2003. Literature survey of the influence of dietary fat composition on methane production in diary cattle. Livestock Prod. Sci., 82: 73–79.

Gill H.S., Shu Q., Leng R.A., 2000. Immunization with Streptococcus bovis protects against lactic acidosis in sheep. Vaccine 18, 2541–2548.

Janssen P.H., Kirs M., 2008. Structure of the archaeal community of the rumen.Applied and Environmental Microbiology 74, 3619–3625.

Joblin K., 1999. Ruminalacetogens and their potential to lower ruminant methane emissions. Australian Journal of Agricultural Research 50, 1307–1313.

Johnson K.A., Johnson D.E., 1995. Methane emissions from cattle.J. Anim. Sci. 73, 2483–2492.

Luo Y., Pfister P., Leisinger T., Wasserfallen A., 2001. The genome of archaealprophage Psi M100 encodes the lytic enzyme responsible for autolysis of Methanothermobacterwolfeii. Journal of Bacteriology 183, 5788–5792.

Martin C., Rouel J., Jouany J. P., Doreau M., Chilliard Y., 2008. Methane output and diet digestibility in response to feeding dairy cows crude linseed, extruded linseed, or linseed oil. J. Anim Sci. 86: 2642-2650.

McAllister T.A., Newbold C.J., 2008. Redirecting rumen fermentation to reduce methanogenesis. Aust. J. Exp. Agric. 48, 7–13.

McAllister T.A., Okine E.K., Mathison G.W., Cheng K.J., 1996. Dietary environmental and microbiological aspects of methane production in ruminants. Can. J. Anim. Sci. 76, 231–243.

McGinn S.M., Beauchemin K.A., Coates T., Colombatto D., 2004. Methane emissions from beef cattle: Effects of monensin, sunflower oil, enzymes, yeast, and fumaric acid. J. Anim. Sci., 82: 3346-3356.

McGinn S.M., Chung Y.-H., Beauchemin K.A., Iwaasa A.D., Grainger C., 2009. Use of corn distillers' dried grains to reduce enteric methane loss from beef cattle. Can. J. Anim. Sci. 89, 409–413.

Moe P.W., Tyrrell H.F., 1979. Methane production in dairy cows. J. Dairy Sci. 62, 1583–1586.

Murphy M.R., Baldwin R.L., Koong L.J., 1982. Estimation of stoichiometric parameters for rumen fermentation of roughage and concentrate diets. J. Anim. Sci. 55, 411–421.

Oliveira S.G., Berchielli T.T., Pedreira M.S., Primavesi O., Frighetto R., Lima M.A., 2007. Effect of tannin levels in sorghum silage and concentrate supplementation on apparent digestibility and methane emission in beef cattle, Anim. Feed Sci. Technol. 135: 236-248.

Pfister P., Wasserfallen A., Stettler R., Leisinger T., 1998. Molecular analysis of Methanobacterium phage psiM2. Molecular Microbiology 30, 233–244.

Reynolds C.K., Tyrrell H.F., Reynolds P.J., 2001. Effects of diet forage to concentrate ration and intake on energy metabolism in growing beef heifers: whole body energy and nitrogen balance and visceral heat production, J. Nutr. 121: 994-1003.

Rosenberg J.N., Oyler G.A., Wilkinson L., Betenbaugh M.J., 2008. A green light for engineered algae: redirecting metabolism to fuel a biotechnology revolution. Curr.Opin.Biotech. 19, 430–436.

Shu Q., Bir S.H., Gill H.S., Duan E., Xu Y., Hiliard M.A., Rowe J.B., 2001. Antibody response in sheep following immunization with Streptococcus bovis in different adjuvants. Veterinary Research Communication 25, 43–54.

Smith P., Martino D., Cai Z., Gwary D., Janzen H., Kumar P., McCarl B., Ogle S., O'Mara F., Rice C., Scholes B., Sirotenko O., 2007. Agriculture. In: Metz, B., Davidson, O.R., Bosch, P.R., Dave, R., Meyer, L.A. (Eds.), Climate Change 2007: Mitigation. Contribution of Working Group III to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge, UK and New York, NY, USA

Stanton T.B., 2007. Prophage-like gene transfer agents – novel mechanisms of gene exchange for Methanococcus, Desulfovibrio, Brachyspira, and Rhodobacter species. Anaerobe 13, 43–49.

Tomkins N.W., Hunter R.A., 2004. Methane mitigation in beef cattle using a patented anti-methanogen. In: Eckard, R.J., Slattery, W. (Eds.), Proceedings of the 2nd Joint Australia and New Zealand Forum on Non-CO2 Greenhouse Gas Emissions from Agriculture. CRC for Greenhouse Accounting, Lancemore Hill, Canberra, 2–9. 0-9579597, October 2003.

Ulyatt M.J., Lassey K.R., Shelton I.D., Walker C.F., 2002. Methane emission from dairy cows and wether sheep fed subtropical grass-dominant pastures in mid-summer in New Zealand. N.Z. J. Agric. Res. 45, 227–234.

Van Kessel J.A.S., Russell J.B., 1996. The effect of pH on ruminalmethanogenesis.FEMS Microbiol. Ecol. 20, 205–210.

Waghorn G.C., Tavendale M.H., Woodfield D.R., 2002. Methanogenesis from forages fed to sheep. Proc. N.Z. Grassl. Assoc. 64, 167–171.

Williams A.G., Coleman G.S., 1988. The rumen protozoa. In: Hobson, P.N., Stewart, C.S. (Eds.), The Rumen Microbial Ecosystem. Springer, New York, NY, USA, 77–129.

Williams Y.J., Popovski S., Rea S.M., Skillman L.C., Toovey A.F., Northwood K.S., Wright A.D., 2009. A vaccine against rumen methanogens can alter the composition of archaeal populations. Applied and Environmental Microbiology 75, 1860–1866.

Woodward S.L., Waghorn G.C., Laboyrie P., 2004. Condensed tannins in birdsfoot trefoil (Lotus corniculatus) reduced methane emissions from dairy cows. Proc. N.Z. Soc. Anim. Prod. 64, 160–164.

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FLORISTIC BIODIVERSITY OF FEEDING GROUND FOR DEERS (DAMA DAMA) BRED ON FARMS

Janusz Kilar, Maria Ruda

Stanislaw Pigon State Higher Vocational School in Krosno, Rynek 1, 38-400 Krosno, Poland
Tel. (+48) 1343 75580, email: jankil@pwsz.krosno.pl, email: m.ruda@pwsz.krosno.pl

Corresponding author email: jankil@pwsz.krosno.pl

Abstract

The aim of the researches was to evaluate the biodiversity of plant life of feeding ground for deers bred for meat. The researches were conducted in 2011-2013. They were carried on the farm for deers, located near Krosno town. The fhytosociological tests were based on the Braun-Blanquet method using six-note scale. In general there were 30 photos taken which were collectively elaborated. It was established that in the composition of feeding ground there were 62 plant species among which there were 11 species of trees and shrubs and 51 plants that contain phytoncides and they are very valuable for health and animal productivity.

Key words*: deers, farmed deer breeding, plant life of feeding ground.*

INTRODUCTION

The consumer behaviour to meat that has been observed in the recent years, indicates that the offer for animal quality and the treatment of meat as functional food has been enhanced. The high level of functional food is characteristic for the wild meat (Florek and Drozd, 2013; Kilar and Ruda, 2014; Kilar, 2013). In many countries the wild meat is considered as the up-scale product (Dzierżyńska-Cybulko and Fruzinski, 1997). The organization of farming production was caused by the demand for the wild meat over its supply. All these things were noticed in some countries in 1980s (Berg and Asher, 2003; Janiszewski and Daszkiewicz, 2010). The pioneers of farming production of deer meat came from New Zealand. Taking Europe into account, most deers bred on farms are in Germany, Ireland and Austria. The first deer farms in Poland came into existence in 1990s (Borys, 2004). Legally, deer farming was authorised in 2001 when red deers (*Cervus elaphus*), Fallow deers *(Dama Dama)* and Sika deers *(Cervus nippon)* were considered as the farm animals (Dz.U. 2001 nr 129 poz. 1438). In Poland in 2013 there were 522 farms where there were about 31000 animals kept (www.wetgiw.gov.pl). Deer farming is particularly aimed at international meat production.

Polish wild meat consumption is only about 0.08 kilograms per year (Borys, 2012). According to the researches (Kilar and Ruda, 2014; Radkowska, 2013; Radkowski and Barabasz-Krasny, 2008; Wolański, 2011), variety of floristic composition of feeding grounds is very important for animals as it gives them many primary nutrient. Also it has a very beneficial effect on their health and on the pro-health properties. A very important group of plants are herbs (Chabuz, 2012; Grzelak, 2013; Radkowska, 2013; Stokłosa, 2007).

All of alkaloids, anthocyanins, phenolic acids, phytosterols, flavonoids, glycosides, essential oils, mineral salts and many different active appetizing substances make the deer female more milky. Also all these substances make the nutrient availability grow and help the body detaxification (Budny, 2012; Grzelak, 2013; Radkowska, 2013). All these important substances are freely used by wild animals.

The feeding ground for deers is limited on account of the geographic location, topography and the farm area (Kilar and Ruda, 2014).

The aim of the researches was to evaluate the biodiversity of plant life of the feeding ground for deers bread for meat.

MATERIALS AND METHODS

The researches were conducted in 2011-2013.

They were carried on the farm for deers, located near Krosno town. The farm was found in 2006. It was made of one headquarters which was 7.0 hectare big. The clay soils area was formed as a gorge with the water course which was 340 meters high above sea level. Plant communities consisted of anthropocentric forest clearing and the rest apple orchard in *Dentario glandulosae-Fagetum*. A herd of deers consists of 50-60 animals among which there is about 40% of adult female deers.

The phytosociological tests were taken in May and October. The tests were based on the Braun-Blanquet method using six-note scale (Braun-Blanquet, 1964). Every time on the feeding ground there were 5 photos taken which covered the area of $50m^2$. In general there were 30 photos taken which were collectively elaborated. The evaluation of the biodiversity of plant life of feeding ground included: species identification, apportionment of the plants from the economic point of view, belonging to the botanical families and to the phytosociological class. Also, this evaluation included the apportionment of the prophylactic properties and medicinal properties (Broda and Mowszowicz, 1996; Danysz and Buczko, 2008; Matuszkiewicz, 2009; Mirek, 2002).

The use value of plants was determined by the number of use value biased on the point method according to Filipek (Filipek, 1973).

This method has ten-point scale. 9-10 points mean a very good value, 7-8 points- just good value, 4-6 points- average value, 1-3 points- low values, 0 points – worthless. If we have from 1 to 3 points in this method, it means that the plants are poisonous. A comparison of floristic composition of the feeding ground and the feeding ground composition for wild deers was made (Krupka, 1990).

RESULTS AND DISCUSSIONS

During the time from the spring to the late autumn, the staple food for deers bred on farms is the plant resources of the feeding pond.

During the winter, animals are fed with supplementary food because from the floristic resources animals can only get some shoots of the trees or shrubs (Janiszewski and Daszkiewicz, 2010). A big floristic biodiversity of feeding ground has the natural behaviour and it has a beneficial effect on the animal productivity and their health (Kilar and Ruda, 2014; Radkowska, 2013; Stokłosa, 2007).

Table 1. Biodiversity and characteristics of plant life of feeding ground for deers bred on farms

Details	The number of species	%
Total, including	62	100.00
• grass	11	17.74
• fabacea	3	4.84
• carex	1	1.61
• herbs and weeds	36	58.07
• trees and shrubs	11	17.74
The degree of coverage		
• above 75%	0	0.00
• 50-75%	0	0.00
• 25-50%	6	9.68
• 5-25%	18	29.03
• to 5%	23	37.10
	15	24.19
Species having value in use for animals:		
• Lwu 9-10	5	8.06
• Lwu 7-8	3	4.84
• Lwu 4-6	8	12.90
• Lwu 3-1	10	16.13
• Lwu 0	34	54.85
• Lwu -1 do -3	2	3.22
Hytoncides including:		
• species with the strong prophylactic and medicinal properties	51	82.26
	27	43.55
• species with the moderate and weak prophylactic and medicinal properties	24	38.71

It was established that in the composition of feeding ground there were 62 vascular plant species (Table 1). All these plants were belonging to 27 botanical families and to 15 phytosociological classes (Table 3).

From the economic point of view, the apportionment of plant life of feeding ground was composed of: 58.07 % of herbs and weeds, 17.74% of grass, 17.74% of trees and shrubs, 4.48% of *Fabacea*, 1.61% of *Carex* (Table 1). Within the botanical families, the grass family was the biggest (11 species).

The Betulaceae, Caryophyllaceae, Poligonaceae, Primulaceae and *Rosaceae* families consisted of 4 kinds of plants. The *Brassicaceae, Fabaceae and Plantaginaceae* families consisted of 3 kinds of plants. The *Asteraceae, Boraginaceae, Lamiaceae* and *Ranunculaceae* families consisted of 2 kinds of plants.All the *Adoxaceae, Balsaminaceae, Compositae, Cyperaceae, Fagaceae, Gentianaceae, Marchantiaceae, Oxalidaceae, Rhamnaceae, Rubiaceae, Salicaceae, Scrophulariaceae, Umbelliferae, Urticaceae* families consisted of 1 kind of plants (Table 2).

Taking into account the phytosociological classes, the biggest number of taxa was in *Molinio-Arrhena Theretea*, *Querco-Fagetea* and *Stellarietea Mediale* falimies (Table 3).

Table 2.The number of botanical plants of the feeding ground for deers bred on farm

	Family	The namber of plants	Structure
1.	*Adoxaceae*	1	1.61
2.	*Asteraceae*	2	3.23
3.	*Balsaminaceae*	1	1.61
4.	*Betulaceae*	4	6.46
5.	*Boraginaceae*	2	3.23
6.	*Brassicaceae*	3	4.84
7.	*Caryophyllaceae*	4	6.46
8.	*Compositae*	1	1.61
9.	*Cyperaceae*	1	1.61
10.	*Fabaceae*	3	4.84
11.	*Fagaceae*	1	1.61
12.	*Gentianaceae*	1	1.61
13.	*Lamiaceae*	2	3.23
14.	*Marchantiaceae*	1	1.61
15.	*Oxalidaceae*	1	1.61
16.	*Plantaginaceae*	3	4.84
17.	*Poaceae*	11	17.74
18.	*Poligonaceae*	4	6.45
19.	*Primulaceae*	4	6.45
20.	*Ranunculaceae*	2	3.23
21.	*Rhamnaceae*	1	1.61
22.	*Rosaceae*	4	6.45
23.	*Rubiaceae*	1	1.61
24.	*Salicaceae*	1	1.61
25.	*Scrophulariaceae*	1	1.61
26.	*Umbelliferae*	1	1.61
27.	*Urticaceae*	1	1.61

The food value of feeding grounds depends on the hydrological conditions, soil conditions and the land use intensity (Wasilewski, 2012). During the time when the researches were conducted, the ceiling of the stocking density factor was not higher than 0.70 DJP per hectare. Which means that the feeding ground was extensively used what is good for biodiversity of plant protection (Chabuz, 2012; Radkowski and Barabasz-Krasny, 2008).

The photosociological imagery analysis shows that among the plants which are part of the feeding ground, dominated plants are: *Pyrus communis L.*, *Cerasus avium (L) Moench,Poa annua L.*, *Trifolium repens L.*, *Cardamine impatiens L.*, and *Malus sylvestris Mill*.

The degree of the plant cover is from 25% to 50%. The researches have shown that a very low share of the plants in the plant life of feeding ground had: *Carpinus betulus L., Salix caprea L., Elymus europaeus L., Poa trivialis L., Oxalis stricta L., Impatiens parviflora DC., Myosotis silvatica (Ehrh.) Hoffm., Marchantia polymorpha L., Primula elatior (L.) Hill., Rumex crispus L., Holosteum umbellatum L., Silene vulgaris (Moench) Garcke., Heracleum sphondylium L.*

Table 3. The number of phytosociological plants of the feeding ground for deer's bred on farms

Phytosociological class	The number of species	%
Agropyretea Intermedio-Repentis	1	1.61
Artemisietea Vilgaris	4	6.45
Betulo-Adenostyletea	3	4.84
Cakiletea Maritimae	1	1.61
Epilobietea Angustifolii	5	8.06
Festuco Brometea	2	3.23
Koelerio glaucae-Corynephoretea canescentis	2	3.23
Magnoliopsida	1	1.61
Molinio-Arrhena Theretea	19	30.65
Montio-Cardaminetea	2	3.23
Nardo-Callunetea	1	1.61
Querco-Fagetea	10	16.13
Rhamno-Prunetea	1	1.61
Stellarietea Mediale	9	14.52
Vaccino-Piceetea	1	1.61

The research results show that the use value was low- only 2.65 points. The use value was higher for typical forage plants – 4.65 points. The small use value of plant life of the feeding ground is caused by the presence of 34 kinds of plants, which have no use value.

Among all the plants of the feeding ground there were two kinds of poisonous plants (*Ranunculus sceleratus L., Cardamine pratensis L.*). According to Table 1, there was only 8.06% of plants that had a very good value and 4.84% of plants that had just a good value. Among plants with a very good value were: *Dactylis glomerata L., Lolium perenne L., Trifolium repens L., Trifolium pretense L., Trifolium hybridum L.* But the plants that had just a good value were: *Agropyron repens (L.) P.B., Poa trivialis L., Alchemilla pastoralis Bus*.

Even though there was a low use value, the plant life of feeding ground was distinguished on account of the big number of phytoncides (Table 1). All kinds of plants according to their prophylactic and medicinal properties are shown in the Table 4.

The plants such as: *Cerasus avium (L) Moench, Salix caprea L., Carpinus betulus L., Taraxacum officinale Web., Cardamine amara L., Veronica chamaedrys L., Primula elatior (L.) Grufb., Mentha aquatica L., Heracleum sphondylium L.* have pro-health properties, antiparastic properties and they have a positive impact on the digestion process.

The researches have shown that in the composition of feeding ground there was no plant life of small shrubs and ferns, forkbeards

and horsetails. There were only some shoots of trees and shrubs noticeable – about 17.75%. The presence of green dicotyledonous plants was about 63.0%. It was three times as much as the presence of these plants in the feeding ground for wild deers (Table 5).

According to the accurate observations of animals that have been done, the poorer floristic feeding ground did not cause any clinical disorders of the animal health and behaviour.

Table 4. The tapes of plants with the prophylactic properties and medicinal properties

Details	Kinds of plants
Plants with the strong prophylactic and medicinal properties	*Cardamine impatiens L., Glechoma hederacea L., Primula elatior (L.) Hill., Heracleum sphondylium L., Ranunculus sceleratus L., Salix caprea L., Centaurium erythraea Rafn., Betula pendula Roth., Sambucus nigra L., Plantago media L., Taraxacum officinale Web., Plantago maior L., Ranunculus repens L., Alchemilla pastoralis Bus., Cardamine pratensis L., Achillea millefolium L., Mentha aquatica L., Cardamine amara L., Veronica chamaedrys L., Polygala vulgaris L., Carpinus betulus L., Primula elatior (L.) Grufb., Lysimachia nemorum L., Fagus sylvatica L., Frangula alnus Mill., Veronica arvensis L., Anagallis arvensis L.*
Plants with the moderate and weak prophylactic and medicinal properties	*Poa annua L., Impatiens parviflora DC., Calamagrostis arundinacea (L.) Roth., Bromus erectus Huds., Poa annua L., Dactylis glomerata L., Alopecurus geniculatus L., Festuca rubra L., Lolium perenne L., Poa trivialis L., Elymus europaeus L.,*

Table 5. The comparison of the feeding ground for wild deers anddeers bred on farm

Details	Wild deers %	Deers bred on farm %
Shoots of trees and shrubs	33.10	17.75
Small shrubs	24.40	0.00
Grass, sedges, sieve plants	19.80	19.35
Green dicotyledonmous plants	20.20	62.90
Ferns, forkbeards, horsetails	2.50	0.00

CONCLUSIONS

Even if the deer farming is very well organised, the freedom to choose both the feeding ground and the floristic biodiversity is limited. The plant life of feeding ground was composed of 62 kinds of vascular plant species, among which there were 11 kinds of trees and shrubs. Even if there was a big floristic biodiversity of feeding ground there were no small shrubs, ferns, forkbeards and horsetails that are very important for the typical wild deer food.

The deficiency of these plants could be replaced to same extend with a big number of phytoncides (51 species) that have a beneficial effect on the animal health and animal-productivity.

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REFERENCES

1. Berg D.K., Asher G., 2003. New developments reproductive technologies in deer. Theriogenology, 59(1): 189-205.
2. Borys B., Bogdaszewska Z., Bogdaszewski M., 2012. Dynamiczny wzrost fermowej hodowli danieli i jeleni w Polsce. Wiadomości Zootechniczne, R.L.(2012), 1: 33-44.
3. Braun-Blanquet J., 1964. Pflanzensoziologie, Grundzuge der Vegetationskundle. 3. Aufl. Springer, Wien-New York.
4. Broda B., Mowszowicz J., 1996. Przewodnik do oznaczania roślin leczniczych, trujących i użytkowych. Wydawnictwo Lekarskie PZWL, Warszawa.
5. Budny A., Kupczyński R., Sobolewski S., Korczyński M., Zawadzki W., 2012. Samolecznictwo i ziołolecznictwo w profilaktyce i leczeniu zwierząt gospodarskich. Acta Scientiarum Polonorum, Medicina Veterinaria, 11(1): 5-24.
6. Chabuz W., Grzywaczewska G., Rysiak A., Cios S., Podolak G., Litwińczuk Z., 2012. Wpływ wypasu lokalnych ras bydła na różnorodność biologiczną łąk i pastwisk Polesia Lubelskiego. Roczniki Naukowe Polskiego Towarzystwa Zootechnicznego, 8(4) : 81-90.
7. Danysz A., Buczko W., 2008. Kompendium farmakologii o farmakoterapii. Wyd. Urban i Partner, Wrocław-Warszawa.
8. Dzierżyńska-Cybulko B., Fruzinski B., 1997. Dziczyzna jako źródło żywności. Państwowe Wydawnictwo Rolne i Leśne, Poznań.
9. Filipek J., 1973. Projekt klasyfikacji roślin łąkowych i pastwiskowych na podstawie liczb wartości użytkowej. Postępy Nauk Rolniczych, 4: 59-68.
10. Florek M., Drozd L., 2013. Związki bioaktywne w mięsie jeleniowatych. Medycyna Weterynaryjna, 69(9): 535-539.
11. Grzelak M., Gaweł E., Barszczewski J., 2013. Wpływ występowania ziół i chwastów na zróżnicowaniu wartości gospodarczej runi łąk. Progress in Plant Protection, 53(1): 182-185.
12. Janiszewski P., Daszkiewicz T., 2010. Zwierzęta łowne. Wydawnictwo Uniwersytetu Warmińsko-Mazurskiego w Olsztynie.
13. Kilar J., Ruda M., 2014. The nutritional value of organic meat from the loin of deer and fallow deer. 6th International Conference on the "Quality and Safety in Food Production Chain", Wrocław, 26-27 June: 72.
14. Kilar J., Ruda M., 2014. Dobrostan pokarmowy jeleni w ekologicznym chowie fermowym. Materiały konferencyjne LXXIX Zjazdu Naukowego PTZ „Systemy produkcji zwierzęcej w

XXI wieku". Siedlce, 259
15. Kilar M., Kilar J., Ruda M., Różański H., 2013. Deer meat as a functional food. I Międzynarodowa Konferencja "Ziołolecznictwo, Biokosmetyki i Żywność funkcjonalna", Krosno 18-19 kwietnia, 131.
16. Krupka J., 1990. Łowiectwo. Państwowe Wydawnictwo Rolnicze i Leśne, Warszawa.
17. Matuszkiewicz W., 2009. Przewodnik do oznaczania zbiorowisk roślinnych Polski. Wydawnictwo Naukowe PWN, Warszawa.
18. Mirek Z., Piekoś-Mirkowa H., Zając M., 2002. Flowering plants and pteridophytes of Poland: a checklist. W. Szafer Institute of Botany Polish Academy of Sciences, Kraków, 442.
19. Radkowska J., 2013. Wykorzystanie ziół i fitogenicznych dodatków paszowych w żywieniu zwierząt gospodarskich. Wiadomości Zootechniczne, R. LP, 4:117-124.
20. Radkowski A., Barabasz-Krasny B., 2008. Zbiorowiska roślinne pastwisk gromadzkich na Pogórzu Bocheńskim. Łąkarstwo w Polsce, 11: 161-170.
21. Stokłosa A., Stępnik K., Barabasz-Krasny B., 2007. Rośliny lecznicze terenów odłogowanych Pogórza Przemyskiego. Annales UMCS Lublin-Polonia, sect. E, vol. LXII(1), 163-173.
22. Wasilewski Z., 2012. Evaluation of botanical composition and quality of grazed sward in three habitat groups. Journal of Research and Applications in Agricultural Engineering, 57(4): 172-176.
23. Wolański P., Trąba Cz., Rogut K., 2011. Różnorodność florystyczna oraz walory krajobrazowe łąk, pastwisk i szuwarów na pogórzu Przemyskim. Zeszyty Problemowe Postępu Nauk Rolniczych, z. 568: 157-169.
24. Ustawa o organizacji hodowli i rozrodzie zwierząt gospodarskich (Dz.U. 2001 nr 129 poz. 1438).
25. www.wetgiw.gov.pl data dostępu 15.02.2015

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EFFECTS OF SELENIUM AND CHROMIUM SUPPLEMENTATION IN THE DIET OF QUAILS ON LIVE PERFORMANCE AND SOME BLOOD PARAMETERS

Osman KUCUK[1], Kerim KILINC[2]

[1]Erciyes University School of Veterinary Medicine, Department of Animal Nutrition and Nutritional Diseases, Kayseri TURKEY 38039,
Phone: +90 505 628 7252, Fax: +90 352 337 27 40, e-mail: osmankucukwy@yahoo.com
[2]Hafik Ilce Gida Tarimve Hayvancilik Mudurlugu, Sivas TURKEY 58760,
Phone: +90 346 841 20 17, Fax: +90 352 337 27 40, e-mail: kerimkilinc@windowslive.com

Corresponding author email: osmankucukwy@yahoo.com

Abstract

The objective of this work was to investigate the effects of supplemental selenium (Se) and chromium (Cr) on live performance and some blood parameters in quails. A total of 90 10-day-old quails (Coturnix coturnix japonica) were used, and the work was terminated after 30 days. The birds were randomly assigned to caging units, 30 birds each. Water and the diets were consumed by the birds ad libitum throughout the experiment. The quails were fed either 1- a basal diet, 2- the basal diet supplemented with either 0.2 ppm Se(SeO_2) or 3- the basal diet supplemented with 0.2 ppm Se plus 500 ppm Cr ($Na_2Cr_2O_7.2H_2O$).Supplemental Se and Se+Cr resulted in a decrease in live weight gains ($P \geq 0.0238$). Supplemental Se decreased the live weight gain but a combination of Se+Cr caused alleviation on this decrease. Feed consumption increased in quails fed a diet supplemented with Se; however, supplementing Se and Cr together decreased the feed consumption but still greater than that of control ($P < 0.0001$). Feed conversion ratio did not change among treatments ($P = 0.3220$). Selenium supplementation alone did not change the serum concentrations of glucose or total protein whereas Se+Cr treatment resulted in an increase in glucose ($P = 0.0036$) but a decrease in total protein concentrations ($P = 0.0189$). Serum cholesterol or triglycerides concentrations remained similar among treatments ($P = 0.2026$). Serum AST enzyme activity decreased with Se supplementation but more with Se+Cr treatments ($P = 0.0587$). Supplementing Se and Se+Cr to the diet of quails resulted in a decrease in serum concentrations of Ca ($P = 0.0009$), P ($P = 0.0720$) and Na ($P < 0.0001$) but no changes in Mg or Cl concentrations ($P \geq 0.2442$). Supplementing Se and Se+Cr to the diet of quails resulted in an increase in serum concentrations of Fe, Cu, Al and Mn. Selenium treatment caused an increase in serum concentration of Zn but Se+Cr treatment resulted in a decrease in Zn concentrations ($P = 0.0027$). As expected, supplementing quail diets with both Se and Cr resulted in increases in serum concentrations of Se and Cr($P < 0.0001$). In conclusion, Se alone or as a combination with Cr supplementation to the diet of quails resulted in a depressed live performance thus are not recommended in the diet.

Key words*: quails, chromium, selenium, performance.*

INTRODUCTION

Trace elements are important in growth performance of animals including poultry. Providing enough trace elements growth is crucial. NRC (1994) has already recommended the dietary concentrations of trace elements for quails. However, supplementing excess amounts and combination of trace elements to the diet of quails are still unknown.

Selenium (Se) as an essential element acts as a highly effective antioxidant with its function (presence) in the active site of the selenoenzyme (glutathione peroxidase, GSH-Px). This enzyme, together with superoxide dismutase and catalase, protects cells against damage caused by free radicals and lipoperoxides (Combs and Combs, 1986).The positive effects of Se supplementation on quail growth have been known, particularly during heat stress (Sahin and Kucuk, 2007).

Chromium (Cr) as an essential element potentiates the action of insulin through its presence in an organometallic molecule called glucose tolerance factor (Anderson, 1987). Dietary chromium supplementation has been reported to have a positive effect on the growth rate, feed efficiency of growing poultry (Cupo and Donaldson, 1987; Lien et al., 1999) as well as on decreasing mortality and altering the

glucose metabolism of chickens (Lien et al., 1999). These beneficial effects of chromium are increased by dietary, physical and hormonal stress (Anderson, 1994; Wright et al., 1994). Supplemental dietary chromium is also recommended by NRC (1994) for animals undergoing environmental stress.

The objective of this study was to evaluate the effects of Se alone or in a combination with Cr supplementation on live performance and some blood parameters of Japanese quails under an intensive production system.

MATERIALS AND METHODS

A total of 90 10-day-old Japanese quails (*Coturnix coturnix japonica*) obtained from Erciyes University Quail Facility, Kayseri, Turkey was used in the study. The birds with equal numbers of males and females were randomly assigned, according to their initial body weights, to three treatment groups, three replicates of 10 birds each. The birds were kept in cages (four birds per subcage of 19 cm x 19 cm x 19 cm).

The birds received either a control diet or control diet supplemented with 0.2 ppm Se (selenium dioxide - SeO_2) or control diet supplemented with both 0.2 ppm Se and 500 ppm Cr (sodium dichromate dehydrate ($Na_2Cr_2O_7.2H_2O$). The study took 30 days. The birds were fed a commercial diet containing 21.5 % HP and 3000 Mcal/kg ME. Ingredients and chemical compositions of the diet are shown in Table 1. The diets were formulated using NRC (1994) guidelines. Small amounts of the basal diet were first mixed with the respective amounts of elements as a small batch, then with a larger amount of the basal diet until the total amount of the respective diets was homogeneously mixed. The diets and fresh water were offered ad libitum. Light was provided all the time (24 h) inside the hen house.

The experiment was conducted between 15 May and 15 June. At weekly intervals, feed intake and body weight were determined on group basis as replicates of each treatment. Weight gain and feed efficiency of groups were then calculated. At the end of the experiment, all birds from each group were slaughtered and blood (5 cc) was collected. Blood samples were centrifuged at 3 000 × *g* for 10 min and serum was collected from Vena brachialis and stored at –20°C for later analysis. Serum samples were thawed at room temperature. On thawed samples Se, Cr, Ca, P, Mg, Fe, Cu, Zn, Na, K, Al, Mn concentrations were measured using ICP/MS (Agilent 7500a series, Berghof Speedwave, Germany). Serum concentrations of total protein, glucose, cholesterol, triglycerides, and enzyme activities of ALT and AST were measured using biochemical analyser (Abbott Diagnostics - Architect, USA).

Chemical analysis of the diets was run using the international procedures of AOAC. The data were analysed by ANOVA using the GLM procedure of SAS. Differences between the means ($P< 0.05$) were determined using Duncan's multiple range test. Most of the values reported for measured parameters in the present study indicated that the data are normally distributed.

Table 1. Ingredients and chemical composition of the basal diet fed to quails*

Feedstuff	**%**
Crude protein	21.50
Ether extract	7.50
Crude fiber	4.00
Crude ash	6.00
Calcium	0.90
Phosphorous	0.50
Lysine	1.30
Methionine	0.50
Trace elements	**ppm**
Manganese	120.00
Zinc	100.00
Selenium	0.30
Iron	40.00
Iodine	1.25
Cupper	16.00
Vitamins	**IU/kg**
Vitamin A	10.000,00
Vitamin D_3	5.000,00
Vitamin E	75.00

*The diet contains 100 ppm antioxidant (Narasin - Maxiban).

RESULTS AND DISCUSSIONS

Quails fed a diet supplemented with Se alone or a combination of Se+Cr resulted a decrease in live weights ($P \geq 0.0238$) (Table 2). Selenium supplementation in the diet of quails resulted in a decrease in live weight but adding Cr to the diet supplemented with Se caused alleviation in decreased live weights. Similarly, feed intake decreased in quails fed a diet supplemented with Se, but adding Cr to the diet supplemented with Se caused alleviation in decreased feed intake ($P < 0.0001$). However, feed conversion ratio did not change upon any supplementation ($P = 0.3220$).

Surplus of recommended amounts of both Se and Cr by NRC (1994) did not support live weight performance in quails. In addition, excess amounts of these elements resulted in a depressed live performance. These results were not expected because the both elements are involved in crucial functions in the metabolism of the quails. Chromium plays important role in carbohydrate metabolism. The oligopeptide low-molecular-weight chromium-binding protein (chromodulin) tightly binds four chromic ions before the oligopeptide obtains the conformation required for binding to the tyrosine kinase active site of the insulin receptor (Sun et al., 2000). The oligopeptide-chromodulin binds chromic ions in response to an insulin-mediated chromic ion flux, and the metal-saturated oligopeptide can bind to an insulin-stimulated insulin receptor, activating the receptor's tyrosine kinase activity. Thus, chromodulin appears to play a role in an auto-amplification mechanism in insulin signalling (Sun et al., 2000). In addition, the release of chromium from chromium picolinate for use in cells requires reduction of the chromic centre, a process that can lead potentially to the production of harmful hydroxyl radicals (Sun et al., 2000). However at the present work, blood glucose concentrations increased upon Cr supplementation of the diet in quails (Table 3).

It was expected to have a greater live performance of quails fed a Se-supplemented diet because Se supplementation in poultry has long been associated with energy metabolism, increased feed conversion ratio, improved reproduction, and improved immune responses. However, as was a case for Cr, Se supplementation decreased the live performance. Selenium is toxic to poultry when used in high doses (< 3–5 mg/kg feed). The dose used at the present work is not toxic enough to reduce live performance. It was also assumed that the Se dose used at the present work was not toxic enough to cause any toxicity but high concentration enough to reduce live performance, namely, feed intake, body weight gain, and feed conversion ratio.

As expected, supplementing both Se and Cr to the diet of quails increased the blood concentrations of Se and Cr ($P < 0.0001$) (Table 3). In addition, supplementing both Se and Cr to the diet of quails increased the blood concentrations of Fe and Cu ($P < 0.0001$). In general, supplementing either of the trace elements influenced the serum concentrations of the elements measured.

Table 2. Live weight performance of quails fed a diet supplemented with Se and Cr

	Treatment*				
Parameter	Control	Se	Se+Cr	SEM	*P*
Initial live weight, gr	24.633	24.300	23.066	0.6507	0.2061
Final live weight, gr	171.133^{a}	159.500^{b}	165.433^{ab}	2.945	0.0238
Live weight gain, gr	146.500^{a}	135.200^{b}	142.366^{ab}	3.037	0.0331
Feed intake, gr	332.833^{c}	340.666^{a}	337.833^{b}	0.643	<0.0001
FCR**	0.4402	0.4131	0.4214	0.013	0.3220

*a, b, c: Means in the same row with different superscripts differ ($P< 0.05$).

**Feed conversion ratio: live weight gain/feed intake.

Table 3. Changes in some serum metabolites and minerals of quails fed a diet supplemented with Se and Cr*

	Treatment**				
Parameter	Control	Se	Se+Cr	SEM	*P*
Glucose, mg/dL	278.166^{b}	278.280^{b}	308.240^{a}	7.145	0.0036
Triglyceride, mg/dL	247.208	304.760	380.000	52.694	0.2026
Cholesterol, mg/dL	192.541	190.640	199.440	5.626	0.2026
Total protein, g/dL	9.520^{a}	8.628^{a}	6.152^{b}	0.863	0.0189
ALT, U/L	5.625	6.000	5.680	0.324	0.6818
AST, U/L	438.916^{a}	386.720^{ab}	315.840^{b}	36.624	0.0587
Ca, ppm	0.0348^{a}	0.0019^{b}	0.0019^{b}	0.005	0.0009
P, mg/dL	10.813^{a}	10.048^{ab}	8.608^{b}	0.695	0.0720
Mg, ppm	0.0212	0.0023	0.0001	0.009	0.2442
Na, ppm	0.1796^{a}	0.000002^{b}	0.000002^{b}	0.009	<0.0001
Cl, mmol/L	114.608	112.080	113.200	1.469	0.4658
Al, ppm	0.005^{b}	8.719^{b}	24.810^{a}	2.909	0.0002
Mn, ppm	0.0003^{b}	0.0530^{b}	0.1798^{a}	0.0274	0.0007
Fe, ppm	0.0298^{c}	44.212^{b}	80.534^{a}	4.177	<0.0001
Cu, ppm	0.0013^{c}	0.463^{b}	0.930^{a}	0.075	<0.0001
Zn, ppm	0.1364^{a}	0.1886^{a}	0.0002^{b}	0.0307	0.0027
Se, ppm	0.0028^{c}	0.1154^{b}	0.3982^{a}	0.036	<0.0001
Cr, ppm	0.0008^{c}	0.2604^{b}	0.4730^{a}	0.028	<0.0001

*Blood samples were taken in quails starved overnight.

**a, b, c: Means in the same row with different superscripts differ ($P< 0.05$).

CONCLUSIONS

Results of the present work showed that NRC (1994) recommendations should be followed for a better performance. Any excess amounts of Se and Cr results in a depressed live performance in quails.

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REFERENCES

Anderson R.A., 1994. Stress effects on chromium nutrition of humans and farm animals. In: Lyons TP.,Jacques, K. A. (eds), Biotechnology in Feed Industry, University Press, Nottingham, 267-274.

Combs G.F., Combs S.B., 1986. The Role of Selenium in Nutrition. Academic Press, London.

Cupo M.A., Donaldson W.E., 1987. Chromium and vanadium effects on glucose metabolism and lipid synthesis in the chick. Poultry Science, 66;120.

NRC, 1994.Nutrient Requirements of Poultry.National Academy Press, Washington, DC.

Lien T.F., Horng Y.M., Yang K.H., 1999. Performance of broilers as affected by supplement of chromium picolinate.British Poultry Science,40(3):357-365.

Sahin K., Kucuk O., 2007. Selenium supplementation in heat-stressed poultry. CAB Reviews, 2:1-10.

Sun Y., Ramirez J., Woski S.A., Vincent J.B., 2000. The binding of trivalent chromium to chromium. Journal Inorganic Chemistry, 5(1):129-136.

Wright A.J., Mowat D.N., Mallard B.A., 1994. Supplemental chromium and bovine respiratory disease vaccines for stressed feeder calves. Canadian Journal of Animal Science, 74:287-295.

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SOIL MITES DIVERSITY FROM POLLUTED GRASSLAND ECOSYSTEMS IN TRASCĂU MOUNTAINS (WESTERN CARPATHIANS – ROMANIA)

Minodora MANU[1], Marilena ONETE[1], Virgil IORDACHE[2]

[1]Romanian Academy, Institute of Biology, Department of Ecology, Taxonomy and Nature Conservation, street Splaiul Independenţei, no. 296, zip code 0603100, PO-BOX 56-53, Tel. 040212219202, Fax 040212219071, Bucharest, Romania.
Email: minodora_stanescu@yahoo.com and marilena_onete@gmail.com
[2]Research Centre for Ecological Services (CESEC), Faculty of Biology, University of Bucharest, Romania.
Email: virgil.iordache@g.unibuc.ro

Corresponding author email: minodora_stanescu@yahoo.com

Abstract

The study was made in 2013 - 2014, in Trascău Mountains, situated in the south-eastern part of the Apuseni Mountains (Western Carpathians), in their southern limit, represented by the Ampoi valley. In order to determine the mite diversity, 300 soil samples were investigated from twelve grassland ecosystems, taking into account the pollution level source (an old chimney plant, which provided heavy metals pollutants: As, Cu, Zn, Pb, Mn, Ni). The altitude of grasslands varied from 464 m to 958 m, and the distance from pollution source from 975 m to 3200 m. In total, 4447 individuals were counted, belonging to the following mite orders: Trombidiformes (5.42 %), Sarcoptiformes (72.65 %) and Mesostigmata (21.92 %).
In order to assess the diversity aspects of edaphically mites, a comparative analyse was made between the natural and anthropogenic ecosystems. The highest mite diversity was recorded in natural area (grassland G12 - on 3146.52 m distance from pollution source), with 639 individuals, belonging to the 14 mite families and Shannon_H index by 1.412. On the opposite is grassland G9 (on 1311.65 m distance from pollution source), with 253 individuals from 8 families and Shannon_H index by 0.573. Dominant mites were oribatids, decomposers of the organic matter, followed by the mesostigmatids (mostly represented by Ascidae, Laelapidae and Rhodacaridae families, which are predators).
The similarity of mite presence and composition was assessed using Jaccard and respectively Bray-Curtis dendrograms. Evidence from Shannon evenness, Shannon-Weaver diversity and Simpson dominance indexes indicate that in the areas with a low taxa diversity, there is a dominance of few species with individuals unequally distributed between plots.Each investigated grassland ecosystems were characterized by characteristically mite diversity. This study represents a valuable argument for using the soil mite fauna as bioindicators.

Key words: acari, diversity, heavy metals, similarity.

INTRODUCTION

Mites are one of the most abundant edaphic fauna groups from terrestrial ecosystems that are directly or indirectly participate on the soil pedogenesis (Walter and Proctor, 2003; Krantz and Walter, 2009).
In grassland the trophic spectrum of the mites are very wide, from polyphagous to the high specialized species, parasites, herbivores, fungivores, microbivores, detritivores, scavengers and omnivores (Walter et al., 1988; Behan-Pelletier and Kanashiro, 2010).
Soil mites are very sensitive to any natural or anthropical disturbances, their being often used as bioindicators (Kardol et al., 2011; Nielsen et al., 2010, 2012).
In Romania, some qualitative and quantitative studies the edaphic microarthropods fauna from natural grassland ecosystems were made only in Moldavian Plain that is placed in the North-East of Romania (Călugăr, 2006 a, b).
Our objective is to establish a comparative diversity analysis of the soil mite fauna from twelve polluted grassland ecosystems from Romania, taking into account of the some environmental variables and heavy metals pollutants from the investigated areas (Pb, Zn, Mn, Cu, As, Ni).

MATERIALS AND METHODS

The present study was made in June, September-2013 and April-2014, in twelve grassland ecosystems situated in Ampoi Valley, from Trascău Mountains, located in the south-eastern part of the Apuseni Mountains (Western Carpathians) (Figure 1). The pollution source is represented by an old chimney plant, situated in the proximity of the Zlatna city, one of the most famous industrial centres in Romania for the extraction of copper, lead, gold and silver, mainly in the communism period (1953-1993).

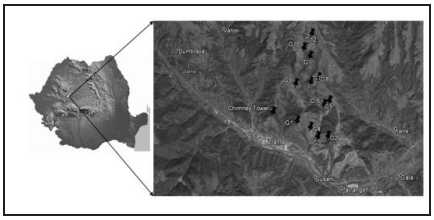


Figure 1. Geographical position of the investigated grassland ecosystems from Trascău Mountains.

The altitude of grasslands varied from 464 m to 958 m, and the distance from pollution source from 975 m (G1) to 3200 m (G12-natural ecosystem). Most of the recorded plant species were xerophytic, hemicryptophyte and perennial. Those species with the overall highest coverage percentage were *Agrostis capillaris, Nardus stricta, Rumex acetosella* and *Trifolium pratense*. The precise local coverage of these species varied greatly with respect to the anthropic impact in the area (overgrazing and pollution) and these species were not uniformly distributed in all investigated plots.

The concentration of the six heavy metals being investigated along the Ampoi valley was mapped using XRF (X-ray fluorescence spectrometer).

In one investigated area by 2500 sq.m. 25 cores were sampled for mite fauna, to a depth of 10 cm with a MacFadyen corer, by 5 cm diameter. The samples (300) were taken randomly. The mites were extracted with a modified Berlese-Tullgren funnel, in ethyl alcohol, clarified in lactic acid and identified to family level, using actual published identification keys.

4442 mites were extracted from the 300 soil cores (296 individuals from Trombidiformes order, 3185 from Sarcoptiformes order and 961 individuals from Mesostigmata).

In order to assess the diversity aspects of edaphically mites, a comparative analyse was made between the natural and anthropogenic ecosystems. Mite diversity (Shannon index), dominance (Simpson's index) and evenness (E index) were calculated using the procedures BioDiversity Pro 2.0, PAST (Hammer et al., 2001). The similarity of mite presence and composition was assessed using Jaccard (q_J) and respectively Bray-Curtis (q_{BC}) dendrograms.

RESULTS AND DISCUSSIONS

The investigated heavy metals pollutants were: arsenic (As), copper (Cu), manganese (Mn), nickel (Ni), plumb (Pb) and zinc (Zn). All values were compared with the admissible normal values of the heavy metals according to the national law (Ministry Order no. 756 from 3 November 1997 concerning the arrangement approval of the environmental pollution assessment). The arsenic concentrations exceed the normal values in all twelve grasslands, following a distance gradient, decreasing from G1 to G12. The same situation was recorded on copper, but with one exception: in G4 was identified one of the highest concentration of this heavy metal. On manganese the normal values were exceeding only in G2, G7, G8, G10 and G11, in the other ecosystems the concentrations decreased till 387.46 mk/kg^{-1}. If we take into consideration nickel and plumb concentrations these were decreasing in ecosystems situated on a high distance from the pollution source. In G5, G10, G11 and G12 the zinc concentrations are lower than the normal value, and in the remaining grasslands that heavy metal recorded increased values, with a maximum at G1 (Table 1).

If we take into consideration the mite fauna, the most abundant group was Sarcoptiformes order, mainly represented by oribatids. Oribatids are soil invertebrates that included many trophycal categories, as macrophytophagous, microphytophagous and panphytophagous species, beeing in the same time saprophagous and second consumers, decomposing the organic matter. They are involved in decomposing processes and in turnover process, making available the organic matter to plants (Walter et al., 1988; Walter and Proctor, 2003; Krantz and Walter, 2009; Nielsen et al., 2012).

Table 1. Average concentrations (mg/kg^{-1}) of heavy metals identified in soil from the grassland ecosystems (n.v. = normal values according to the Romanian law M.O. no.756/1997; ± = standard deviation)

Grassland	As	Cu
G1	55.31 (± 1.86)	576.3 (± 71.32)
G2	23.22 (± 2.62)	135.55 (± 61.54)
G3	24.38 (± 2.74)	147.17 (± 71.51)
G4	23.37 (± 2.95)	305.64 (± 49.16)
G5	15.68 (± 5.99)	91.49 (± 29.87)
G6	13.03 (± 5.80)	100.66 (± 64.64)
G7	21.04 (± 2.12)	156.35 (± 76.52)
G8	24.38 (± 2.62)	80.1 (± 27.54)
G9	10.04 (± 4.73)	63.63 (± 21.62)
G10	12.07 (± 3.01)	52.16 (± 16.19)
G11	10.89 (± 4.52)	46.56 (± 12.44)
G12	7.36 (± 5.57)	22.11 (± 11.36)
N.v.	5	20
Grassland	Ni	Pb
G1	34,13 (± 8.82)	421.12 (± 71.62)
G2	109.72 (± 12.52)	155.24 (± 54.27)
G3	73.74 (± 11.45)	167.23 (± 56.22)
G4	125.80 (± 22.80)	278.96 (± 36.03)
G5	109.06 (± 13.01)	102.49 (± 34.19)
G6	127.23 (± 16.37)	105.16 (± 48.22)
G7	162.82 (± 27.02)	110.68 (± 51.67)
G8	125.94 (± 19.76)	69.76 (± 16.52)
G9	105.25 (± 24.51)	71.16 (± 17.41)
G10	102.56 (± 23.04)	57.63 (± 15.40)
G11	121.83 (± 19.73)	36.88 (± 9.04)
G12	100.45 (± 18.40)	28.21 (± 4.62)
N.v.	20	20
Grassland	Mn	Zn
G1	616.34 (± 81.77)	211.69 (± 18.72)
G2	920.68 (± 95.62)	161.12 (± 15.13)
G3	896.74 (± 57.78)	204.18 (± 18.76)
G4	869 (± 51.07)	224.57 (± 19.87)
G5	387.46 (± 69.58)	99.66 (± 13.32)
G6	454.91 (± 77.03)	121.57 (± 21.02)
G7	1156.17 (± 451.65)	162.83 (± 16.12)
G8	1344.38 (± 511.34)	121.09 (± 20.98)
G9	672.78 (± 85.73)	119.4 (± 20.56)
G10	1071.34 (± 437.92)	90.83 (± 14.35)
G11	911.17 (± 137.47)	91.27 (± 13.91)
G12	854.91 (± 115.33)	74.49 (± 8.41)
N.v.	900	100

If we taking into account the numerical densities of the soil mites, the species from Mesostigmata order were on the second place, mainly represented by Ascidae, Lealapidae and Rhodacaridae families. They are predators, feeding on immature of oribatids or other soil invertebrates. These invertebrates are frequently found in anthropic ecosystems, as: urban parks, spoilt areas, industrial and derelict areas. Species from Trombidiformes order had the lowest number of individuals, being represented by predators species from Bdellidae family (feeding with arthropods eggs, nematodes), Cunaxidae family (feed on microarthropods in soil, plant debris, moss, or straw), Trombidiidae, and Tydeidae families (feeding with small mites that scavenge or feed on fungi on plant surfaces) (Walter and Proctor, 2003; Krantz and Walter, 2009) (Table 2).

In total, 4442 individuals were counted, belonging to the following soil mite orders: Trombidiformes (5.42%), Sarcoptiformes (72.65%) and Mesostigmata (21.92%).

According to other studies from the natural grasslands, the most abundant species are prostigmatids, followed by oribatids and mesostigmatids (Battigelli and McIntyre, 1999; Battigelli et al., 2003; Osler et al., 2008; Behan-Pelletier and Kanashiro, 2010). Due to the anthropic impact (heavy metal pollution), in the present study the situation is different, the dominant species being oribatids-Sarcoptiformes order, followed by mesostigmatids-Mesostigmata order and the last by the prostigmatids-Trombidiformes order.

Making a comparison between grasslands, the highest mite diversity was recorded in natural area G12 (situated on 3146.52 m distance from pollution source), with 639 individuals, belonging to the 14 mite families and Shannon_H index by 1.412. On the opposite is G9 (on 1311.65 m distance from pollution source), with 253 individuals from 8 families and Shannon_H index by 0.573. The highest dominance index was recorded in G10 and G12, less polluted areas. On opposite are the G3, G5 and G9 ecosystems, where the Ni, As, Pb, Cu and Zn exceed the normal values. The evenness index indicate that in the areas with a low taxa diversity, there is a dominance of few species with individuals unequally distributed between investigated areas (Figure 2).

In order to make a comparison between soil mite populations, some similarity indexes were established. If we take into consideration the numerical abundance of the all mites, the Bray-Curtis index of similarity showed us that these invertebrates were grouped as following: those from G1-G2-G10-G11; G3-G4-G6-G12 and G5-G7-G8-G9.

Table 2. Numerical abundance of the soil mite fauna from investigated grassland ecosystems.

Systematic group		G1	G2	G3	G4	G5	G6	G7	G8	G9	G10	G11	G12
Order	Trombidiformes												
Suborder	Prostigmata												
Family	Tydeidae	62	49	24	1	4	2	29	10	5	0	4	0
	Trombidiidae	0	0	0	1	4	4	0	2	1	1	1	16
	Cunaxidae	0	0	0	1	6	0	0	0	0	2	3	4
	Bdellidae	0	0	0	0	0	2	0	0	0	0	0	0
	Total	62	49	24	3	14	8	29	12	6	3	8	20
Order	Sarcoptiformes												
Suborder	Oribatida	122	141	398	311	220	455	239	260	219	108	157	310
Family	Acaridae	0	0	0	19	0	128	0	0	0	23	27	42
	Glycyphagidae	0	0	0	0	5	0	0	0	0	1	0	0
	Total	122	141	398	330	225	583	239	260	219	132	184	352
Order	Mesostigmata												
Suborder	Gamasina												
Systematic group		G1	G2	G3	G4	G5	G6	G7	G8	G9	G10	G11	G12
Family	Parasitidae	14	10	11	0	0	0	1	3	1	2	1	7
Family	Ascidae	7	11	7	20	4	6	33	18	3	12	7	21
Family	Phytoseiidae	1	1	0	0	0	0	1	3	0	0	3	0
Family	Macrochelidae	0	0	0	0	1	0	0	0	3	0	0	2
Family	Eviphididae	0	0	0	0	0	1	0	0	0	0	0	0
Family	Laelapidae	25	13	23	77	7	31	18	24	20	46	102	206
Family	Pachylaelapidae	5	1	4	1	0	1	0	0	0	0	0	1
Family	Zerconidae	0	0	1	0	0	3	11	0	0	0	1	0
Family	Veigaiidae	3	0	4	0	0	0	2	2	0	0	0	2
Family	Rhodacaridae	1	4	1	9	16	9	1	8	1	9	3	16
Suborder	Uropodina												
Family	Trachytidae	0	0	2	0	0	1	3	0	0	0	0	2
Family	Uropodidae	0	0	11	0	0	0	0	0	0	1	0	3
Family	Oplitidae	0	0	0	0	0	0	0	0	0	3	1	7
	Total	56	40	64	107	28	52	70	58	28	73	118	267
	Total mites	240	230	486	444	272	649	338	340	253	218	321	651

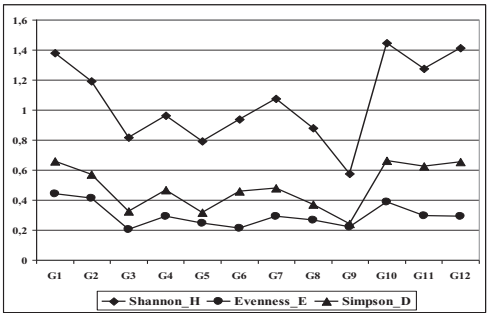
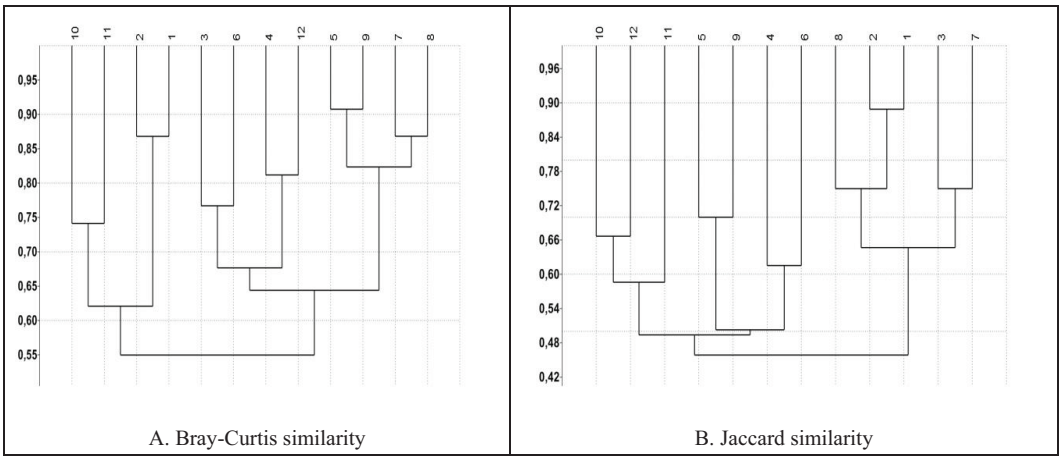


Figure 2. The diversity (Shannon_H index), the dominance (Simpson_D index) and the eveness (E) of the soil mite populations from investigated grassland ecosystems.

The highest Bray-Curtis similarity index was obtained between populations from G1-G2 (q_{BC} = 0.94); G5-G9 (q_{BC} = 0.82), G7-G8 (q_{BC} = 0.84), G4-G12 (q_{BC} = 0.69) and G10-G11 (q_{BC} = 0.78) (Figure 3A). The increased similarities between less and most polluted grasslands, could be explained through the abundance of the prostigmatids – order Trombidiformes, especially of mites from Tydeidae family (predatory, fungivorous and scavenging invertebrates). It is possible that the presence of the high heavy metals concentrations to determine increasing of the soil acidity, favorable environment for fungi development or other parasites invertebrates development, that constitute the trophic reservoir for these mites.

Taking into discussion the presence/absence of the mite systematic groups, there were classified in three groups: invertebrates from G10-G11-G12, from G4-G5-G6-G9 and G1-G2-G3-G7-G8. The highest value of the

Jaccard similarity index where obtain between populations from G1-G2 (q_J = 0.88), G5-G9 (q_J = 0.70) and G10-G12 (q_J = 0.68) (Figure 3B). These groupings demonstrated that heavy metals influence the soil mite composition, on distance gradient. Mites systematic groups from the grasslands situated closed to the pollution source (G1, G2, G3, G4) are characterized by a lower representation in comparison with those from ecosystems situated on a distance from chimney tower (as G10, G11, G12).



A. Bray-Curtis similarity B. Jaccard similarity

Figure 3. Similarity dendrograms of the investigated systematic mite groups from investigated grassland ecosystems.

CONCLUSIONS

Each investigated grassland ecosystems were characterized by specifically heavy metals concentrations and by characteristically mite taxa diversity. The concentrations of heavy metals are much higher in soil of ecosystems near to the pollution source and lower on the distance. All heavy metals exceed the admissible legal values concentrations in all investigated ecosystems, except the natural grassland.

The most abundant group was Sarcoptiformes order, mainly represented by oribatids, follwed by Mesostigmata and Trombidiformes orders.

Evidence from Shannon evenness, Shannon diversity and Simpson dominance indexes indicated that in the areas with a low taxa diversity, there is a dominance of few taxa with individuals unequally distributed between plots. The mite diversity increased in grasslands situated on a higher distance from the pollution sourse. The influence of the heavy metal pollution is highlighted by the affinity between mites groups from different ecosystems. Mites systematic groups from the grasslands situated closed to the pollution source are characterized by a lower representation in comparison with those from ecosystems situated on a distance from chimney tower.

Modifications of the structural parameters of the mite populations and their composition represent useful arguments for their usage as bioindicators.

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REFERENCES

Battigelli J.P., McIntyre G.S., 1999. Effects of long-term grazing on abundance and diversity of soil mesofauna. In: Krzic M.., Broesma K., Thompson D., Bomke A.(Eds.), Effects of long-term grazing on soil quality in southern British-Columbia. Edited by British Columbia. -report no.3 (April 1998-1999). Beef cattle industry development fund (project no.58), 25-30.

Battigelli J.P., McIntyre G.S., Broesma K., Krzic M., 2003. Impact of cattle grazing on prostigmatid mite densities in grassland soil from southern interior British Columbia. Canadian Journal of Soil Science , 83:533–5.

Behan-Pelletier V.M., Kanashiro D., 2010. Acari in grassland soils of Canada. In: J.D. Shorthouse, K.D. Floate KD (Eds), Arthropods of Canadian Grasslands. Vol. 1: Ecology and Interactions in Grassland Habitats (B.S.C.), 137-166.

Călugăr A., 2006 a. Qualitative and quantitative studies upon the edaphic microarthropods fauna in some grassland ecosystems from Moldavia Plain (Romania). Complexul Muzeal de Ştiinţele Naturii "Ion Borcea" Bacău, Studii şi Comunicări, 21: 230-231 (in Romanian).

Călugăr A., 2006 b. On the gamasid fauna (Acari:Gamasina) from the grassland ecosystems from Moldavia Plain (Romania). Complexul Muzeal de Ştiinţele Naturii „Ion Borcea" Bacău, Studii şi Comunicări, 21: 231-235 (in Romanian).

Hammer Ř., Harper D.A.T., Ryan P.D., 2001. PAST: Paleontological statistics software package for education and data analysis. Palaeontologia Electronica, 4 (1): 1-9.

Kardol P., Reynolds W.N., Norby N.J., Classen A.T., 2011. Climate change effects on soil microarthropod abundance and community structure. Applied Soil Ecology, 47: 37– 44.

Krantz W., Walter D.E., 2009. A Manual of Acarology. Texas Tech University Press, Lubbock Texas.

Nielsen U.N., Osler G.H.R., Campbell C.D., Burslem F.R.P., Van der Wal R., 2010. The influence of vegetation type, soil properties and precipitation on the composition of soil mite and microbial communities at the landscape scale. Journal of Biogeography, 37 (7): 1317-1328.

Nielsen U.N., Osler G.H.R., Campbell D.C., Burslem D.F.R.P., Van der Wal R., 2012. Predictors of fine-scale spatial variation in soil mite and microbe community composition differ between biotic groups and habitats. Pedobiologia, 55 (2): 83-91.

Osler G.H.R., Harrison L., Kanashiro D.K., Clapperton M.J., 2008. Soil microarthropods assemblages under different arable crop rotation in Alberta, Canada. Applied Soil Ecology, **38**: 71-78.

Walter D.E., Hunt H.W., Elliot T.E., 1988. Guilds or functional groups? An analysis of predator arthropods from a short grass steppe soil. Pedobiologia, 31:247-260.

Walter D.E., Proctor H.C., 2003. Mites: Ecology, Evolution and Behaviour. Life at a Microscale. Springer – Verlag, second edition.

*** Romanian Waters, Forests and Environmental Protection Ministry Order no. 756 from 3 November 1997 concerning the arrangement approval of the environmental pollution assessment.

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EFFECTS OF BACTERIOCIN AND ORGANIC ACIDS ON GROWTH PERFORMANCE OF JAPANESE QUAILS

Mursel OZDOGAN, Ahmet Onder USTUNDAG

Adnan Menderes University Faculty of Agriculture, Department of Feed and Animal Nutrition, South Campus, Aydın, Turkey, Phone: +90256 772.70.23, Fax: +90256 772.72.33, E-mail: mozdogan@adu.edu.tr,austundag@adu.edu.tr

Corresponding author e-mail: mozdogan@adu.edu.tr

Abstract

The aim of this study was investigate the effects of bacteriocin and organic acids on growth performance of Japanese quails. 600-day-old Japanese quails of mixed sex were randomly divided into six experimental groups. Each group included five replicates of 20 chicks per pen. Chicks were fed a control diet, 150 mg/kg bacteriocin, 300 mg/kg bacteriocin, 3 g/kg organic acid, 150 mg/kg bacterocin + 3 g/kg organic acid (Selacid® GreenGrowth MP), 300 mg/kg bacteriocin + 3 g/kg organic acid for 35 days. Active ingredients of Selacid® GreenGrowthMPweresorbicacid, formicacid, aceticacid, lacticacid, propionicacid, ammoniumformate, citricacid 1,2-propanediol, coconut/palmkernelfattyaciddistillate, silicondioxide (SiO2). There were no effects of dietary treatments on body weight, body weightgain, feedintakeandfeedconversionratio of Japanesequails.

Key words: *bacteriocin, organic acid, growth performance, quail.*

INTRODUCTION

Maintaining microflora balance and gut health of chicks is one of the major issues of modern poultry nutrition to prevent diseases by controlling the proliferation of potentially pathogen microorganism (Józefiak et al., 2010; Jothi et al., 2012). Therefore, antibiotics have been widely used as growth promoters and therapeutic treatments for more than 50 years in animal nutrition (Dibner and Richards, 2005; Diez-Gonzalez, 2007). But, the appearance of antibiotic resistant bacteria and residual antibiotics in meat caused to ban of antibiotics. Ban of antibiotics in 2006 and increasing demand of organic production have increased interest in searching for alternative to antibiotics (Dahiya et al., 2006; Shin et al., 2008; Tatsadjieu et al., 2009). Probiotics, prebiotics, organic acids, essential oils and plant extracts, bacteriocins, antimicrobial peptides, bacteriophages and feed enzymes take place among these alternatives (Joerger, 2003; Józefiak et al., 2007; Shin et al., 2008; Alloui et al., 2013). Bacteriocins, which are one of these alternatives, are ribosomally synthesized antimicrobial substances of proteinaceous character and are active against bacteria more or less related to the producing bacteria (Klaenhammer, 1993; Cigánková et al., 2004; Gillor et al., 2005, 2008). Bacteriocin received much attention because of their wide antibacterial spectrum and their potential application in foods and feeds for controlling spoil age and pathogenic microorganism (Cleveland et al., 2001; Stern et al., 2006, Rihakova et al., 2009; Józefiak et al., 2010; Musikasang et al., 2012). As food preservative, Nisin is a bacteriocin produced by certain strains of *Lactococcus lactis subsp. Lactis* and widely used in the world uptodate (Kišiyadová et al., 2003; Ogunbanwo et al., 2003; Li et al., 2005). Organic acids are another alternative feed additives to antibiotics (Soltan, 2008; Hermans et al., 2010; Menconi et al., 2013). Organic acids are considered to be any organic carboxylic acid, including fatty acids and amino acids, of the general structure R-COOH and widely used in animal nutrition as feed acidifers (DibnerandButtin, 2002; Ricke, 2003). They reduce feed buffering capacity and decrease pH of feed, crop and intestinal contents and thus inhibit the growth of pathogen bacteria in food, gastrointestinal tract and also improve the solubility of minerals though increase digestive enzymes activity (Yesilbag ve Colpan, 2006; Liem et al., 2008; Housmand et al., 2011; Swiatkiewics and Arczewska-Wlosek 2012 a, b). Despite the reare many

studies that researched the effects of organic acids on performance of quails, studies that investigated the effects of bacteriocins on performance of quails are limited. Therefore, the objective this study was to determine the effects of bacteriocin and organic acids on performance of Japanese quails.

MATERIALS AND METHODS

Experimental procedures were approved by Institutional Animal Care and Use Committee of Adnan Menderes University.

BIRDS, DIETS AND MANAGEMENT

In this study, 600-day-old Japanese quail chicks (*Coturnix coturnix japonica*) of mixed sex were used. Chicks were weighted and randomly divided into six experimental groups. Each group included five replicates of 20 chicks per pen (6x5x20). The experiment was lasted for 35 days. Chicks were fed a control diet, 150 mg/kg bacteriocin (B150), 300 mg/kg bacteriocin (B300), 3 g/kg organic acid(Selacid® GreenGrowth MP) (OA), 150 mg/kg bacterocin + 3 g/kg organic acid (B150+OA), 300 mg/kg bacteriocin + 3 g/kg organic acid (B300+OA) for 35 days. Active ingredients of Selacid® GreenGrowth MP weresorbicacid, formicacid, aceticacid, lacticacid, propionicacid, ammoniumformate, citricacid 1,2-propanediol, coconut/palm kernel fatty acid distillate, silicon dioxide (SiO2). Nisin was used as bacteriocin in this study and it was microencapculated according to Stern et al., (2006). The diets formulated to meet requirements of quail according to the NRC (1994) in Table 1.

Table 1 Ingredients and chemical composition of experimental diets

Ingredients (%)	Control	B150	B300	OA	B150+OA	B300+OA
Corn	47.90	47.89	47.87	47.70	47.70	47.70
Soybean meal	45.5	45.5	45.5	45.5	45.49	45.47
Vegetable oil	3.7	3.7	3.7	3.6	3.6	3.6
Bacteriocin	0	0.015	0.030	0	0.015	0.030
Organic acid	0	0	0	0.3	0.3	0.3
Dicalcium phosphate	0.65	0.65	0.65	0.65	0.65	0.65
Calcium carbonate	1.4	1.4	1.4	1.4	1.4	1.4
Salt	0.3	0.3	0.3	0.3	0.3	0.3
DL-Methionin	0.2	0.2	0.2	0.2	0.2	0.2
Vitamin premix[1]	0.25	0.25	0.25	0.25	0.25	0.25
Mineral premix[2]	0.1	0.1	0.1	0.1	0.1	0.1
Nutrient Composition (%)						
Dry matter	87.55	87.53	87.52	87.28	87.26	87.25
Crude protein	23.97	23.97	23.97	23.95	23.95	23.94
Crude fiber	4.26	4.26	4.26	4.26	4.26	4.26
Ether extract	5.78	5.78	5.78	5.68	5.68	5.68
Ash	5.84	5.84	5.84	5.83	5.83	5.83
Metabolizable energy (kcal/kg)	2897.72	2897.23	2896.74	2882.36	2882.03	2881.70
Calcium	0.87	0.87	0.87	0.87	0.87	0.87
Available phosphorus	0.30	0.30	0.30	0.30	0.30	0.30
Methionine	0.56	0.56	0.56	0.56	0.56	0.56
Methionine + Cystine	0.95	0.95	0.95	0.95	0.95	0.95
Lysine	1.33	1.33	1.33	1.33	1.33	1.33

[1] Each 2.5 kg vitamin premix contained: 15000000 IU Vit. A, 3000000 IU Vit. D_3, 50000 mg Vit. E, 5000 mg Vit. K_3, 3000 mg Vit. B_1, 6000 mg Vit. B_2, 5000 mg Vit. B_6, 30 mg Vit. B_{12}, 50000 mg Vit. C, 25000 mg Niacin, 12000 mg Cal.D-Pantothenate, 75 mg D-Biotin, 1000 mg Folic Acid.

[2] Each 1 kg mineral premix contained: 80000 mg Mn, 60000 mg Fe, 60000 mg Zn, 5000 mg Cu, 1000 mg I, 200 mg Co, 150 mg Se, 200000 mg Choline Cloride %60.

The quails were allowed access to the feed and water *ad libitum* during the experimental period. The chemical composition of the diets was determined based on the methods of Association of Official Analytical Chemists (AOAC, 1997). During the experiment, the body weight and feed consumption of quails were recorded weekly. Body weight gain was determined by taking body weight differences between weeks.

Feed conversion ratio (feed consumed per 1 g of body weight gain) was calculated.

STATISTICAL ANALYSIS

Data were analysed by ANOVA using the GLM procedure with SAS 8 software. The differences among the means were tested using Duncan's multiple range tests. All statements of significance were based on a probability of $P<0.05$.

RESULTS AND DISCUSSIONS

Effects of bacteriocin and organic acids on growth performance of Japanese quails were given in Table 2. Bacteriocin supplementation slightly increased performance of quails. But, there were no statistically differences between body weight, body weight gain, feed consumption and feed conversion ratio in the groups ($P>0.05$). Studies investigated the effects of bacteriocin on performance of quails could not be found. However, there are many studies conducted with broilers and laying hens. Many studies reported positive effects of bacteriocin on performance of broilers (Wang et al., 2011; Jozefiak, 2013; Jothi 2012) and laying hens (Wang et al., 2014;Loh et al., 2014). Our results are in agreement with several reports indicating that bacteriocin addition had no effects on performance of broiler and laying hens (Ogunbanwo et al., 2004; Chen et al., 2012; Guo et al., 2012; Józefiak et al., 2012). Józefiak et al., (2011) reported that despite the liquid bacteriocin had no effect on performance of broilers, lyophilized non-encapsulated bacteriocin improved performance of broilers.

Table 2 Effects of bacteriocin and organic acids on growth performance of Japanese quails

	Diets	Week 1	Week 2	Week 3	Week 4	Week 5
Body Weight (g)	Control	22.85±1.53	63.12±7.19	102.51±6.00	138.69±5.09	169.45±5.95
	B 150	23.46±1.98	63.32±2.18	102.52±8.27	139.21±1.26	171.00±7.22
	B 300	23.64±2.59	63.47±1.27	102.68±3.76	139.12±4.34	170.43±3.58
	OA	23.93±2.30	63.81±9.54	102.47±5.28	139.10±3.36	170.39±6.40
	B 150+OA	23.17±0.93	63.52±2.26	102.13±2.33	138.81±1.58	170.18±3.67
	B 300+OA	23.12±2.55	63.96±1.89	103.67±3.74	141.28±3.29	171.60±4.80
Body Weight Gain (g/day/bird)	Control	2.11±0.22	5.76±1.12	5.63±0.60	5.17±0.39	4.40±0.24
	B 150	2.20±0.28	5.69±0.23	5.60±1.29	5.24±1.28	4.54±1.09
	B 300	2.23±0.37	5.69±0.39	5.60±0.52	5.21±0.31	4.48±0.31
	OA	2.27±0.33	5.70±1.25	5.52±1.02	5.23±0.48	4.47±0.59
	B 150+OA	2.16±0.13	5.76±0.33	5.52±0.51	5.24±0.39	4.48±0.43
	B 300+OA	2.15±0.37	5.83±0.47	5.67±0.60	5.37±0.22	4.33±0.10
Feed Consumption (g/day/bird)	Control	2.86±0.42	8.29±0.10	17.59±0.15	19.33±1.67	20.97±2.57
	B150	2.88±0.40	8.39±0.27	17.86±0.49	18.94±2.43	20.76±2.82
	B 300	2.86±0.53	8.41±0.24	17.67±0.43	18.76±3.25	20.75±3.79
	OA	2.85±0.27	8.36±0.21	17.65±0.57	18.34±4.37	20.74±1.87
	B 150+OA	2.82±0.20	8.27±0.10	17.73±0.63	18.61±2.77	20.87±2.78
	B 300+OA	2.66±0.24	8.22±0.20	17.62±0.40	18.42±0.58	20.64±1.18
Feed Conversion Ratio	Control	1.36±0.25	1.48±0.28	3.15±0.32	3.75±0.33	4.76±0.39
	B150	1.32±0.22	1.48±0.08	3.36±0.96	3.76±0.92	4.68±0.63
	B 300	1.30±0.27	1.48±0.12	3.18±0.34	3.62±0.68	4.67±1.02
	OA	1.28±0.23	1.54±0.43	3.28±0.54	3.53±0.91	4.69±0.61
	B 150+OA	1.31±0.13	1.44±0.08	3.23±0.28	3.55±0.48	4.70±0.82
	B 300+OA	1.26±0.24	1.41±0.10	3.13±0.29	3.43±0.21	4.76±0.23

Values are means ± standard deviation (SD).

Additionally, Józefiak et al., (2013) investigated the effects of bacteriocin supplementation at 100 IU, 300 IU, 900 IU and 2700 IU on broiler chicks and reported that only 900 IU and 2700 IU levels of bacteriocin positively affected the performance of broilers. In this study, organic acid supplementation did not affect the body weight, body weight gain, feed consumption and feed conversion ratio of quails ($P>0.05$). Similarly, several studies reported that organic acids had no effects on performance of quails (Abdel-Mageed, 2012;

Fazilat et al., 2014; Yusuf et al., 2015). Ocak et al., (2009) showed that addition of malic acid did not affect the feed efficiency. But, it increased body weight gain and feed consumption of quails. Another study reported that weight gains and feed efficiency of quails were improved with butyric acid supplementation. However, feed intake only numerically decreased with butyric acid (Salmanzadeh, 2013). Peyman et al., (2014) showed that organic acid supplementation improved body weight, body weight gain, feed intake and feed efficiency. Our results showed that combination of organic acids and bacteriocin had no effects on performance of quails. Similar results were observed by Çakır et al., (2008). They compared the effects of combined probiotic-prebiotic mixture and organic acid supplementation and reported that dietary treatments did not affect performance of quails. However, Ghosh et al., (2007) observed that performance of quails was improved with organic acid-prebiotic mixture.

CONCLUSIONS

In conclusion, single and combined dietary supplementation with bacteriocin and organic acid had no beneficial effects on quail performance in present study. However, performance characteristics numerically improved with dietary treatments. Various results have been observed in studies conducted with organic acids and bacteriocins in poultry. Thus, it is needed to more studies conducted under different conditions to understand the mode of action of bacteriocin and organic acid on performance of poultry.

REFERENCES

Abdel-Mageed M.A.A., 2012. Effect of using organic acids on performance of Japanese quail fed optimal and sub-optimal energy and protein levels 2.butyric acid. Egyptian Poultry Science Journal, 32(III): 625-644.

Alloui M.N., Szczurek W., Świątkiewicz S., 2013. The usefulness of prebiotics and probiotics in modern poultry nutrition: a review. Annals of Animal Science, 13(1): 17-32.

AOAC, 1997. Official methods of analysis. 16th ed. Association of Official Analytical Chemists, Washington, D.C.

Chen C.Y., Yu C., Chen S.W., Chen B.J., Wang H.T., 2012. Effect of yeast with bacteriocin from rumen bacteria on growthperformance, caecal flora, caecal fermentation and immunity function of broiler chicks. Journal of Agricultural Science, 151: 287-297.

Cigánková V., Lauková A., Guba P., Nemcová R., 2004. Effect of Enterocin a on the instantinal epithelium of Japanese Quails infected by *Salmonella* duesseldorf. Bulletin of the Veterinary Institute in Pulawy, 48: 25-27.

Cleveland J., Montville T.J., Nes I.F., Chikindas M.L., 2001. Bacteriocins, safe, natural, antimicrobials for food preservation. International Journal of Food Microbiology, 71: 1-20.

Çakır S., Midilli M., Erol H., Şimsek N., Çınar M., Altıntas A., Alp H., Altıntas L., Cengiz Ö, Antalyalı A., 2008. Use of combined probiotic-prebiotic, organicacid and avilamycin in diets of Japanesequails. Revue de Médecine Vétérinaire, 159(11): 565-569.

Dahiya J.P., Wilkie D.C., Van Kessel A.G., Drew M.D., 2006. Potential strategies for controlling necrotic enteritis in broiler chickens in post-antibiotic era. Animal Feed Science and Technology, 129: 60-88.

Dibner J.J., Buttin P., 2002. Use of organic acids as a model to study the impact of gut microflora on nutrition and metabolism. The Journal of Applied Poultry Research, 11: 453-463.

Dibner J.J., Richards J.D., 2005. Antibiotic growth promoters in agriculture: history and mode of action. Poultry Science, 84: 634-643.

Diez-Gonzalez F., 2007. Applications of bacteriocins in livestock. Current Issues in Instentinal Microbiology, 8: 15-24.

Fazilat H., Kheiri F., Faghani M., 2014. Effects of using commercial Globacid® acidifier supplementation on growth performance and some haematological parameters in Japanese quail (Coturnix japonica). Research Opinions In Animal & Veterinary Sciences, 4(11): 622-625.

Ghosh H.K., Halder G., Samanta G., Paul S.K., Pyne S.K., 2007. Effect of dietary supplementation of organic acid and mannan oligosaccharide on the performance and gut health of Japanese quail (*Coturnix coturnix japonica*). Asian Journal of Poultry Science 1(1): 1-7.

Gillor O., Nigro L.M., Riley M.A., 2005. Genetically engineered bacteriocins and their potential as the next generation of antimicrobials. Current Pharmaceutical Design, 11: 1067-1075.

Gillor O., Etzion A., Riley M.A., 2008. The dual role of bacteriocins as anti-and probiotics. Applied Microbiology and Biotechnology, 81: 591-606.

Guo Y., Jia G., Yu Z., Zhang R., 2012. Effects of bacteriocin-like substance on performances and egg quality of laying hens. Journal of Animal and Veterinary Advences, 11(13): 2276-2279.

Hermans D., Martel A., Van Deun K., Verlinden M., Van Immerseel F., Garmyn A., Messens W., Heyndrickx M., Haesebrouck F., Pasmans F., 2010. Intestinal mucus protects Camphylobacter jejuni in ceca of colonized broiler chickens against the bactericidal effects of medium-chain fatty acids. Poultry Science, 89: 1144-1155.

Housmand M., Azhar K., Zulkifli I., Bejo M.H., Kamyab A., 2011. Effects of nonantibiotic feed additives on performance, nutrient retention, gut pH, and intestinal morphology of broiler fed different levels of energy. The Journal of Applied Poultry Research, 20: 121-128.

Joerger R.D., 2003. Alternatives to antibiotics: bacteriocins, antimicrobial peptides and bacteriophages. Poultry Science, 82: 640-647.

Jothi V.V., Anandapandian K.T.K., Shankar, T., 2012. Bacteriocin production bacteria from curd and its field application to poultry. Archives of Applied Science Research, 4(1): 336-347.

Józefiak D., Kaczmarek S., Bochenek M., Rutkowski A., 2007. A note on effect of benzoic acid supplementation on the performance and microbiota population of broiler chickens. Journal of Animal and Feed Sciences, 16: 252-256.

Józefiak D., Sip A., Kaczmarek S., Rutkowski A., 2010. The effects of *Carnobacterium divergens* AS7 bacteriocin on gastrointestinal microflora *in vitro* and nutrient retention in broiler chickens. Journal of Animal and Feed Sciences, 19: 460-467.

Józefiak D., Sip A., Rawski M., Steiner T., Rutkowski A., 2011. The dose response effects of liquid and lyophilized *Carnobacterium divergens* AS7 bacteriocin on the nutrient retention and performance of broiler chickens. Journal of Animal and Feed Sciences, 20: 401-411.

Józefiak D., Sip A., Rutkowski A., Rawski M., Kaczmarek S., Wołuń-Cholewa M., Engberg R.M., Højberg O., 2012. Lyophilized *Carnobacterium divergens* AS7 bacteriocin preparation improves performance of broilerchickens challenged with *Clostridium perfringens*. Poultry Science 91:1899-1907.

Józefiak D., Kierończyk B., Juśkiewicz J., Zduńczyk Z., Rawski M., Długosz J., Sip A., Højberg O., 2013. Dietary nisin modulates the gastrointestinal microbial ecology and enhancesgrowth performance of the broiler chickens. PLoS ONE 8(12): e85347. doi:10.1371/journal.pone.0085347.

Kišiyadová S., Siroka P., Lauková A., 2003. Effects of nisin on two cultures of Rumen ciliates. Folia Microbiologica, 48(3): 408-412.

Klaenhammer T.R.,1993. Genetics of bacteriocins produced by lactic acid bacteria. FEMS Microbiology Reviews, 12: 39-85.

Li T., Tao J., Hong F., 2005. Study on the inhibition effect of nisin. The Journal of American Science, 1(2): 33-37.

Liem A., Pesti G.M., Edwards Jr. H.M., 2008. The effect of several organic acids on phytate phosphorus hydrolysis in broiler chicks. Poultry Science, 87: 689-693.

Loh T.C., Choe D.W., Foo H.L., Sazili A.Q., Bejo M.H., 2014. Effects of feeding different postbiotic metabolite combinations produced by *Lactobacillus plantarum* strains on egg quality and production performance, faecal parameters and plasma cholesterol in laying hens. BMC Veterinary Research, 10: 149.

Menconi A., Reginatto A.R., Londero A., Pumford N.R., Morgan M., Hargis B.M., Tellez G., 2013. Effect of organic acids on *Salmonella typhimurium* infection in broiler chickens. International Journal of Poultry Science, 12(2): 72-75.

Musikasang H., Sohsomboon N., Tani A., Maneerat S., 2012. Bacteriocin-producting lactic acid bacteria as a probiotic potential from Thai indigenous chickens. Czech Journal of Animal Science, 57(3): 137-149.

NRC, 1994. Nutrient requirements of poultry, 9. Revised Edition. National Research Council. National Acedemy Press, Washington, D. C.

Ocak N., Erener G., Altop A., Kop C., 2009. The effects of malic acid on performance and some digestive tract traits of Japanese quails. Journal of Poultry Science, 46: 25-29.

Ogunbanwo S.T., Sanni A.I., Onilude A.A., 2003. Characterization of bacteriocin produced by *Lactobacillus plantarum* F1 and *Lactobacillus brevis* OG1. African Journal of Biotechnology, 2(8): 219-227.

Ogunbanwo S.T., Sanni A.I., Onilude A.A., 2004. Influence of bacteriocin in the control of *Escherichia coli* infection of broiler chickensin Nigeria. World Journal of Microbiology & Biotechnology, 20: 51-56.

Peyman F., Yahya E., Habib A.S., Naser M.S., Alireza A., 2014. Effects of organic acids supplement on performance and gut parameters in male Japanese quail (Coturnix Coturnix). Biological Forum – An International Journal, 6(2): 127-134.

Ricke S.C., 2003. Perspectives on the use of organic acids and short chain fatty acids as antimicrobials. Poultry Science, 82: 632-639.

Rihakova J., Petit V.W., Demnerova K., Prevost H., Rebuffat S., Drider D., 2009. Insights into structure-activity relationships in the C-terminal region of divercin V41, a class IIa bacteriocin with high-level antilisterial activity. Applied and Environmental Microbiology, 75: 1811-1819.

Salmanzadeh M., 2013. Evaluation of dietary butyric acid supplementation on small intestinal morphology, performance and carcass traits of Japanese quails. Revue de Médecine Vétérinaire, 164(10): 481-485.

SAS, 1999. The SAS System SAS Institute Inc., Cary, NC, USA, Version 8 Copyright © 1999.

Shin M.S., Han S.K., Ji A.R., Kim K.S., Lee W.K., 2008. Isolation and characterization of bacteriocin-producing bacteria from the gastrointestinal tract of broiler chickens for probiotic use. Journal of Applied Microbiology, 105: 2203-2212.

Soltan M.A., 2008. Effect of dietary organic acid supplementation on egg production, egg quality and some blood serum parameters in laying hens. International Journal of Poultry Sciences 7(6): 613-621.

Stern N.J., Svetoch E.A., Eruslanov B.V., Perelygin V.V., Mitsevich E.V., Mitsevich I.P., Pokhilenko V.D., Levchuk V.P., Svetoch O.E., Seal B.S., 2006. Isolation of a Lactobacillus salivarius strain and purification of its bacteriocin, which is inhibitory to

Camphylobacter jejuni. Antimicrobial agents and Chemotherapy, 50(9): 3111-3116.

Świątkiewicz S., Arczewska-Wlosek A., 2012a. prebiotic fructans and organic acids as feed additives improving mineral availability. World's Poultry Science Journal, 68: 269-279.

Świątkiewicz S., Arczewska-Wlosek A., 2012b. Bone quality characteristics and performance in broiler chickens fed diets supplemented with organic acids. Czech Journal of Animal Science, 57(4): 193-205.

Tatsadjieu N.L., Njintang Y.N., Kemgang Sonfack T., Daoudou B., Mbofung C.M.F., 2009. Characterization of lactic acid bacteria producing bacteriocins against chicken *Salmonella enterica* and *Escherichia coli*. African Journal of Microbiology Research, 3(5): 220-227.

Wang H.T., Yu C., Hsieh Y.H., Chen S.W., Chen B.J., Chen C.Y., 2011. Effects of albusin B (bacteriocin) of Ruminococcus albus 7 expressed by yeast on growth performance and intestinal absorption of broiler chickens-its potential role as an alternative to feed antibiotics. Journal of the Science of Food and Agriculture. DOI 10.1002/jsfa.4463.

Wang H.T., Shin W.Y., Chen S.W., Wang S.Y., 2014. Effects of yeast with bacteriocin from rumen bacteria on laying performance, blood biochemistry, faecalmicrobiota and egg quality of of laying hens. Journal of Animal Physiology and Animal Nutrition. DOI: 10.1111/jpn.12262.

Yesilbag D., Colpan I., 2006. Effects of organic acid supplemented diets on growth performance, egg production and quality and on serum parameters in laying hens. Revue de Médecine Vétérinaire, 157(5): 280-284.

Yusuf M.S., Mahmoud M.M.A., Samy H.M., Ibrahim M.T., 2015. Effect of lactose, yeast and organic acids mixture supplementation on laying performance of Japanese quails (*Coturnix coturnix japonica*). Global Animal Science Journal, 2(1): 1233-1247.

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USAGE POSSIBILITIES OF MULBERRY LEAVES IN POULTRY NUTRITION

Ahmet Onder USTUNDAG, Mursel OZDOGAN

Adnan Menderes University Faculty of Agriculture, Department of Feed and Animal Nutrition, South Campus, Aydın, Turkey, Phone: +90256 772.70.23, Fax: +90256 772.72.33, E-mail: austundag@adu.edu.tr, mozdogan@adu.edu.tr

Corresponding author e-mail: austundag@adu.edu.tr

Abstract

Mulberry is a popular medicinal plant belongs to family Moraceae and genus Morus. Genus Morus (Mulberry) is an example that contains more than 150 species, Morusalba L. (White mulberry) is dominant specie among them. The leaves of mulberry are mainly used as food for the silkworms and they are some times eaten as vegetable or used as cattle fodder in different parts of the world. Mulberry leaves contain moisturizer from 71.13 to 76.68%, protein from 4.72 to 9.96%, fat from 0.64 to 1.51% and carbohydrates from 8.01 to 13.42%. While in dried mulberry leaves the moisture content decreases and it ranged from 5.11 to 7.24%, from 15.31 to 30.91% for protein, from 2.09 to 4.93% for fat and from 9.70 to 29.64% for carbohydrates. Also, they are very good source of ascorbic acid, β-carotene, antioxidant components, which includes rutin. Mulberry leaves are nontoxic natural therapeutic agents known to possess antidiabetic, antimicrobial, antimutagenic, antioxidant, anticancer, anxiolytic, anthelmintic, antistress, immunomodulatory, hypocholesterolemic, nephroprotective, hepatoprotectiveactivities. The purpose of this review is to explicate the usage possibilities of mulberry leaves in poultry nutrition by revealing the important Pharmacological activities.

Key words: *mulberry leaves, pharmacological activities, usage possibilities, poultry, nutrition.*

INTRODUCTION

Poultry industry is very important sector that provides the cheapest animal protein source for human consumption within the shortest production period. Poultry industry is highly dependent on the feed price because of feed costs have a major proportion ranging between 60-70% of poultry production costs. In view of these circumstances, alternative feed sources seeking instead of especially expensive protein sources like soybean meal and fish meal have accelerated in recent years. Mulberry leaves have a great potential as an alternative protein source for poultry industry due to rich protein, minerals, metabolizable energy contents and negligible anti-nutritional factors like tannic acid (Saddul et al., 2004; Srivastava et al., 2006; Al-Kirshi et al., 2010; Simol et al., 2012; Kamruzzaman et al., 2012; Olmo et al., 2012). Besides the nutritive value, mulberry leaves are nontoxic natural therapeutic agents known to possess antidiabetic, antimicrobial, antimutagenic, antioxidant, anticancer, anxiolytic, anthelmintic, antistress, immunomodulatory, hypocholesterolemic, nephroprotective, hepatoprotective activities (Yang et al., 2012; Devi et al., 2013). The purpose of this review is to explicate the usage possibilities of mulberry leaves in poultry nutrition by revealing the important pharmacological activities.

CLASSIFICATION OF MULBERRY

Mulberry belongs to the genus *Morus* contains 16 species family of Moraceae and 11 species are found in China. Genus Morus is one of such example that consists of over 150 species, among these *Morus alba* L. is dominant (Srivastava et al., 2006, Imran et al., 2010). Classification of mulberry has beenshown in Table 1.

Table 1 Classification of mulberry

Kingdom	Plantae – Plants
Subkingdom	Tracheobionta – Vascular plants
Superdivision	Spermatophyta – Seed plants
Division	Magnoliophyta – Flowering plants
Class	Magnoliopsida – Dicotyledons
Subclass	Hamamelididae
Order	Urticales
Family	Moraceae – Mulberry family
Genus	Morus L. – mulberry
Species	Morus alba L. – white mulberry

*USDA, NRCS. 2015. The PLANTS Database. (http://plants.usda.gov, 19January 2015)

NUTRITIONAL VALUE OF MULBERRY LEAVES

Mulberry leaves contain significant levels of protein with good amino acid profile, carbohydrates, fats, minerals, fibers, metabolizable energy and vitamins such as β-carotene and ascorbic acid (Saddul et al., 2004; Srivastava et al., 2006; Butt et al., 2008; Al-Kirshi et al., 2010). Srivastava et al. (2006) reported that fresh mulberry leaves contain 71.13-76.68% moisture, 4.72-9.96% crude protein, 4.26-5.32% total ash, 8.15-11.32% neutral detergent fiber (NDF), 0.64-1.51% crude fat, 8.01-13.42% carbohydrate, 69-86 kcal/100 g energy, 160-280 mg/100 g ascorbic acid, 10.000-14.688 µg/100 gβ-carotene, 4.70-10.36 mg/100 g iron, 0.22-1.12 mg/100g zincand 380-786 mg/100 g calcium. Also it was reported that dried mulberry leaves contain 5.11-7.24% moisture, 15.31-30.91% crude protein, 14.59-17.24% total ash, 27.60-36.66% NDF, 2.09-4.93% crude fat, 9.70-29.64% carbohydrate, 113-224 kcal/100 g energy, 100-200 mg/100 g ascorbic acid and 8438-13.125 µg/100 gβ-carotene, 19.00-35.72 mg/100 g iron, 0.72-3.65 mg/100 g zincand 786.66-2226.66 mg/100 g calcium. Composition of mulberryleaves is summarized in Table 2.

Table 2 Composition of MulberryLeaves (Dryweightbasis)

Mouisture, %	5.11-10.75
Crude protein, %	15.31 - 30.91
Crudefat, %	2.09 - 6.90
Crude fiber, %	9.9 - 13.85
Total ash, %	8.91 - 11.81
Carbohydrates, %	9.70 - 39.70
AcidDetergent Fiber (ADF), %	17.33 - 28.00
NeutralDetergent Fiber (NDF), %	19.38 - 35.77
AcidDetergent Lignin (ADL), %	3.4 - 8.10
Hemicellulose, %	2.5 - 12.80
Ascorbicacid, mg/100 g	100 - 200
β-carotene, mg/100 g	8.44 - 13.13
Iron, mg/100 g	19.00 - 35.72
Zinc, mg/100 g	0.72 - 3.65
Calcium, mg/100 g	786.66 - 2226.66
Phosphorus, mg/100 g	970
Magnesium, mg/100 g	720
AntinutritionalFactors	
Oxalates, mg/100 g	183
Phytates, mg/100 g	156
Tannicacid, %	0.13 - 0.36

*Yen et al., 1996; Saddul et al., 2004; Srivastava et al., 2006; Butt et al., 2008; Lin and Lai, 2009; Guven, 2012; Iqbal et al., 2012; Al-Kirshi et al., 2013.

PHYTOCHEMISTRY OF MULBERRY

Mulberry contains various phytochemicals such as alkaloids, anthocyanins, flavonoids, saponins, stilbenes, triterpens (lupeol), sterols (β-Sitosterol), coumarinsand phenolic acids (Zhishen et al., 1999;Omidiran et al., 2012; Thabti et al., 2012; Ahmad et al., 2013; Chen et al., 2013; Devi et al., 2013; Lakshmi et al., 2013; Ramesh et al., 2014). 1-deoxynojirimycin (DNJ), an alkaloid component found in leaves (Oku et al., 2006; Nuengchamnong et al., 2007; Nakagawa et al., 2010). The predominant anthocyanins in mulberry are cyanidin 3-rutinoside and cyanidin 3-glucoside (Du et al., 2008; Sarikaphuti et al., 2013). Resveratrol, Oxyresveratrol and Mulberroside A are stilbenes found in mulberry (Chung et al., 2003; Song et al., 2009; Zhou et al., 2013; Ramesh et al., 2014). Identified major phenolic acids in the mulberry leaves are chlorogenic acid, caffeic acid, vanillic acid, p-hydroxybenzoic acid, p-coumaric acid, sinapic acid, protocatechuic acid and ferulic acid (Memon et al., 2010; Radojković et al., 2012; Flaczyk et al., 2013). Flavonoids exist widely in the plants. Mulberry leaves contain rutin, izoquercitrin (quercetin 3-β-D-glucoside), quercetin-3-O-glucoside, quercetin-3-O-rhamnoside-7-O-glucoside, quercetin-3,7-D-O-β-D-glucopyranoside, quercetin-3-O-(6-malonyl)-β-D-glucopyranoside, quercetin-3-O-glucoside-7-O-rhamnoside, kaempferol-7-O-glucoside, kaempferol-3-O-glucopyranosyl-(1,6)-β-D-glucopyranoside (Astragalin), kaempferol-3-O-(6-malonyl) glucoside (Kim et al., 1999; Katsube et al., 2006; Katsube et al., 2009; Song et al., 2009; Flaczyk et al., 2013; Thabti et al., 2012). Also, Yang et al. (2011) isolated new arylbenzofuran, 3',5'-dihydroxy-6-methoxy-7-prenyl-2-arylbenzo-furan from *Morus alba var. multicaulis* Perro. (Moraceae) white and a total of 89 Diels-Alder-type adducts have been isolated from Chinese Morus plants (Yang et al., 2014).

PHARMACOLOGICAL PROPERTIES OF MULBERRY

Various pharmocological activities such as antimicrobial, antioxidant, antidiabetic, hypocholesterolemic, hepatoprotective activity and immunomodulatory activity of mulberry have been reported.

ANTIMICROBIAL ACTIVITY

Mulberry shows strong antimicrobial activity against pathogens due to contains substances like kuwanon C, mulberrofuran G, mourin and albanol B (Park et al., 2003; Sohn et al., 2004; Yang and Lee, 2012). Previous studies conducted in vitro and in vivo shown that various fractions of mulberry had antimicrobial effect against *Staphylococcus aureus, B. subtilis, B. cereus, Escherichia coli, Streptococcus faecalis, Mycobacterium smegmatis, Streptococcus mutans, Streptococcus sobrinus, Streptococcus sanguis, Porpyromonas gingivalis, A. tamari, P. vulgaricus, Pseudomonas aeruginosa, A. niger, F. oxysporum, P. oxalicum,* and some mold species (Ayoola et al., 2011; Manjula and Shubha, 2011; Omidiran et al., 2012; Anis et al., 2012; Kostić et al., 2013; Salem et al., 2013).

ANTIOXIDANT ACTIVITY

There are many methods used to evaluate the antioxidant activities of biological samples including DPPH (1,1-diphenyl-2-picrylhydrazyl Scavenging Activity), ABTS[2,2'-azinobis-(3-ethylbenzthiazoline-6-sulphonic acid) radical cation scavenging capacity], FRAP (Ferric Ion Reducing Antioxidant Power), SSA (Superoxide Radical Scavenging Activity) and HSA (Hydroxyl Radical Scavenging Activity) (Imran et al., 2010; Zou et al., 2012; Iqbal et al., 2012). Mulberry is a good source of polyphenolic compounds especially flavanoids and among the flavanoids quercetin 3-(6-malonylglucoside) is most important for antioxidant potential (Katsube et al., 2006; Butt et al., 2008). A strong correlation between free radical scavenging and the phenolic contents has been reported for mulberry (Yen et al., 1996; Zhishen et al., 1999; Enkhmaa et al., 2005; Bae and Suh, 2007; Arabshahi-Delouee and Urooj, 2007; Imran et al., 2010; Radojković et al., 2012; Zou et al., 2012; Chao et al., 2013; Flaczyk et al., 2013)

ANTIDIABETIC ACTIVITY

Diabetes in general is a syndrome characterized by high blood glucose level and altered insulin metabolism (Butt et al., 2008). 1-deoxynojirimycin (DNJ) and its derivatives isolated from mulberryhave significant α-glycosidase inhibitors activity and therefore suppress the response of both blood glucose and insulin secretion resulting in a decrease of blood glucose level (Oku et al., 2006; Nuengchamnong et al., 2007; Nakagawa et al., 2010; Sarikaphuti et al., 2013). Results of studies conducted in diabetic human and mice indicated that mulberry decreased the blood glucose level (Kimura et al., 2007; Park et al., 2009; El-Sayyad et al., 2011; Nakamura et al., 2011; Mohammad and Naik, 2012; Banu et al., 2014).

HYPOCHOLESTROLEMIC AND ANTIATHEROGENIC ACTIVITY

Hyperlipidemia is lipid metabolism disorder characterized as high level serum triglyceride and cholesterol (Liu et al., 2009). High triglyceride and cholesterol levelshave been identified as a risk factor for atherosclerosis and cronary heart disease (hypotriglycemic). Although low high density protein (HDL) and oxidative modification of low density lipoprotein (LDL) are associated with increased cronary artery disease (Toth, 2004; Enkhmaa et al., 2005; Liu et al., 2009). Many studies indicated that flavonoids and anthocyanins contents in mulberry help to prevent atherosclerosis and cronary heart disease via scavenging the radicals, inhibition LDL oxidation and decreasing blood triglyceride and cholesterol levels (Zhishen et al., 1999; Chen et al., 2005; Enkhmaa et al., 2005; Katsube et al., 2006; Du et al.,2008; Liu et al., 2009; Yang et al., 2010; Zeni and Molin, 2010; Valacchi et al., 2014).

HEPATOPROTECTIVE ACTIVITY

The liver is the major organ controlling all the biochemical pathways and hepatotoxins such as aflatoxin impair the liver function (Muhammad et al., 2012). Mulberrycontains flavonoids, coumarine and stilbene that possess hepatoprotective activity (Oh et al., 2002). It was reported that mulberry had hepatoprotective potential against hepatotoxicity induced by carbon tetrachloride (CCL_4) (Zeni and Molin, 2010; Hogade et al., 2010; Hussein et al., 2010)

IMMUNOMODULATORY ACTIVITY

Immune system is the main regulatory system controlling homeostasis of the body and has animportant role in the progression of entire life from birth to death (Awais and Akhtar, 2012). Different methods such as clearance test, cyclophosphamide induced neutropenia, neutrophil adhesion test, effect on serum immunoglobulins, mice lethality test and indirect haemagglutination testare used for evaluate to effects of mullberry on the immun system (Devi et al., 2013; Sharma et al., 2013). Kim et al. (2000) reported that polysaccharide isolated from mulberry had immunomodulatory activity. Also other studies indicated that aqueous and methanolic extracts of mulberry leaves increased serum immunoglobulin levels and decreased mortality rate (Venkatachalam et al., 2009; Bharani et al., 2010; Hou et al., 2011).

USE OF MULBERRY LEAVES IN POULTRY NUTRITION

Although mulberry leaves generallyuse to feed the silkworms,many researchers have studied it as an alternative food source for animals due to the high fiber content (Saurabh Bajpai et al., 2012; Simol et al., 2012; Sujathamma et al., 2013; Vijeyan et al., 2014). Several studies have shown that mulberry leaves can be used to nutrition of cattle (Saddul et al., 2005; Vu et al., 2011; Huyen et al., 2012; Tan et al., 2012; Zhou et al., 2012), sheep (Liu et al., 2001; Tudaro et al., 2007; Yulistiani et al., 2008; Kandylis et al., 2009), goats (Omar et al., 1999; Azim et al., 2002; Kouch et al., 2003), rabbits (Deshmukh et al., 1993; Prasad et al., 2003; Bamikole et al., 2005) and fish (Mondal et al., 2012; Sheikhlar et al., 2014). Mulberry leaves powder have also been used to feed poultry (Simol et al., 2012). Digestibility of mulberry leaves is very high by ruminants (Saddul et al., 2005; Todaro et al., 2007; Huyen et al., 2012). However, digestibility of mulberry leaves dry matter is poor (35-37%) by poultry due to the high neutral detergent fiber (NDF) content. Despite poor utilization of mulberry leaves dry matter, crude protein and ether extract are highly digested (73% and 88%, respectively) by poultry (Al-Kirshi et al., 2013). Therefore, various studies were conducted to assess the effects different levels of mulberry leaves powder on performance of broilers (Mulla et al., 2003; Chowdary et al., 2009; Olmo et al., 2012; Simol et al., 2012; Has et al., 2013; Panja, 2013; Islam et al., 2014), layers (Lokaewmanee et al., 2009; Al-Kirshi et al., 2010; Kamruzzaman et al., 2012; Olteanu et al., 2012; Panja, 2013) and quails (Hermana et al., 2014). Different results were observed in studies conducted with broilers. Mulla et al. (2003) reported that broiler performance was negatively affected by supplementation of mulberry leaf meal at 2% of diet. Olmo et al. (2012) and Has et al. (2013) observed similar results with addition of mulberry leaf meal at 10, 20 and 30 % of diet. Panja (2013) showed that there was no significant improvement of body weight gain, feed intake and feed conversion ratio (FCR) in broilers supplemented with mulberry leaves at 0, 0.5, 1.0, 1.5 and 2.0 % of diet. However, Islam et al. (2014) observed that supplementation of mulberry leaf meal between 2.5 and 3.5% significantly improved the broiler performance and decreased serum total cholesterol and triglyceride levels. Similarly, it was reported that the highest body weight was observed in 10% mulberry leaf meal addition (Chowdary et al. 2009). Additionally, Simol et al. (2012) reported that mulberry leaf addition up to 30% decreased starter and grower feed cost (24.82 and 26.09%, respectively) without any adversely effect.

The results of studies conducted with layers and quails indicated that mulberry leaves supplementation up to 10% did not affect the productive performance and egg quality. Also mulberry leaves decreased yolk cholesterol and increased pigmentation of egg yolk (Lokaewmanee et al., 2009; Al-Kirshi et al., 2010; Kamruzzaman et al., 2012; Olteanu et al., 2012; Panja, 2013; Hermana et al., 2014).

CONCLUSIONS

Reducing the feed prices which make up the majority of production costs plays key role for the poultry industry. In this context, mulberry leaves have a great potential. Mulberry leaves can be used instead of expensive protein

sources such as soybean meal and fish meal used in poultry diets in limited levels.

Using mulberry leaves as an alternative protein source instead of expensive protein sources like soybean meal and fish meal in poultry diets plays an important role for poultry industry due to it reduces feed costs. Studies conducted with poultry indicated that addition of mulberry leaves are possible by up to 10% in poultry diets without any adversely effect on performance of poultry.

REFERENCES

Ahmad, A., Gupta, G., Afzal, M., Kazmi, I., Anwar, F. 2013. Antiulcer and antioxidant activities of a new steroid from Morus alba. Life Sciences, 92:202-210.

Al-Kirshi, R.A., Alimon, A.R., Zulkifli, I., Sazili, A.Q., Zahari, W.M., Ivan, M., 2010. Utilization of mulberry leaf meal (Morus alba) as protein supplement in diets for laying hens. Italy Journal of Animal Science, 9: e51.

Al-Kirshi, R.A., Alimon, A., Zulkifli, I., Atefah, S., Wan Zahari, M., Ivan, M. 2013. Nutrirional digestibility of mulberry leaves (Morus alba).Italian Journal of Animal Science, volume 12:e36.

Anis, S., Bhargava, T., Upadhyay, H. 2012. A review on phytotherapy by Morus Alba. International Journal of Pharmaceutical and Chemical Sciences, 1(4): 1563-1566.

Arabshahi-Delouee, S., Urooj, A. 2007. Antioxidant properties of various solvent extractsof mulberry (Morus indica L.) leaves. Food Chemistry, 102:1233-1240.

Awais, M.M., Akhtar, M. 2012. Evaluation of some sugarcane (*Saccharum officinarum* L.) extracts for immunostimulatory and growth promoting effects in industrial broiler chickens. Pakistan Veterinary Journal, 32(3): 398-402.

Ayoola, O.A., Baiyewu, R.A., Ekunola, J.N., Olajire, B.A., Egunjobi, J.A., Ayeni, E.O., Ayodele, O.O. 2011. Phytoconstituent screening and antimicrobial principles of leaf extracts of two variants of Morus alba (S_{30} and S_{54}). African Journal of Pharmacy and Pharmocology, 5(19): 2161-2165.

Azim, A., Khan, A.G., Ahmad, J., Ayaz, M., Mirza, I.H. 2002. Nutritional evaluation of fodder tree leaves with goats. Asian-Australasian Journal of Animal Sciences, 15(1): 34-37.

Bae, S.H., Suh, H.J. 2007. Antioxidant activities of five different mulberry cultivars in Korea. LWT- Food Science and Technology, 40: 955-962.

Bamikole, M.A., Ikhatua, M.I., Ikhatua, U.J., Ezenwa, I.V. 2005. Nutritive value of mulberry (*Morus* Spp.) leaves in the growing rabbits in Nigeria. Pakistan Journal of Nutrition, 4(4): 231-236.

Banu, S., Jabir, N.R., Manjunath, N.C., Khan, M.S., Ashraf, G. Md., Kamal, M.A., Tebrez, S. 2014. Journal of Biological Sciences, http://dx.doi.org/10.1016/j.sjbs.2014.04.005.

Bharani, S.E.R., Asad, M., Dhamanigi, S.S., Chandrakala, G.K. 2010. Immunomodulatory activity of methanolic extract of *Morus alba* Linn. (Mulberry) leaves. Pakistan Journal of Pharmaceutical Sciences, 23(1): 63-68.

Butt, M.S., Nazir, A., Sultan, T.S., Schroën, K. 2008. Morus alba L. nature's functional tonic. Trends in Food Science & Technology 19: 505-512.

Chao, P.Y., Lin, K.H., Chiu, C.C., Yang, Y.Y., Huang, M.Y., Yang, C.M. 2013. Inhibitive effects of mulberry leaf-related extracts on cell adhesion and imflammatory response in human aortic endothelial cells. Evidence-Based Complementary and Alternative Medicine, Volume 2013, Article ID 267217, 14 pages.

Chen, C.C., Liu, L.K., Hsu, J.D., Huang, H.P., Yang, M.Y., Wang, H.Y. 2005. Mulberry extract inhibits the development of atherosclerosis in cholesterol-fed rabbits. Food Chemistry, 91: 601-607.

Chen, Y.C., Tien, Y.J., Chen, C.H., Beltran, F.N., Amor, A.C., Wang, R.J., Wu, D.J., Mettling, C., Lin, Y.L., Yang, W.C. 2013. Morus alba and active compound oxyresveratrolexert anti-inflammatory activity via inhibition ofleukocyte migration involving MEK/ERK signaling. BMC Complementary and Alternative Medicine, 13:45.

Chowdary, N.B., Rajan, M.V., Dandin, S.B. 2009. Effect of poultry feed supplemented with mulberry leaf powder on growth and development of broiler. The IUP Journal of Life Sciences, 3(3): 51-54.

Chung, K.O., Kim, B.Y., Lee, M.H., Kim, Y.R., Chung, H.Y., Park, J.H., Moon, J.O. 2003. In-vitro and in-vivo anti-inflammatory effect of oxyresveratrol frol Morus alba L. Journal of Pharmacy and Pharmacology, 55: 1695-1700.

Deshmukh, S.V., Patnak, N.N., Takalikar, D.A., Digraskar, S.U. 1993. Nutritional effect of mulberry (*Morus alba*) leaves as sole ration of adult rabbits. World Rabbit Science, 1(2): 67-69.

Devi, B., Sharma, N., Kumar, D., Jeet, K. 2013. Morus alba linn: A phytopharmacological review. International Journal of Pharmacy and Pharmaceutical Sciences, 5(2): 14-18.

Du, Q., Zheng, J., Xu, Y. 2008. Composition of anthocyanins in mulberry and their antioxidant activity. Journal of Food Composition and Analysis, 21: 390-395.

El-Sayyad, H.I.H., El-Sherbiny, M.A., Sobh, M.A., Abou-El-Naga, A.M., Ibrahim, M.A.N., Mousa, S.A. 2011. Protective effects of *Morus alba* leaves extract on ocular functions of pups from diabetic and hypercholestrolemic mother rats. International Journal of Biological Sciences, 7(6): 715-728.

Enkhmaa, B., Shiwaku, K., Katsube, T., Kitajima, K., Anuurad, E., Yamasaki, M., Yamane, Y. 2005. Mulberry (*Morus alba* L.) leaves and their major flavonol quercetin 3-(6-malonylglucoside) attenuate atherosclerotic lesion development in LDL receptor-deficient mice. Journal of Nutrition, 135: 729-734.

Flaczyk, E., Kobus Cisowska, J., Przeor, M., Korczak, J., Remiszewski, M., Korbas, E., Buchowski, M. 2013. Chemical characterization and oxidative properties of Polish variety of Morus alba L. leaf aqueous extracts from the labrotory and pilot-scale processes. Agricultural Sciences, 4(5B): 141-147.

Freddie Simol, C., Alek Tuen, A., Hazid Ahmad Khan, H., Chubo, J.K., King, P.J.H., Ong, K.H. 2012. Performance of chicken broilers fed with diets substitued with mulberry leaf powder. African Journal of Biotechnology, Vol. 11(94): 16106-16111.

Guven, I. 2012. Efect of species on nutritive value of mulberry leaves. Kafkas Üniversitesi Veteriner Fakültesi Dergisi, 18(5): 865-869.

Has, H., Yunianto, V.D., Sukamto, B., 2013. The effectivity of fermented mulberry leaves with Rumen liquor as broiler feed on finel body weight, dry matter and crude fiber digestibility, and metabolic energy. Animal Production, 15(3): 173-179.

Hermana, W., Toharmat, T., Sumiati, Manalu, W. 2014. Performances and egg quality of quail offered feed containing sterol from katuk (*Sauropus androgynus*) and mulberry (*Morus alba*) leaf meal. International Journal of Poultry Science, 13(3): 168-172.

Hogade, M.G., Patil, K.S., Wadgar, G.H., Mathapati, S.S., Dhumal, P.B. 2010. Hepatoprotective activity of *Morus alba* (Linn.) leaves extract against carbon tetrachloride induced hepatotoxicity in rats. African Journal of Pharmacy and Pharmacology, 4(10): 731-734.

Hou, R.H., Liao, S.T., Liu F., Zou, Y.X., Deng, Y.Y. 2011. Immunomodulatory effect of polysaccharides from mulberry leaves (PML) in mice. Journal of Food Science, 32(13): 280-283.

Hussein, M.Sh., El-Tawil, O.S., Yassin, N.E.H., Abdou, K.A. 2010. The protective effect of *Morus alba* and *Calendula officinalis* plant extracts on carbon tetrachloride-induced hepatotoxicity in isolated rat hepatocytes. Journal of American Science, 6(10): 762-773.

Huyen, N.T., Wanapat, M., Navanukraw, C. 2012. Effect of mulberry leaf pellet (MUP)supplementation on rumen fermentation and nutrient digestibility in beef cattle fed onrice straw-based diets. Animal Feed Science and Technology, 175:8-15.

Imran, M., Khan, H., Shan, M., Khan, R., Khan, F. 2010. Chemical composition and antioxidant activity of certain Morus species. Journal of Zhejiang University-SCIENCE B (Biomedicine & Biotechnology), 11(12): 973-980.

Iqbal, S., Younas, U., Sirajuddin, Chan, K.W., Sarfraz, R.A., Uddin, Md. K. Proximate composition and antioxidant potential of leaves from three varieties of mulberry (Morus sp.): A comparative study. International Journal of Molecular Science, 13: 6651-6664.

Islam, M.R., Siddiqui, M.N., Khatun, A., Siddiky, M.N.A., Rahman, M.Z., Bostami, A.B.M.R., Selim, A.S.M. 2014. Dietary effect of mulberry leaf (Morus alba) meal on growth performance and serum cholesterol level of broiler chickens. SAARC Journal of Agriculture, 12(2): 79-89.

Kamruzzaman, M.d., Rahman, M.S., Asaduzzaman, M.d., Zaminur Rahman, M.d., 2012. Significant effect of mulberry leaf (Morus alba) meal in the reduction of egg-yolk cholesterol. Bangladesh Research Publications Journal, 7(2): 153-160.

Kandylis, K., Hadjigeorgiou, I., Harizanis, P. 2009. The nutritive value of mulberry leaves (*Morus alba*) as a feed supplement for sheep. Tropical Health and Production. 41: 17-24.

Katsube, T., Imawaka, N., Kawano, Y., Yamazaki, Y., Shiwaku, K., Yamane, Y. 2006. Antioxidant flavonol glycosides in mulberry (Morus alba L.) leaves isolated based on LDL antioxidant activity. Food Chemistry, 97: 25-31.

Katsube, T., Tsurunaga, Y., Sugiyama, M., Furuno, T., Yamasaki, Y. 2009. Effect of air-drying temperature on antioxidant capacity and stability of polyphenolic compounds in mulberry (Morus alba L.) leaves. Food Chemistry, 113: 964-969.

Kim, S.Y., Gao, J.J., Lee, W.C., Ryu, K.S., Lee, K.R., Kim, Y.C. 1999. Antioxidative flavonoids from the leaves of Morus alba. Archives of Pharmacal Research, 122: 81-85.

Kim, H.M., Hart, S.B., Lee, K.H., Lee, C.W., Kim, C.Y., Lee, E.J. 2000. Immunomodulating activity of a polysaccharide isolated from Mori Cortex Redicis. Archives of Pharmacal Research, 23(3): 240-242.

Kimura, T., Nakagawa, K., Kubota, H., Kojima, Y., Goto, Y., Yamagishi, K. 2007. Food-grade mulberry powder enriched with 1-deoxynojirimycin suppresses the elevation of postprandial blood glucose in humans. Journal of Agricultural and Food Chemistry, 55: 5869-5874.

Kostić, D.A, Dimitrijević, D.S., Mitić, S.S., Mitić, M.N., Stojanović, G.S., Zivanović, A.V. 2013. A survey on macro- and micro-elements, phenolic compounds, biological activity and use of Morus spp. (Moraceae). Fruits, 68: 333-347.

Kouch, T., Preston, T.R., Ly, J. 2003. Studies on utilization of trees and shrubs as the sole feedstuff by growing goats; foliage preferences and nutrient utilization. Livestock Research for Rural Development, 15(7).

Lakshmi, P., Ganapaty, S., Bharathi, K.M., 2013. Chemical and biological examination of leaves of morus indica. International Reseach Journal of Pharmacy, 4(5): 173-177.

Lin, H.Y. and Lai, L.S. 2009. Isolation and viscometric characterization of hydrocolloidsfrom mulberry (Morus alba L.) leaves. Food Hydrocolloids, 23: 840-848.

Liu, J.X., Yao, J., Yan, B., Yu, J.Q., Shi, Z.Q. 2001. Effects of mulberry leaves to replace rapeseed meal on performance of sheep feding on ammoniated rice straw diet. Small Ruminant Research, 39: 131-136.

Liu, L.K., Chou, F.P., Chen, Y.C., Chyau, C.C., Ho, H.H., Wang, C.J. 2009. Effects of mulberry (Morus alba L.) extracts on lipid homeostasis in vitro and in

vivo. Journal of Agricultural and Food Chemistry, 57: 7605-7611.

Lokaewmanee, K., Mompanuon, S., Khumpeerawat, P., Yamauchi, K. 2009. Effects of dietary mulberry leaves (*Morus alba* L.) on egg yolk color. Journal of Poultry Science, 46: 112-115.

Manjula, A.C., Shubha. 2011. Screening of antibacterial activity of total soluble protein of mulberry varieties. International Journal of Current Pharmaceutical Research, 3(2): 60-61.

Memon, A.A., Memon, N., Luthria D.L., Bhanger, M.I., Pitafi, A.A. 2010. Phenolic acids profiling and antioxidant potential of mulberry (Morus laevigata W., Morus nigra L., Morus alba L.) leaves and fruits grown in Pakistan. Polish Journal of Food and Nutrition Sciences,60: 25-32.

Mohammad, J., Naik, P.R. 2012. The histopathologic effects of Morus alba leaf extract on the pancreas of diabetic rats. Turkish Journal of Biology. 36: 211-216.

Mondal, K., Kaviraj, A., Mukhopadhyay, P.K. 2012. Effects of partial replacement of fishmeal in the diet by mulberry leaf meal on growth performance and digestivie enzyme activites of İndian minor carp *Labeo bata*. International Journal of Aquatic Science, 3(1): 72-83.

Muhammad, D., Chand, N., Khan, S., Sultan, A., Mushtaq, M., Rafiullah. 2012. Hepatoprotective role of milk thistle (*Silybum marianum*) in meat type chicken fed aflatoxin B_1 contaminated feed. Pakistan Veterinary Journal, 32(3): 443-446.

Mulla, J., Shivakumar, M.C., Naik, D.G. 2003. Effect of feding different leaf meal on performance and carcass characteristics of broiler. Karnataka Journal of Agricultural Sciences, 16(2): 288-290.

Nakagawa, K., Ogawa, K., Higuchi, O., Kimura, T., Miyazawa, T., Hori, M. 2010. Determination of iminosugars in mulberry leaves and silkworms usinghydrophilic interaction chromatography–tandem mass spectrometry. Analytical Biochemistry 404: 217-222.

Nakamura, S., Hashiguchi, M., Yamaguchi, Y., Oku, T. hypoglycemic effects of Morus alba leaf extract on postprandial glucose and insulin levels in patients with type 2 diabetes treated with sulfonylurea hypoglycemic agents. Journal of Diabetes and Metabolism, 2: 9.

Nuengchamnong, N., Ingkaninan, K., Kaewruang, W., Wongareonwanakij, S., Hongthongdaeng, B. 2007. Quantitative determination of 1-deoxynojirimycin in mulberryleaves using liquid chromatography–tandem mass spectrometry. Journal of Pharmaceutical and Biomedical Analysis 44: 853-858.

Oh, H., Ko, E.K., Jun, J.Y., Oh, M.H., Park, S.U., Kang, K.H. 2002. Hepatoprotective and free radical scavenging activities of prenylflavonoids coumarin and stilbene from *Morus alba*. Planta Medica, 68: 932-934.

Oku, T., Yamada, M., Nakamura, M., Sadamori, N., Nakamura, S. 2006. Inhibitory effects of extractives from leaves of Morus alba on humanand rat small intestinal disaccharidase activity. British Journal of Nutrition, 95: 933-938.

Olmo, C.,Martínez, Y., León, E., Leyva, L., Nuñez, M., Rodríguez, R., Labrada, A., Isert, M., Betancur, C., Merlos, M., Liu, G., 2012. Effect of mulberry foliage (Morus alba) meal on growth performance and edible portions in hybrid chickens. International Journal of Animal and Veterinary Advences, 4(4): 263-268.

Olteanu, M., Panaite, T., Ciurescu, G., Criste, R.D., 2012. Effect of dietary mulberry leaves on performance parameters and nutrient digestibility of layin hens. Indian Journal of Animal Science, 82(8).

Omar, S.S., Shayo, C.M., Udén, P. 1999. Voluntary intake and digestibility of mulberry (*Morus alba*) diets by growing goats. Torpical Grasslands, 33: 177-181.

Omidiran, M.O., Baiyewu, R.A., Ademola, I.T., Fakorede, O.C., Toyinbo, E.O., Adewumi, O.J., Adekunle, E.A., 2012. Phytochemical analysis, nutritional composition and antimicrobial activities of White mulberry (Morus alba). Pakistan Journal of Nutrition, 11(5): 456-460.

Panja, P., 2013. The effects of dietary mulberry leaves (Morus alba L.) on chicken performance, carcass, egg quality and cholesterol content of meat and egg. Walailak Journal of Science and Technology, 10(2): 121-129.

Park, J.M., Bong, H.Y., Jeong, H.I., Kim, Y.K., Kim, J.Y., Kwon, O., 2009. Postprandial hypoglycemic efffect of mulberry leaf in Goto-Kakizaki rat and counterpart control Wistar rats. Nutrition Research and Practise, 3(4): 272-278.

Park, K.M., You, J.S., Lee, H.Y., Baek, N.I., Hwang, J.K., 2003. Kuwanon G: an antibacterial agent from the root bark of Morusalba against oral pathogens. Journal of Ethnopharmacology, 84: 181-185.

Prasad, R., Misra, A.K., Sankhyan, S.K., Mishra, A.S., Tripathi, M.K., Karim, S.A., Jakhmola, R.C., 2003. Growth performance and cecal fermentation in growing rabbits fed on diets containing graded levels of mulberry (*Morus alba*) leaves. Asian-Australasian Journal of Animal Sciences, 16(9): 1309-1314.

Radojković, M.M., Zeković, Z.P., Vidović, S.S., Kočar, D.D., Mašković, P.Z., 2012. Free radical scavenging activity and total phenolic and flavonoid content of mulberry (Morus spp. L.,*Moraceae*) extracts. Hemijska Industrija, 66(4): 547-552.

Ramesh, H.L., Sivaram, V., Yogananda Murthy, V.N., 2014. Antioxidant and medicinal properties of mulberry (Morus sp.): A review. World Journal of Pharmaceutical Research, 3(6): 320-343.

Saddul, D., Jelan, Z.A., Liang, J.B., Halim, R.A., 2004. The potential of mulberry (Morus alba) as a fodder crop: The effect of plant maturity on yield, persistence and nutrient composition of plant fractions. Asian-Australasian Journal of Animal Science, 17(12): 1657-1662.

Saddul, D., Jelan, Z.A., Liang, J.B., Halim, R.A., 2005. Evaluation of mulberry (Morus alba) as potential feed supplement for ruminants: The effect of plant maturity on in situ disappearance and in vitro

intestinal digestibility of plant fractions.Asian-Australasian Journal of Animal Science, 18(11): 1569-1574.

Salem, M.Z.M., Aly, H., Gohar, Y., El-Sayed, A.W., 2013. Biological activity of extracts from *Morus alba* L., *Albizzia lebbeck* (L.) Benth. and *Casuarina glauca* Sieber against the growth of some pathogenic bacteria. International Journal of Agricultural and Food Research, 2(1): 9-22.

Sarikaphuti, A., Nararatwanchai, T., Hashiguchi, T., Ito, T., Thaworanunta, S., Kikuchi, K., Oyama, Y., Maruyama, I., Tancharoen, S., 2013. Preventive effects of Morus alba L. anthocyanins on diabetes in Zucker diabetic fatty rats. Experimental and Therapeutic Medicine, 6: 689-695.

Saurabh Bajpai, BhaskaraRao, A.V., Muthukumaran, M., Nagalakshmamma, K., 2012. History and active pharmacokinetic principles of mulberry: A review. IOSR Journal of Pharmacy, 2(4): 13-16.

Sharma, V., Chand, S., Singh, P., 2013. Mulberry: A most common and multi-therapeutic plant. International Journal of Advenced Research, 1(5): 375-378.

Sheikhlar, A., Alimon, A.R., Daud, H., Saad, C.R., Webster, C.D., Meng, G.Y., Ebrahimi, M., 2014. White mulberry (*Morus alba*) foliage methanolic extract can alleviate *Aeromonas hydrophila* infection in African catfish (*Clarias gariepinus*). The Scientific World Journal, Article ID 592709, 8 pages.

Simol, C.F., Tuen, A.A., Khan, H.H.A., Chubo, J.K., King, J.H., Ong, K.H., 2012. Performance of chicken broilers fed with diets substituted with mulberry leaf powder. African Journal of Biotechnology, 11(94): 16106-16111.

Sohn, H.Y., Son, K.H., Kwon, C.S., Kwon, G.S., Kang, S.S., 2004. Antimicrobial and cytotoxic activity of 18 prenylated flavonoids isolated from medicinal plants: Morus alba L., Morus mongolica Schneider, Broussnetia papyrifera (L.) Vent, Sophora flavescens Ait and Echinosophora koreensis Nakai. Phytomedicine, 11: 666-672.

Song, W., Wang, H.J., Bucheli, P., Zhang, P.F., Wei, D.Z., Lu, Y.H., 2009. Phytochemical profiles of different mulberry (Morus sp.) Species in China. Journal of Agricultural and Food Chemistry, 57: 9133-9140.

Srivastava, S., Kapoor, R., Thathola, A., Srivastava, R.P., 2006. Nutritional quality of leaves of some genotypes of mulberry (Morus alba). International Journal of Food Science and Nutrition, 57: 305-313.

Sujathamma, P., Savithri, G., Kavyasudha, K., 2013. Value addition of mulberry (Morus spp). International Journal of Emerging Technologies in Computational and Applied Sciences,5(4): 352-356.

Tan, N.D., Wanapat, M., Uriyapongson, S., Cherdthong, A., Pilajun, R., 2012. Enhancing mulberry leaf meal with urea by pelleting to improve rumen fermentation in cattle.Asian-Australasian Journal of Animal Science, 25(4): 452-461.

Thabti, I., Elfalleh, W., Hannachi, H., Ferchichi, A., Campos, M.D.G., 2012. Identification and quantification of phenolic acids andflavonol glycosides in Tunisian Morus species by HPLC-DADand HPLC–MS. Journal of Functional Foods, 4: 367-374.

Toth, P.P., 2004. High-density lipoprotein and cardiovascular risk. Circulation, 109:1809-1812.

Tudaro, M., Sinacori, A., Marinaro, G., Alicata, M.L., Giaccone, P., 2007. Palatability and in vivo digestibility of mulberry leaves (*Morus latifolia* CV. Kokusou 21) in sheep feding. Journal of Animal and Veterinary Advences, 6(4): 509-512.

USDA, NRCS. 2015. The PLANTS Database, (http://plants.usda.gov, 19January 2015).

Valacchi, G., Belmonte, G., Miracco, C., Eo, H., Lim, Y., 2014. Effect of combined mulberry leaf and fruit extract on liver and skin cholesterol transporters in high fat diet-induced obese mice. Nutrition Research and Practice, 8(1): 20-26.

Venkatachalam, V.V., Kannan, K., Ganesh, S., 2009. Preliminary immunomodulatory activities of aqueous extract of *Morus alba* Linn. International Journal of Chemical Sciences, 7(4): 2233-2238.

Vijayan, K., Raju, P.J., Tikader, A., Saratchnadra, B., 2014. Biotechnology of mulberry (Morus L.) - A review. Emirates Journal of Food and Agriculture, 26(6): 472-496.

Vu, C.C., Verstegen, M.W.A., Hendriks, W.H., Pham, K.C., 2011. The nutritive value of mulberry leaves (*Morus alba*) and partial replacement of cotton seed in rations on the performance of growing vietnamese cattle. Asian-Australasian Journal of Animal Science, 24(9): 1233-1242.

Yang, X.Y., Yang, L., Zheng, H., 2010. Hypolipidemic and antioxidant effects of mulberry (*Morus alba* L.) fruit in hyperlipidaemia rats. Food and Chemical Toxicology, 48: 2374-2379.

Yang, Z.G., Matsuzaki, K., Takamatsu, S., Kitanaka, S., 2011. Inhibitory effects of constituents from *Morus alba* var. *multicaulis* on differentiation of 3T3-L1 cells and nitric oxide production in RAW264.7 cells. Molecules, 16: 6010-6022.

Yang, J.Y., Lee, H.S., 2012. Evaluation of antioxidant and antibacterial activities of morin isolated mulberry fruits (*Morus alba* L.). Journal of the Korean Society for Applied Biological Chemistry, 55: 485-489.

Yang, N.C., Jhou, K.Y., Tseng, C.Y., 2012. Antihypertensive effect of mulberry leaf aqueous extract containing c-aminobutyric acid in spontaneously hypertensive rats. Food Chemistry, 132: 1796-1801.

Yang, Y., Tan, Y.X., Chen, R.Y, Kang, J., 2014. The latest review on the polyphenols and their bioactivities of Chinese Morus plants. Journal of Asian Natural Products Research, 16(6): 690-702.

Yen, G.C., Wu, S.C., Duh, P.D., 1996. Extraction and identification of antioxidant components from the leaves of mulberry (Morus alba L.). Journal of Agricultural and Food Chemistry, 44:1687-1690.

Yulistiani, D., Jelan, Z.A., Liang, J.B., 2008. Degradability of mulberry (*Morus alba*) and rice bran in the Rumen of sheep fed different diets. Indonesian Journal of Animal and Veterinary Sciences, 13(4): 264-272.

Zeni, A.L.B., Molin, M.D., 2010. Hypotriglyceridemic effect of *Morus alba* L., Moraceae, leaves in hyperlipidemic rats. Brazilian Journal of Pharmacognasy, 20(1): 130-133.

Zhishen, J., Mengcheng, T., Jianming, W., 1999. The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. Food Chemistry, 64: 555-559.

Zhou, B., Meng, Q,X., Ren, L.P., Shi, F.H., Wei, Z., Zhou, Z.M., 2012. Evaluation of chemical composition, in situ degradability and in vitro gas production of ensiled and sun-dried mulberry pomace. Journal of Animal and Feed Sciences, 21: 188-197.

Zhou, J., Li, S., Wang, W., Guo, X., Lu, X., Yan, X., Huang, D., Wei, B., Cao, L., 2013. Variations in the levels of mulberroside A, oxyresveratrol, and resveratrol in mulberries in different seasons and during growth. The Scientific World Journal, Volume 2013, Article ID 380692, 7 pages.

Zou, Y., Liao, S., Shen, W., Liu, F., Tang, C., Chen, C.Y.O., Sun, Y., 2012. Phenolics and antioxidant activity of mulberry leaves depend on cultivar and harvest month in Southern China. International Journal of Molecular Science. 13: 16544-16553.

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CIHATEUP FEMALE DUCKS PERFORMANCE GROWTH BY VARIOUS PROTEIN- ENERGY RATIONS GIVEN AT WATER MINIM HOUSING SYSTEM

Tuti WIDJASTUTI[1], Wiwin TANWIRIAH[1], ABUN and Sjafril DARANA[1]

[1]Faculty of Animal Husbandry, Padjadjaran University,
Phone +620227798241, Fax : +620227798212

Corresponding author email: tuti_widjastuti@yahoo.com

Abstract

Cihateup duck is one of the local duck which coming from Cihateup village, Tasikmalaya District, West Java- Indonesia. This bird is very good for laying ducks with production of about 280-300 eggs. Some constraints because of limited agricultural land, as well as agricultural intensification, resulting land fallow period after harvest has in a short and limited and commonly full of pollution with pesticides. That situation makes duck productivity going down or low. For this condition requires much effort to overcome the real problem by maintaining ducks used ration and water efficiently as a priority. The research were divided into two phases, where the first phase was done to determine the efficiency of protein utilization value (EUP) dietary by means of excreta collection, and to define the requirement standard of protein and energy during growth. Twelve *female ducks at 8 weeks old were used by using Scott et al. (1982) formula and carried out in two week. The second phase was aimed to evaluate the protein and energy diet levels during growth period. The protein in energy diet 300 kcal/kg was standard requirement and 2% of protein in energy diet just the same was evaluated. A Completely randomized design with four replications was used in this experiment. The parameters were feed consumption, body weight gain , feed conversion for the growth period, and age at first egg laid. Feeding trial was used 48 female Cihiateup ducks at 3 weeks old, and was carried out up until 20 weeks. The result indicated that efficiency utilization protein (EUP) dietary as big as 54.14%. The Protein and Energy requirement of female Cihateup ducks for starter, grower I and grower II phase were respectively need 17.75 percent of protein, 2,454.57 Kcal/kg; 11.79 percent of protein, 2,122.81 Kcal/kg; 7.17percent of protein and 2,207.30 Kcal/kg. Based with the two phase period, it can be concluded that female Cihateup ducks can be raised with the low energy (2,200 kcal/kg ME) and low protein (12.0%) in the growing (20 weeks) diet without any ill-effects on feed intake, body weight gain, feed conversion and the age of first lay.*

Key words : *Cihateup ducks, efficiency utilization protein (EUP), age of first lay ,water minim housing system.*

INTRODUCTION

Cihateup duck is one of the local duck which coming from Cihateup village, Tasikmalaya District, West Java- Indonesia. This bird is very good for laying ducks with production of about 280-300 eggs. Cihateup duck is namely mountainous duck because it can adapt to cool temperature and survive in high land (Wulandari et al., 2005). Some constraints because of limited agricultural land, as well as agricultural intensification, resulting land fallow period after harvest has in a short and limited and commonly full of pollution with pesticides. That situation makes duck productivity going down or low. For this condition requires much effort to overcome the real problem

by maintaining ducks used ration and water efficiently as a priority. Susanti and Prasetyo (2009) state that a local duck productivity can improved by improving the genetic, feed quality and management. Improving of genetic quality is considered was ways due to its permanent impacts. Although it is generally accepted that growth of female ducks are influenced by the dietary protein and energy rations (Leeson and Summers, 2001; Cheeke, 2005; Karman et al, 2008). To compose an efficient diet, firstly has to make the metabolizable energy and the protein for rearing purpose must be known. Energy is the control of feed consumption, meaning the ration energy will determine in feed intake. If the energy ration high then the consumption of ration is low, on the contrary if the energy ration is low so the consumption of ration is high. That is why the energy level of the ration will determine the level of nutrients composition such as protein substances. Therefore between energy and protein ration should be balance (Scott et al., 1982; Leeson and Summers, 2001). Similar results were observed by Fan et al. (2008), who performed a 42 days trial on ducks and evaluated the energy level which best fits for growth parameters of the ducks, showed increase in body weight with increase in energy from 2600 to 3100 kcal/kg. The result showed that the feed intake is affected by ambient temperature. This is due to thermostatically mechanism that can control income and expenditure of energy into and out of the body, in order to maintain a stable body temperature. Therefore energy is used in different climates, and its efficiency will be different (Soeharsono, 1976; Sturkie, 1976; Daghir, 2008). Ducks are kept in the tropics generally consume less feed than ducks kept in sub-tropical regions. However, low feed consumption was not significant due to the high content of energy ration. This is related to the volume of ration which first stimulates crop distension, so that feed consumption stopped despite energy intakes of protein and protein is still lack. The protein is essential organic substances and essential for growth and production (Pack, 2002). Therefore, the accuracy of the protein that is used both for maintenance, tissue growth and hair growth should be calculated by knowing the value of the efficiency of the use of protein in the ration. The balance of protein - energy is influenced by the environment, and the amount of ducks needs. Ration with a high energy level must be followed by a high protein, minerals and vitamins in order that be balance. Ketaren and Prasetyo (2002) reported that the nutritional requirements for laying ducks in the growth phase the age of 1-16 weeks tend to be low at about 85-100% of the recommendation of 15 percent. The balance of metabolizable energy and protein within ration determine the growth rates. Thus, the same benefit economically will be gained. Therefore the preparation of rations based on energy-protein needs can be a useful benchmark for duck farmers and livestock food industry in formulating rations for local Ducks especially Cihateup on water minim housing system.

MATERIALS AND METHODS

The research were divided into two phases, where the first phase was done to determine the efficiency of protein utilization value (EUP) dietary by means of excreta collection, and to define the requirement standard of protein and energy during growth. Twelve female ducks at 8 weeks old were used by using Scott et al. (1982) formula and carried out in two week.

$$EUP = \frac{PIP - (PEP - PEK)}{EUP} \times 100\%$$

EUP = Efficiency utilization of dietary protein (%)
PIP = Protein Intake from treatment ration (g)
PIK = Protein intake from corrected ration (g)
PEP = The amount of excreta protein derive from basal ration (g)
PEK = The amount of excreta protein derive from corrected ration (g)

The protein requirement by day using Scott et al. (1982) formula recommended as follows:

The Grower phase :

$$\frac{(A \times W \times 6{,}25) + (DG \times PC) + (DG \times F \times PF)}{EUP}$$

Explanation:
A = Endogenous Nitrogen excreta of the grower ducks (mg/kg body weight)
W = Body weight (kg)
DG = Daily gain of body weight (g)
PC = Protein content of carcass (%)
EUP = Efficiency utilization of dietary protein (%)
F = Percentage of feather from body weight (%)
PF = Protein content of feathers (%)

The metabolizable energy requirement for Cihateup ducks by using the Scott et al. (1982)
The Grower phase: Energy for their maintenance + activity energy + tissue production energy
1. Energy for maintenance: 83 x $W^{0.75}$: 0.82 Kcal
2. Energy activity: on cage system 37% of the energy for maintenance,
3. Energy to produce tissue: body gain x 1.5 Kcal.
To determine protein and energy in ration
Protein Requirement:

$$\frac{Requirenment\ of\ the\ protein\ (g)}{Feed\ intake\ perday} x100\%$$

Energy Requirement:

$$\frac{Requirenment\ of\ the\ energy\ (g)}{Feed\ intake\ perday} x100\%$$

Second experiment was done as the basis of the results from experiment 2. The diets were composed on the basis of energy-protein levels, standard and over the standard requirement (energy differences 300 kcal/kg and 2 percent protein). A Completely Randomized Design (CRD) was used in this experiment with 4 treatments, P1 (2200 kcal/kg ME and 12% protein), P2 (2200 Kcal/kg ME and 14% protein), P3 (2500 kcal/kg ME and 12% protein) and P4 (2500 kcal/kg and 14% protein), each treatment is repeated five times. Then the data was analyzed by Random Simple Test (Stell and Torrie, 1989). The research used 48 female Cihateup ducks at 3 weeks old, and was carried out up until 20 weeks. The variable analysis were feed intake, body weight gain , feed conversion for the growth period, and age of first lay.

RESULTS AND DISCUSSIONS

The Result of Research - First Experiment

The results of determination of the efficiency protein utilization, the energy and protein requirements on growth phases of female Cihateup ducks are shown at Table 1.

Table 1. Efficiency Protein Utilization Value, Energy and Protein Requirements for Female Cihateup on Growth Phases

Description	Growth Phase
Efficiency Protein Utilization (EUP) %	54.14
Energy Requirement/day (Kcal)	140.88
Energy Ration (Kcal/Kg)	2207.30
Protein requirement/day (g)	7.17
Protein Ration (%)	12

Table 1 shows that the efficiency of protein utilization value (EUP) for Cihateup female grower phase is 54.14 percent. EUP value Cihateup grower phase have the lowest UEP value of pekin ducks is 55% (reported by Scott and Dean, 1991), Muscovy ducks 58.65 (Wiwin Tanwiriah, 2011), Leghorn chicken is 61% (Scott et al., 1982). EUP value reflects the amount of protein utilization efficiency of nitrogen that can retention by ducks for growth per day, feather growth and replacement of nitrogen lost (Leeson and Summers, 2001). The difference between the value of EUP Cihateup with pekin ducks and leghorn chickens, because the results of more rigorous selection in the chicken and pekin ducks are done continuously. So the degistibility pekin ducks and mucsovy ducks is higher than Cihateup ducks. The result by using the method of Scott et al. (1982), the need for energy of female Cihateup on growth is 140.88 kcal/ bird/day, with slight restriction of energy, below the *ad libitum* rate, as a means of controlling excessive fat deposition in ducks. The level of energy in the ration 2,200 kcal/kg is enough to Cihateup female ducks grower period. These results agree with the opinion of Scott and Dean (1991) feed conversion are not normally expected in poultry when weight gain is reduced, since less gain relative maintenance requirement reduces efficiency of gain. For protein per day in a period of growing is 7.17 g/bird/day, For protein per day in a period of growing is 7.17 g/bird/day, with feed intake 150 g/head/day, it needs protein in ducks rations for growth phase is 12 percent. According to Khajarern and Khajarern (1987) recommended 10-15% protein for growing Thai ducks from 3 to 10 weeks of age. The recommendation of Shafiuddin (1985) was 17% protein for growing Bangladesh ducks, and that of Sainsbury (1980) was 15% for growing ducks. Ketaren and Prasetyo (2002) that the nutritional requirements for laying ducks in the growth phase the age of 1-16 weeks tend to be low at about 85-100% of the recommendation of 15 percent. According to Leeson and Summers (2001), the light weight breeds also require less protein per day for maintenance and so chicken need a somewhat lower overall daily protein intake than do on medium breeds.

The Results of Second Experiment (Feeding Trial)

The results of the influence of energy-protein levels for Growing (3 – 20 weeks) female Cihateup Ducks are shows at Table 2. It can be seen in the Table 2, that female Cihateup ducks in the growing stage the body weight gain was slow and feed intake was very high, so the feed 'conversion ratio was very high. This result was similar to that obtained by Thongwittaya et al. (1991), who reported that the most rapid growth rate of female Khaki Campbell ducks was observed up to 4 weeks of age, the lowest from 13 to 16 weeks of age, and the feed conversion ratio increased with an increase in age. Feed intake was not different among the treatments. It means that energy-protein level (2,200 – 2,500 Kcal ME/ 12-14% protein) did not influence diet palatability and ducks appetite. According to Cole (1996), the feed intake is influenced by age, body weight, level of production, environmental temperature and nutrient content of the feedstuff. Lesson and Summers (2001) reported that poultry eat to get energy required and they will stop to eat when energy needed is achieved. Rasyaf (2003) states that the palatability determine of the feed intake. The level of consumption of ration greatly affect the performance of livestock production and reflects the level of palatability of a ration that is consumed.

Table 2. The Effects of Dietary Energy Level on Feed intake, Body Weight Gain and The Feed Conversion Ratio for Growing (3 – 20 weeks) Female Cihateup Ducks

Variable	Ratio			
	P1 (2200:12)	P2 (2200:14)	P3 (2500:12)	P4 (2500:14
Feed Intake (kg/period)	13.48 [a]	14.96 [a]	13.19 [a]	14.54 [a]
Daily gain (g/period)	834.80 [a]	963.50 [a]	891.70[a]	928.2 [a]
FCR (kg/period)	16.15[a]	15.53[a]	14.80[a]	15.67[a]
age at first egg laid (day)	154 [a]	151 [a]	151 [ab]	150 [b]

Note: The similar superscript in the same row show non significant difference (P>0.05)

Analysis of variance showed that energy-protein into the ration was not significantly (P>0.05) effect on daily gain weight. The body weight gain after the age of 12 weeks ducks began to decline. This is because at that age there is a wing feathers growth and process of maturation reproductive organs such as ovaries and oviduct. This gives an indication that the ducks were fed 12 % protein at 16 weeks of age as well as with ducks fed 14 % protein. Thus, using 2,200 kcal/kg ME and 12 % protein in the ration to achieve normal growth ranging from the age of 3 - 20 weeks. The results of the present study are in line to the findings of Yung et al. (2001) and Wu et al. (2005), who reported that non-significant (P>0.05) difference was observed in weight gain, feed consumption and feed conversion (FCR) with increasing the protein and energy ratio in commercial breeder pullet diets at growing phase.

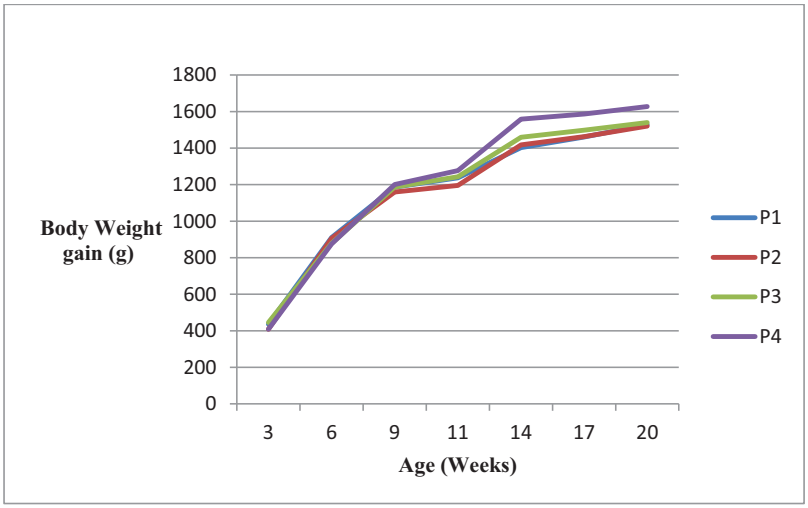

Figure 1. Growth Female Cihateup Ducks

Analysis of variance showed that energy-protein into the ration was also not significantly (P>0.05) effect on feed conversion ratio. In growing stage, the body weight gain was slow and feed intake was very high, so the feed 'conversion ratio was very high. The result was parallel on feed consumption and daily gain weight those was no significant different among the treatment. Pan et al. (1978) reported that

there was no significant difference in body weight gain and feed efficiency among growing Tsaiya ducks fed diets of different protein levels. The results of the present study are in line to the findings of Yung et al. (2001) and Wu et al. (2005), who reported that non-significant ($P>0.05$) difference was observed in weight gain, feed consumption and FCR with increasing the protein and energy ratio in commercial breeder pullet diets at growing phase.

As shown in Table 2, the age at first lay of female Cihateup duck was 150 – 153 days. Cihateup ducks to begin lay earlier than Tegal Ducks 168.8 days (Subiharta et al., 2002). Analysis of variance showed that energy-protein level in the ration was significantly ($P<0.05$) effect on the age of fist lay. By adding the 2,200 kcal/kg ME and 12 percent protein in the ration of Cihateup duck still gave a good result on the age of first lay (154 day) than if adding 2,500 kcal/kg ME and 14 percent protein in the ration (150 day). This is because if the pullets mature earlier, can be lay smaller eggs at start of egg production and the pullets lay at lower rate and over a shorter period after they begin their production cycle (Bell and Weaver, 2002).

CONCLUSIONS

1. The result indicated that efficiency utilization protein (EUP) dietary as big as 54.14%. The Protein and Energy requirement of female Cihateup ducks for starter, grower I and grower II phase were respectively need 17.75 percent of protein, 2,454.57 Kcal/kg; 11.79 percent of protein, 2,122.81 Kcal/kg; and 7.17 percent of protein and 2,207.30 Kcal/kg.
2. Based with the two phase period, it can be concluded that female Cihateup ducks can be raised with the low energy (2,200 kcal/kg ME) and low protein (12%) in the growing (20 weeks) diet without any ill-effects on feed intake, body weight gain, feed conversion and the age of first lay.

REFERENCES

Bell D.D., W.D. Weaver, 2002. Commercial Chicken Meat and Egg Production. Fifth Edn., Kluwer Academic Publisher.

Cheeke P.R., 2005. Applied Animal Nutrition. Feed and Feeding. Third Edition. Pearson Education, Inc. Upper Saddle River. New Jersey.

Daghir N.J., 2008. Poultry Production in Hot Climates. 2nd Edn., Printed and bound in the UK at The University Press, Cambridge, 101

Fan P.H., Xie M., Wang W.W., Hou S.S., Huang W., 2008. Effects of dietary energy on growth performance and carcass quality of white growing pekin ducks from two to six weeks of age. Poult. Sci., 87:1162-1164

Karman Z., M. Sarwar, M. Nisa, M.A. Nadeem, S. Mahmood, M.E. Barbar, S.Ahmed, 2008. Effect of low protein diet having constant energy-to-protein ratio on performance and carcass characteristics of broiler Chicken from to Thirty-Five Days Age. Poult Sci 87 : 468-474

Ketaren P.P., L.H. Prasetyo, 2000. Productivity of MA ducks in Ciawi and Cirebon. Proceeding of the National seminar on animal husbandry and veterinary. Livestock research centers, research institute and agricultural development, agricultural development.

Khajarern J., S. Khajarern, 1987. Poultry feeds and feeding. Khon Kacn Univivercity, 360.

Leeson S., J.D. Summers, 2001. Nutrition of the Chicken. 4 th Edition. University Brooks, Canada.

Pan C.M., X.C.Tai, J.C.Chen, H.H.Huang, T.F. Shen, 1978. The Protein and metabolizable energy requirements of growing ducks. Taiwan Livest. Res. 11:1-10

Pack M., 2002. Amino Acid in Animal Nutrition, Publishing House Coral Sanivet, Bucharest.

Rasyaf M., 2003. Beternak Itik Komersial. Yogyakarta. Kanisius.

Sahoo G., P. Panda, M. Mishra, S. C. Sahoo, 1985. Scudy of growth in Khaki Campbell ducks.Ind. J. Poult. Sci. 20:220-222

Sainsbury D., 1980. Poultry Health and Management. Grada Publishing Ltd. London, 365

Scott M.L., M.C. Nesheim, 1982. Nutrion of Chicken. 3th Edition Published by M.L. Scott and Associated. Ithaca. New York.

Scott M.L., W.F. Dean, 1991. Nutrion and Management of Ducks. ML Scott of Ithaca. New York

Shafiuddin A.I., 1985. Duck production Bangladesh. Proc. Duck Prod. Workshop. Bogor, November 18-22.

Soeharsono, 1976. Respon Broiler Terhadap Berbagai Kondisi Lingkungan. Disertasi, Fakultas Peternakan Universitas Padjadjaran.

Stell R., J.H. Torrie, 1989. Principleand Procedures of Statistics, Mc-Grow-Hill Book Company, New York, Toronto, London.

Sturkie, 1976. Avian Phisiology. 3th Ed. Comstok Publishing Assocates, A. Devision of Cornell University

Subbiharta L.H., Prasetyo Y.C., Rahardjo D., Pramono B., Budiharto Hartono, I.M Usawati, 2002. Perbibitan Itik Tegal Hasil Seleksi. Laporan Tahunan. BPTP Jawa Tengah

Susanti T., L.H. Prasetyo, 2009. Pendugaan parameter genetic sifat-sifat produksi telur itik Alabio. Prosiding Seminar Nasional Teknologi Peternakan and Veteriner. Bogor. 11-12 November 2008. Pusat Penelitian dan Pengembangan Peternakan Bogor, 588 – 610.

Thongwittaya I., P. Pleusamran, N. Choktaworn, I. Tasaki, 1992. Energy and protein requirements of Kaki Campbell X Thai Native Growing Ducks. AJAS. 5 (2) : 357 - 363

Wulandari W.A., 2005. Kajian Karakteristik biologis itik Cihateup. Thesis. Sekolah Pascasarjana Institut Pertanian Bogor.

Wiwin Tanwiriah, 2011. Male muscovy ducks (Cairina moschata) performance given ration at varios energy/protein rations under different housing system. Disertasi. Program Pascasarjana Universitas Padjadjaran.

Wu G., M.M. Bryant, R.A. Voitle, D.A. Roland, 2005. Effect of dietary energy on performance and egg composition of bovans white and dekalb white hens during phase 1. Poult Sci., 84: 1610-1615.

Yung L.C., C. Chuanhuang, C. Yenghow, H. Jennchung, C. Mingtasao, L. Dengcheng, 2001. Effects of different dietary protein and energy levels on the growth performance, blood characteristics and sensory panels of caponized Taiwan country chicken cockerels during finishing period. J. Chinese Soc. Anim. Sci., 30: 81-91.

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OPPORTUNITIES TO IMPROVE THE EFFICIENCY OF REPRODUCTION OF FARM ANIMALS

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

Academy of Sciences of Moldova, 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

The method of long-term preservation of mammalian semen in deep-frozen condition provides great opportunities for development and improvement of the system of reproduction of farm animals. Using this method it is possible to check the breeders on the quality of offspring so as to maximum use the improvers. This allows to perform the large-scale genetic selection in animal husbandry, which significantly increases the rate of mass improvement of breeding and productive qualities of animals. However, the existing cryotechnology not provide maximum preservation of the biological integrity of the reproductive cells. Comprehensive research has shown the possibility of increasing the efficiency of cryopreservation by improving of synthetic mediums and the development of optimum process parameters cryopreservation.

Key words*: synthetic mediums, cryopreservation, spermatozoa, efficiency of reproduction, farm animals.*

INTRODUCTION

The most important condition of the dynamic growth of livestock production, along with the providing a full feeding and increasing the productivity of animals, is the intensification of reproduction of the herd, the effective use of the biological capacity of a female livestock and high-value breeders on the basis of wide application of the method of artificial insemination. This method, and especially the possibility of long-term seed storage in deep-frozen state allows to radically accelerate the tempo of evaluation of breeders at quality of offspring, to rational use their genetic potential and in the shortest possible time to increase the potential productivity of the herds, to save and restore the gene pool of rare and endangered species. Further improvement of the method of freezing the semen of animals provides the reduction of losses of spermatozoa during freezing - thawing, increasing of the safety of their functional activity and, consequently, the effectiveness of insemination, as well as elimination of certain technological difficulties. The solution of these problems requires an in-depth study of the mechanisms of cryodamage and cryoprotection of sexual cells, the study of protective properties of the individual components of the synthetic mediums which are used for dilution of the seed and improve of existing cryotechnology. On this concentrated the attention of researchers working in different fields of knowledge (Blesbois et al., 2007; Zamfirescu et al., 2010).

Based on the above, the aim of the research was to explore the possibilities of increasing the efficiency of cryopreservation and the use of seed in artificial insemination of farm animals.

MATERIALS AND METHODS

The object of the study was the semen of the bulls of Black and White breed, the rams of Ţigaie breed, the boars of Large White breed, the roosters of Rhode Island Red breed and seed of carp. The optimal composition and concentration of components of cryoprotective mediums for freezing sperm of animals was determined by the method of consecutive rows (Милованов, 1962).

The semen was frozen in the form of granules with volume of 0,1 - 0,2 ml and in polymer

straws of 0,25 ml. Thawing was performed in water bath at 40 °C or using aerodynamic device.

The amount of cholesterol was determined by the method Ilka (Благоразумова, 1965), at the wavelength of 665 nm with a spectrophotometer SF - 26. The content of loosely bound cholesterol was determined by the formula:

$$X = A*B/C*D$$, where:

X – the amount of cholesterol in μg per one billion of cells,
A – data obtained from the calibration curve,
B – multiplicity of the dilution of seed,
C – the sperm concentration (billion),
D – the volume of the diluted seed, ml.

The activity of aspartate transaminase (AST) were determined by the method of Paskhinna (Пасхина, 1959). Data were obtained on the spectrophotometer SF - 46 at a wavelength of 530 nm. Calculation of AST activity was performed according to the formula:

$$T = A*B/C*D*0,1$$, where:

T – the activity of AST, in IU per 1 billion of spermatozoa,
A - multiplicity of the dilution of seed,
B - the activity of AST was found in the table,
C - the sperm concentration (billion),
D – the volume of the diluted plasma in the sample, ml
0,1 – the volume of plasma required for research.

Study of morphological changes in the acrosome of the spermatozoa was performed using phase-contrast microscopy by the use of interference microscope LPI - 5. Microscopy was performed under immersion at 1500 of times magnification. Smears were fixed with 1% solution of sodium fluoride. The experimental data was processed by the method of variation statistics using the Student's t-test.

RESULTS AND DISCUSSIONS

Raising of the effectiveness of cryopreservation of semen of animals is possible by improving of the cryoprotective agents and the use of effective technological methods. However, they can be developed on the basis of experimental studies, performed at different levels of organization of biological objects using substances distinguished by their mechanism of action.

One of the promising approaches in this direction is the introduction in the composition of the mediums of such components, which provide the possibility of formation of new bio-complexes between components of plasma membranes and synthetic medium. In this regard, we studied the effect of amino acids belonging to different groups, on the functional indices of thawed spermatozoa of the rooster after freezing it in synthetic mediums (Борончук et al., 2008; Фурдуй et al., 2013), which contained amino acids with acidic side chains (aspartic acid), basic side chains (arginine), hydrophobic (valine) and neutral (alanine) (table 1).

Table 1

Effect of exogenous amino acids on the quality of the thawed semen of the rooster

Name of amino acids	Motility of thawed gametes, points
Alanine	3,8 ± 0,14
Arginine	4,4 ± 0,12*
Aspartic acid	3,9 ± 0,21
Valine	4,6 ± 0,11*
Medium without amino acids (control)	3,9 ± 0,11

*The difference is statistically authentic

The data presented in the table 1 show that after freezing semen of the rooster in medium which contain examinee amino acids the motility of spermatozoa varies in the range of 3,8 ± 0,7 to 4,6 ± 0,11 points.

It is known that the surface of the spermatozoa carries a negative electric charge, so the efficiency of amino acid easier to explain from the point of view of their classification, according to the state of residue of amino acids in a protein chain. In this case, amino acids are classified into polar and non-polar. Arginine and aspartic acid, according to this classification refers to polar amino acids. They are able to form hydrogen bonds, acting thus on the structure of water in the cryopreservation process. However, in the physiological range pH is 6 – 8, arginine has a positive charge but aspartic acid - negative. Based on these considerations, the positive effect of arginine may be due to the formation of bio-complexes with components of plasma membranes carrying electronegative charge. Aspartic acid cannot form such biocomplexes which explains its lower efficacy.

Alanine and valine are non-polar amino acids. The different effectiveness of these amino acids can be explained by the fact that valine has a non-polar residue that is inside the protein globule, and at alanine there is no clear distribution of amino acid residues in different parts of the protein molecule (Balan, 2013; Наук, 1991).

Previously, in our laboratory it was shown that steroid glycosides kapsikoside and purpureatoside, possess antioxidant capacity in the cryopreservation of sperm of the bull.

Therefore, we considered it appropriate to investigate a series of steroid glycosides as antioxidants in the cryopreservation of sperm of the different species of animals (table 2).

Table 2

The influence of steroid glycosides on the motility of frozen–thawed gametes of different species of animals

Name of steroid glycosides	Motility of thawed gametes, points
The sperm of the bull	
Petumoside – 2	3,8 ± 0,12*
	3,4 ± 0,10
Rusticoside	3,8 ± 0,12*
	3,4 ± 0,10
Melangoside	3,7 ± 0,18
	3,6 ± 0,07
Lilia – H	4,2 ± 0,28
	3,6 ± 0,33
Trioside – Lilia	4,1 ± 0,11
	3,8 ± 0,22
Asparagoside - H	4,4 ± 0,11*
	3,5 ± 0,11
The sperm of the rooster	
Petumoside – 2	5,8 ± 0,21*
	4,8 ± 0,20
Strophantine	4,7 ± 0,31
	4,2 ± 020
Lilia – H	6,1 ± 0,17*
	5,2 ± 0,12
Trioside – Lilia	6,3 ± 0,23*
	5,4 ± 0,17
Asparagoside - H	6,7 ± 0,18*
	5,9 ± 0,17
The sperm of the boar	
Petumoside – 2	3,7 ± 0,34*
	2,5 ± 0,29
The sperm of the carp	
Balconoside	4,2 ± 0,20
	3,8 ± 0,21
Melangoside	3,3 ± 0,21
	3,8 ± 0,20
Petumoside – 2	4,7 ± 0,74
	3,8 ± 0,14
Rusticoside	4,2 ± 0,20
	3,8 ± 0,21

*The difference is statistically authentic in comparison with the experimental variant. The numerator provides the data of the experimental variants, the denominator - the data of control variants.

From the data presented in table 2 follow that the steroid glycosides increases the quality of the thawed semen of the bull, rooster and boar. Thus, the use of the drug Lilia – H allows to increase the motility of thawed spermatozoa of the rooster by 17,3% compared to the control variant, where the antioxidants were not used. However, some glycosides exhibit high protective properties, as shown above, while others, such as Melangoside, at the cryopreservation of sperm of the bull is less effective. It should be noted that the optimal concentration of drugs varies in the range of 0,015 – 0,312 mg. per 100 ml. of the medium, even within the semen of the same species (the semen of the bull). While experimenting with the semen of other species of animals the concentration of substances is also changing. Also noteworthy is the fact that some steroid glycosides (Lilia - H) is effective in the cryopreservation of sperm of some species of animals (rooster), in another case (Asparagoside - H) other animal species (bull). But if there are cases (Petumoside – 2) when the drugs are effective in the cryopreservation of sperm of several species of animals (bull, rooster, boar), still it is different in the cryopreservation of semen of various species of animals (Petumoside – 2 is more effective in the freezing of seed of the boar).

Steroid glycosides were tested by us in the cryopreservation of seed of the carp. It was found that the tested antioxidants do not have a significant protective effect, which may be explained by the fact that their efficiency in the cryopreservation is associated with different hydrophobic–hydrophilic interactions of molecules of steroids. More hydrophobic steroid glycosides have less biological activity (Давыдов, 1986), as well as features of seed of the carp and used cryopreservation techniques.

Since in the process of cryopreservation is disturbed the stability of bond of protein-cholesterol complexes and occur the changes of acrosome of the most labile structures, then one would assume that this is accompanied by loss of enzymes. These enzymes include glutamic-aspartic transaminase, localized in the mitochondria. Leakage of this enzyme into the extracellular space indicates at the serious intracellular damage. These indicators play an important role in maintaining of the functional state of the spermatozoa and can be used to predict of their fertilizing ability. In this regard, we investigated the possibility of stabilizing of these indicators through applying of different cryopreservation techniques (table 3).

The data presented in table 3 indicate a better stabilization of morphological indicators and safety of protein–cholesterol complexes in the case of freezing semen in plastic straws.

Table 3

The indicators of sperm of the ram which were cryopreserved in a different ways

№ of indicators	Method of packing	
	granules	mini straw
I	The content of intact acrosome, %	
	44,0 ± 1,23	48,4 ± 1,09*
II	The content of loosely bound cholesterol, µg/billion	
	328,5 ± 17,94	417,4 27,81*
III	The activity of glutamic-aspartic transaminase IU (billion)	
	144,8 ± 24,87	152,4 ± 27,09

*The difference is statistically authentic between the methods of cryopreservation

What concerns the activity of glutamic–aspartic transaminase, we observed a similar trend. Improving the efficiency of cryopreservation of semen can be explained by the fact that the cylindrical shape of the packaging is more preferable, since it influences the formation of crystals, thus preventing significant morphological and biochemical changes in thawed spermatozoa (Андреев et al., 2014).

In separate production experiments were found that fertility (e.g. cows) is higher by 5,5% in the variant where the sperm of the bulls was frozen in plastic straws in comparison with the packaging in the form of granules. Summarizing the results of the conducted research it can be concluded that the prospect of research in the field of cryopreservation of reproductive cells is purposeful synthesis of components of cryoprotective medium.

CONCLUSIONS

The researches allow making the following conclusions:

1. Increasing the effectiveness of artificial insemination of farm animals is possible by improving of the cryoprotective medium and optimization of the technological methods that would contribute to a more complete manifesttation of the cryoprotective properties of new components of synthetic mediums.
2. Best cryoresistance of semen and reducing of the damaging effects of low temperatures can be achieved by the use of new cryoprotective agents, the regulation of lipid peroxidation, the use of polar compounds and through creating favorable conditions for cryopreservation of seed of animals.
3. Stabilization of resistance of protein-cholesterol complexes, morphological structures, activity of glutamic-aspartic transaminase and motility of spermatozoa of semen of the ram breeders are better achieved with the use of cryopreservation technology in polymer straws.

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REFERENCES

Balan I., 2013. Teoria şi practica crioconservării spermei de cocoş în tehnologia reproducerii descendenţilor sănătoşi. Autoref. dr. hab. în biologie. Chişinău, 35.

Blesbois E., et al., 2007. Semen cryiopreservation for ex – situ management of genetic divercity in chickens; creation of the French Avian Cryobanc. Poultri Science, Vol. 86 (3), 555–564.

Zamfirescu S., Anghel A. , 2010. Researches regarding the ultrastructural modifications of sperm cells before and after friezing in different media. Lucrări ştiinţifice, Seria Zootehnie, Vol. 53 (15), 67–74.

Андреев А.А., Садыкова Д.Г., Тихомирова А.Н., Фирсова А.В., 2014. Термомеханическое растрескивание и формирование микрочастиц льда при охлаждение криозащитных сред до температур жидкого азота. Теоретические и практические аспекты современной криобиологии. Сыктывкар, 15–18.

Благоразумова М.А., 1965. Липопротеидные комплексы в крови человека и животных. Москва. 85–81.

Борончук Г.В., Балан И.В., 2008. Структурно – функциональные и биохимические изменения в биологических системах при криоконсервации. Кишинёв, 632.

Давыдов В.Я., 1986. Связь биологической активности сердечных гликозидов с их гидрофильно – гидрофобными свойствами и структурой молекул. VII Всесоюзный симпозиум по межмолекулярному взаимодействию и конформациям молекул. Пущино, 135.

Милованов В.К., 1962. Биология воспроизведения и искусственного осеменения животных. М., 696.

Наук В.А., 1991. Структура и функция спермиев сельскохозяйственных животных при криоконсервации. Кишинёв, 200.

Пасхина Т.С., 1959. Определение глутамино – аланиновой и глутамино – аспарагиновой аминотрансфераз в сыворотке человека. М., 12.

Фурдуй Ф.И., Балан И.В., Чокинэ В.К., Борончук Г.В., 2013. Температурозависимые реакции в процессе криконсервации. Кишинёв, 608.

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DIMINISHING BUFFALO COWS OVARIAN ACTIVITY DURING STABULATION USING HORMONAL PRODUCTS BASED ON PROSTAGLANDIN

Adrian BOTA[1], Florin GRIGORE[1], Gheorghe Emil MARGINEAN[2]

[1]Research and Development Station Sercaia for Buffalo Breeding, 2 Street Field, Sercaia, 507195, Brasov County, Romania, Tel /Fax: 40268245890, Email: scdcb.sercaia@yahoo.cm
[2]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: adrianbota68@yahoo.com

Abstract

There is considered that if buffalo cow fertilization has not occurred before entering the barn to be protected against the cold weather of the winter she will get in anestrous state and new heats will delay for a long time. The present paper refers to the results of a prostaglandin $PGF_{2\alpha}$ derivate treatment of 44 cases of after calving anestrous in buffalo cows registered during the housing period in the last 4 years. The buffalo cows aged between 6 and 14 years. The time interval from the former last heat was over 90 days. The treatment consists of intra muscular injection 2 ml of one of cloprostenol derivates. After the first injection 15 buffalo cows out of 44 ones came in heat within 5 days. All of them were two times inseminated artificially at a 12 hours interval. Out of them 12 buffalo cows became pregnant. The other 29 buffalo cows have received a second doze of prostaglandins 11 days after the first treatment. All of them have received blind insemination after 48 and 72 hours from the second treatment. This time 9 buffalo cows became pregnant. Result of the treatment has to be considered as a good one since 24 cows out of 44 treated cases (54, 55%) became pregnant. Perhaps 14 buffalo cows didn't react to the treatment. Nevertheless the price of the 73 doses of prostaglandin used for the treatment is lower compared to the feeding cost saved by shortening the buffalo cows what became pregnant.

Key words*: anestrous, artificial insemination, buffalo cows, calving interval, prostaglandins.*

INTRODUCTION

Calves period of buffaloes, which lasts five months a year, is diminishing consequences of their reproductive function, manifested primarily by the absence of heat. Because at this time there are large fluctuations in temperature and humidity, animals have no possibility of movement and nutrition is poor in terms of quality, it was found that about 80% of heat are discrete not conclusive as to the cow (Bogdan et al., 1981; Ilinca et al., 1993).

MATERIALS AND METHODS

The work was done over four years and consisted in the administration of hormonal based $PGF_{2\alpha}$ who followed there sumption of sexual cycle, with the advent of heat followed artificial insemination or natural service using luteolytic effect of prostaglandin. Following transrectal examination were identified 44 anestrus buffaloes with a period of more than 90 days after calving, aged 6 to 14 years old and a very good state of repair. The protocol consisted of $PGF_{2\alpha}$ administration, tracking the occurrence of heat and artificial insemination or natural service. The buffaloes showed no heat, yet received a dose of $PGF_{2\alpha}$ 11days after the first inoculation was followed occurrence heat, the heat not demonstrating blind artificial seeding is performed at 72 hours and to 96 hours.

RESULTS AND DISCUSSIONS

Ovarian response following treatment with prostaglandins is shown in table 1. Although detection of ovarian formations is more difficult than the buffalo cows because of the small size of the ovaries, following a correct diagnose is by transrectal examination, it can act to revive hormonal ovarian function and thus to obtain gestation and during this

period of the year. Also it was found that animals disrepair maintenance, those with advanced age or those who have experienced various problems in the post-partum, did not respond positively to this treatment. Buffalo bull daily presence around the female, is beneficial, even if it has allow libido (Braselli et al, 1997; Vidu et al., 2011).

Table 1. Ovarian response following the treatment with prostaglandins

Year	Buffaloes treated	Drugs used	The first administration of $PGF_{2\alpha}$		A second injection of $PGF_{2\alpha}$				Total gestation	Birth rate %
			Artificial insemination	Pregnancy	Artificial insemination	Pregnancy	Artificial insemination to 72-96 hours	Pregnancy		
2011	13	Flavoliz	3	3	-	-	10	3	6 (46%)	67
2012	15	Prosolvin	4	4	2	2	9	2	8 (53%)	72
2013	6	Enzaprost	2	2	-	-	4	1	3 (50%)	90
2014	10	Prosolvin	6	3	2*	1	2	-	4 (40%)	87
Total	**44**	**-**	**15**	**12**	**4**	**3**	**25**	**6**	**21 (48%)**	**-**

*** - natural service**

CONCLUSIONS

Use preparations based on PGF2α helps increase the percentage of gestation and hence living products and increasing the milk produced.

Method is beneficial especially for households where buffaloes are usually sexual inactivity in winter, the cost of treatments accessible. Method helps to extend artificial insemination, especially in private breeders accustomed only natural service, usually during this period.

REFERENCES

Braselli S.P. et al, 1997. Ovarian folicular by nomeres during the estrous cycle in buffalo. Technology, 44(8).

Bogdan A.T. et al., 1981. Livestock reproduction. Scrisul romanesc Publishing House, Craiova.

Creta V. et al., 1993. Some indices of breeding and their influence on buffalos milk production. Lucr. Stiintifice USAMV, seria C, Vol XXII, Bucharest.

Ilinca N. et al., 1993. Research on the introduction of the poliovulatiei to buffalo cows. Lucr. Stiintifice taurine, vol. 14, Bucharest.

Vidu Livia, Udroiu Alina, Popa R., Băcilă V., Popa Dana, 2011. Research on the influence of season on quantitative and qualitative milk buffalo, International Scientific Symposium: „Tradition, performance and efficiency in animal husbandry"-60 years of animal science higher education in Moldova, aprilie 14-15, vol. 56, seria Zootehnie, Iaşi, 88-91.

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NON-ANIMAL MACROMOLECULES AS AN ALTERNATIVE TO BOVINE SERUM ALBUMIN IN THE BULL SPERM CAPACITATION MEDIUM: PRELIMINARY RESULTS

Monica-Elena BURAC[1], Manuela PASCAL[1], Adriane WOEHL WENIGERKIND[2], Alin BÎRȚOIU[1]

[1]University of Agronomic Sciences and Veterinary Medicine of Bucharest, Faculty of Veterinary Medicine 59 Mărăşti Blvd, District 1, 011464, Bucharest, Romania, Phone: +4021.318.25.64, Fax: + 4021.318.25.67, Email: monicaburac@yahoo.com, manuelastanescu@hotmail.com, birtoiu_vet@yahoo.com
[2] Besamungsverein Neustadt a.d. Aisch e.V., 17-27 Karl-Eibl-Straße, 91402, Neustadt a.d. Aisch, Germany, Phone: +49 09161 787 0, Fax: + 49 09161 787 250, Email: info@bvn-online.de

Corresponding author email: monicaburac@yahoo.com

Abstract

The aim of this paper was to compare two non-animal macromolecules (methyl cellulose and hydroxyethyl-starch) as an alternative to bovine serum albumin supplement in the bull sperm capacitation medium. Attention was paid to the rate of penetrated oocytes and to the polyspermic fertilization in order to assess the effect of these substances on bull semen capacitation.435 class one and two oocytes were matured in TCM -199supplemented with bovine serum albumin (3mg/ml) and FSH (0.88 mg/ml) for 24 hours at 38.5°C, 5% CO_2 in saturated humidity. 21 straws from one single bull ejaculate were used. Sperm motility was assessed using Sperm Vision 3.7® (Minitübe, Germany) before the separation process. Sperm preparation was done on a commercial density gradient medium (BoviPure®, Nidacon, Sweden). Three groups were formed: the bovine serum albumin group (BSA at 6mg/ml), the methyl cellulose group (MC at 0.1mg/ml) and the hydroxyethyl-starch group (HES at 10mg/ml). Matured oocytes were co-incubated for 18 hours with the sperm (38.5°C, 5% CO_2 in saturated humidity). After 18 hours, the oocytes were denuded, fixated and stained with aceto-orcein in order to assess the penetration and pronuclear formation.77.80%±14.82% of the oocytes in the BSA group were not-penetrated, 22.20%±14.82% were penetrated and3.37%±2.37% were with polyspermy. In the MC and HES group respectively, 71.18%±21.66% and 60.23% ± 23.04% of the oocytes were not-penetrated, 28.82%±21.66% and 39.77%±23.04% werepenetrated and8.71%±6.3% and 13.74%±8.87% were with polyspermy. The physical characteristics of methyl cellulose and hydroxyethyl-starch were considered suitable for the in vitro production; they are all white powder, colourless and odourless in aqueous solution. The results showed that, there are no significant differences ($p < 0.05$) between methyl cellulose and bovine serum albumin regarding the penetration rate and the polyspermic fertilization, thus making methyl cellulose suitable to be used as an alternative supplement to the capacitation medium for bull semen.

Key words*: bovine serum albumin, bull sperm, capacitation medium, non-animal macromolecules.*

INTRODUCTION

Bovine serum albumin (BSA) is extensively added to all the mediums for in vitro fertilization. Its role is to prevent cells from sticking to themselves or to the plastic ware and to provide nitrogenous substrate for cellular metabolism (Matson and Tardif, 2012). Additionally, the bovine serum albumin induces the efflux of cholesterol from the sperm membrane, the first crucial step in the sperm capacitation process (Naseer, 2014). In human medicine, animal or human blood-derived proteins are avoided in all the culture media for assisted reproductive technologies. This is based on the desire of excluding any risk of infection with hepatitis or Creutzfeldt-Jakob disease or with other viruses and infective agents that may appear in the process. Researchers in human medicine have considered plant-derived products such as enzymes for removing the cumulus cells (Parinaud and Vieitez, 1998) or culture medium supplements (Parinaud and Milhet, 1998; Parinaud and Milhet, 1999). Nevertheless, the cost of these plant-derived proteins must be

taken into consideration as it is substantially lower (a very important aspect for veterinary medicine and in vitro embryo production).
This study aims to investigate the efficiency of two different non-animal macromolecules as a supplement to capacitation medium for bull sperm cells and to compare these supplements efficacy to bovine serum albumin. In order to assess the performance of those non-animal macromolecules, the fertilization rate was taken into consideration (attention was paid to the rate of penetrated oocytes and to the polyspermic fertilization). Also, the physical characteristics of the supplements and their potential acceptability were studied.

MATERIALS AND METHODS

Chemicals and reagents

All chemicals were purchased from Sigma-Aldrich (St. Louis, MO, USA), unless stated otherwise.

Collection and maturation of the oocytes

A total of 300 bovine ovaries were collected from adult cows in a local slaughterhouse and transported in NaCl 0.9% solution at 35°C to the laboratory. Only follicles between 2 and 8 mm diameter were aspired using 18G needles attached to a vacuum pump in 50 ml tubes. The sediment was taken out using a Pasteur pipette and transferred into a screened Petri dish (90 mm diameter) with modified PBS-Dulbecco with Ca^{2+} and Mg^{2+} (mPBS, Biochrom®, Berlin) supplemented with glucose (1 mg/ml), pyruvate (27.7 mg/ml), penicillin (0.02 mg/ml), streptomycin (0.04 mg/ml), heparin (0.01 mg/ml) and bovine serum albumin (0.3 mg/ml). The cumulus oocyte complexes (COCs) were classified as described by Leibfried and First (Leibfried and First, 1979) and onlyclass one and twoCOCs, with compact layers of cumulus cells and homogeneous cytoplasm, were selected and washed in mPBS. The selected COCs were washed twice with TCM-199 supplemented with bovine serum albumin (3 mg/ml), pH 7.4, then transferred to a 4-well dish (NUNC, Thermofisher, USA) containing 400µl TCM-199 supplemented with bovine serum albumin (3 mg/ml) and FSH (0.88 mg/ml), for washing. Finally, they were placed into the final 4-well dish with the same medium for maturation (24 hours at 38.5°C, 5% CO_2 and saturated humidity).

Sperm preparation

During the experiment 21 straws from a single bull ejaculate were used (0.25 ml straws, 15 x 10^6 sperm per straw). The experiment was repeated 7 times in 7 different days. Sperm motility was assessed before the sperm separation process using a Computer-Assisted-Sperm-Analysis system (Sperm Vision 3.7®, Minitübe, Germany) and the mean motility of the sperm was ranged at 60%.
Motile sperm was obtained using a commercial density gradient medium (BoviPure®, Nidacon, Sweden) and all the protocol was followed according to the producers recommendations. Briefly, for each sperm sample three Eppendorf tubes were prepared with 40%, 80% BoviPure and the wash one containing 1000µl BoviWash. These tubes were incubated for at least 30 minutes in 38°C. Afterwards, using a sterile pipette, 500µl BoviPure 40% were carefully transferred on top of the bottom layer of 500µl BoviPure 80%. The content of the thawed and cleaned sperm straw was slowly emptied onto the BoviPure gradient. The Eppendorf tube was centrifuged at 300 x g for 15 minutes at room temperature. After centrifugation, the supernatant was removed. The remaining pallets were transferred to the wash tube containing 1000µl of BoviWash solution and centrifuged again at 300 x g for 5 minutes. The supernatant was removed, leaving the pallet. The pallet was diluted using the capacitation medium to a final concentration of $1x10^6$sperm/ml in the IVF drop. After this procedure the sperm concentration was determined using an Improved Neubauer haemocytometer as described previously by Mahmoud et al. (1997).
Three groups were formed: the bovine serum albumin group (BSA), the methyl cellulose group (MC) and the hydroxyethyl starch group (HES).

In vitro fertilization

Modified Tyrode-Lactate medium (Bavister and Yanagimachi, 1977) containing heparin, gentamycin and hypotaurine-epinephrine was used for the *in vitro* fertilization and it will be further referred as modified TALP. The

capacitation/fertilization medium was supplemented, depending on the experimental group, with: 1) BSA (6 mg/ml) and sodium pyruvate (0.22 mg/ml); 2) MC (0.1 mg/ml) and sodium pyruvate (0.22 mg/ml); 3) HES (10 mg/ml) and sodium pyruvate (0.22 mg/ml). After maturation, the recovered COCs were washed in 200µl drops of modified TALP and then transferred into the 60µl drops of the same medium for the fertilization (Haenisch Woehl, 2003).
Fertilization was carried out at 38.5°C under 5% CO_2 in 100% humidified air for 18 hours.

Assessment of sperm penetration of oocytes *in vitro*

After 18 hours of co-incubation, the fertilized oocytes were denuded in 400µl modified D-PBS by repeated pipetting and then washed three times.
The denuded oocytes were mounted, fixed for 48 hours at 4°C in ethanol-glacial acetic acid (3:1), stained with 1% (v/v) orcein in 45% (v/v) acetic acid and examined under phase-contrast microscopy at a magnification of 200 or 400x (Nikon Eclipse E400) in order to assessthe sperm penetration of oocytes.
Oocytes that had been penetrated were identified when an enlarged sperm head or male pronucleus with its accompanying sperm tail was present in the oocytes. Oocytes with more than two pronuclei and a clear second polar body, but without a sperm tail, were also considered penetrated (polyspermy).

Statistical analysis

Values for penetrated oocytes, not-penetrated oocytes and polyspermy were evaluated using analysis of variance (One Way ANOVA) in the IBM® SPSS® Statistics Version 21 software program.

RESULTS AND DISCUSSIONS

The values of penetrated oocytes, not-penetrated oocytes and penetrated oocytes with polyspermy (mean ± standard deviation) for the three groups are given in Table 1 and Figure 1 and 2.

Table 1: Comparative values of penetrated oocytes,not-penetrated oocytes and polyspermic fertilization (mean ± standard deviation)

Capacitation medium supplement	Penetrated oocytes	Not-penetrated oocytes	Polyspermy
BSA	22.20 ± 14.82	77.80 ± 14.82	3.37 ± 2.37
MC	28.82 ± 21.66	71.18 ± 21.66	8.71 ± 6.3
HES	39.77 ± 23.04	60.23 ± 23.04	13.74 ± 8.87

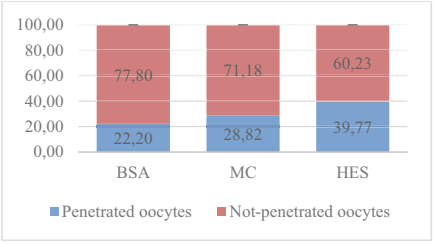


Figure 1. Comparative values of penetrated oocytes and not-penetrated oocytes

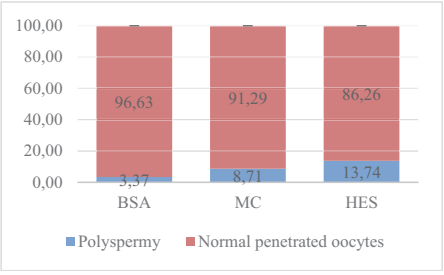


Figure 2. Comparative values of polyspermic fertilization and penetrated oocytes with no polyspermy

The penetrated oocytes (Figure 3) did not differ significantly ($p < 0.05$) between the methyl cellulose (37/150, 28.82 ± 21.66%) and thehydroxyethyl-starch group (48/134, 39.77 ± 23.04%) compared with the bovine serum albumin group (34/149, 22.20 ± 14.82%) as showed in Table 2.
The not-penetrated oocytes (Figure 3) did not differ significantly ($p < 0.05$) in the methyl cellulose group (113/150, 71.18 ± 21.66%) and in the hydroxyethyl-starch group (86/134, 60.23 ± 23.04%) compared with the bovine serum albumin group (115/149, 77.80 ± 14.82%) as showed in Table 2.

Table 2: Effect of the capacitation medium supplement on the penetrated / not-penetrated oocytes

Capacitation medium supplement		Mean difference
BSA	MC	-,06616
	HES	-,17573
MC	BSA	,06616
	HES	-,10956
HES	BSA	,17573
	MC	,10956

*The mean difference is significant at the 0.05 level

The polyspermic fertilization (Figure 3) was significantly higher ($p<0.05$) in the hydroxyethyl-starch group (15/134, 13.74%± 8.87%) compared with the bovine serum albumin group (5/149, 3.37 ± 2.37%), but it did not differ significantly ($p < 0.05$) between the methyl cellulose group (12/150, 8.71 ± 6.3%)and the bovine serum albumin group (5/149, 3.37 ± 2.37%) as showed in Table 3.

Table 3: Effect of the capacitation medium supplement on the polyspermic penetration of the oocytes

Capacitation medium supplement		Mean difference
BSA	MC	-,05338
	HES	-,10373*
MC	BSA	,05338
	HES	,05035
HES	BSA	,10373*
	MC	,05035

*The mean difference is significant at the 0.05 level

The results of the present study show that there are non-animal macromolecules than can substitute bovine serum albumin from the capacitation medium without damaging the sperm cells and the oocytes. Unfortunately, when referring to the rate of polyspermy, only methyl cellulose is considered to give the same results as bovine serum albumin.

Attempts have been made in the past in order to substitute BSA with other non-animal proteins in several culture media. One of these attempts was made by Biggers and his collaborators (Biggers and Summers, 1997) who have investigated the effect of replacing BSA with polyvinyl alcohol (PVA) and/or amino acids on mouse zygote development. They concluded that PVA could not substitute completely BSA in the mouse embryo culture medium. They followed the blastocyst rate during their experiments and observed that blastocyst development was only slightly less than with BSA, but the rate of partial hatching was significantly less. Substitution of BSA with PVA lowered the overall response but did not lead to major perturbation.

The present study was focused on the penetration of the oocytes in order to avoid the oocyte influence on the fertilization and assess only the ability of BSAs replacements to induce sperm capacitation. The substances used in this study in order to substitute BSA from the capacitation medium were used in human medicine for short term culture of human sperm at the same concentrations (Matson and Tardif, 2012) giving encouraging results and only the best of them were chosen for bull sperm capacitation.

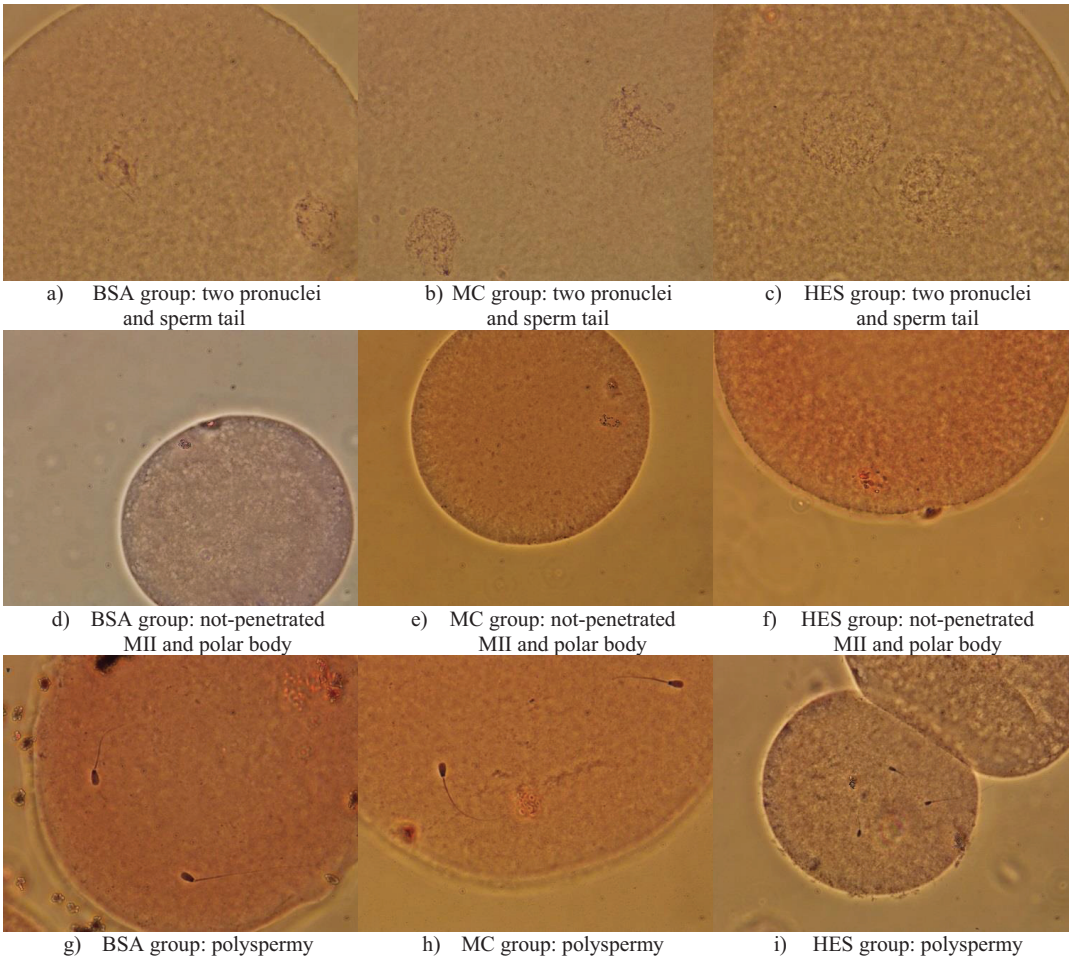
The physical characteristics of methyl cellulose and hydroxyethyl-starch were considered suitable for clinical use; they are all white powder, colourless and odourless in aqueous solution.

Matson and Tardif concluded in their study that hydroxyethyl-starch had poor solubility and had to be centrifuged resulting in a saturated solution of undefined concentration (Matson and Tardif, 2012). In the present study, this problem had been solved by sterile filtration (0.2µm, 5.7cm^2 filter) of the medium containing these supplements before their use (Insufil, Fresenius Kabi, Germany). This poor solubility aspect was noticed only regarding hydroxyethyl-starch.

Additionally, both methyl cellulose and hydroxyethyl-starch did not significantly modify the mediums pH (initially 7.8) after 3hours of incubation before being used for the fertilization (BSA – pH 7.93, MC – 7.96 and HES – 8.03).

Protein-free medium have been used before but only in human assisted reproductive technologies. SMART1® medium (Parinaud, Milhet et al. 1998) was developed in order to support fertilization and it was shown to give good fertilization rates and hence supported capacitation and avoided premature acrosome reaction. Another series of media, the ART-7 series (Ali and Shahata, 2000) ensured a sperm survival rate of approximately 80% and has been shown to support both fertilization and embryo development. It contained undefined supplements, but these were likely to include methyl cellulose (Ali, 2009).

There are no data available at this moment for protein-free medium designed for assisted reproductive technologies in veterinary medicine, so that our results cannot be compared with other studies results. These preliminary results will be completed with more than one bull ejaculate in order to avoid the ejaculate effect and individual influence on the results.



a) BSA group: two pronuclei and sperm tail
b) MC group: two pronuclei and sperm tail
c) HES group: two pronuclei and sperm tail
d) BSA group: not-penetrated MII and polar body
e) MC group: not-penetrated MII and polar body
f) HES group: not-penetrated MII and polar body
g) BSA group: polyspermy
h) MC group: polyspermy
i) HES group: polyspermy

Figure 3. Morphological analysis of the oocytes. Oocytes were stained with aceto-orcein dye to analyse chromatin configuration: penetrated oocytes (the presence of both male and female pronucleus and the sperm tail), not-penetrated oocytes (MII stage and the presence of the polar body) and polyspermic fertilization (the presence of more than one sperm inside of the oocyte or more than two pronuclei)

CONCLUSIONS

In conclusion, no significant differences were observed between methyl cellulose and bovine serum albumin regarding the penetrated oocytes and the polyspermic fertilization. Thus MC is suitable to be used as a supplement in the capacitation medium for bull sperm.

The present study showed no significant differences between hydroxyethyl-starch and bovine serum albumin regarding the penetrated oocytes, but found a significant difference when the polyspermic fertilization was taken into consideration, thus suggesting that hydroxyethyl-starch may have a negative effect on the penetration of the oocytes.

Further studies are needed in order to assess the real efficiency of these non-animal macromolecules on the embryo development when added as supplements in bull sperm capacitation medium.

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REFERENCES

Ali J., 2009. Protein-free gamete handling and culture media products. United States: Cellcura Inc. US 2009/0226879 A1: 1-16.

Ali J., Shahata M.A., 2000. Formulation of a protein-free medium for human assisted reproduction. Hum Reprod 15(1): 145-156.

Bavister B.D., Yanagimachi R., 1977. The effects of sperm extracts and energy sources on the motility and acrosome reaction of hamster spermatozoa in vitro. Biol Reprod 16(2): 228-237.

Biggers J.D., Summers M.C., 1997. Polyvinyl alcohol and amino acids as substitutes for bovine serum albumin in culture media for mouse preimplantation embryos. Hum Reprod Update 3(2): 125-135.

Haenisch Woehl A., 2003. Untersuchungen zum Einfluss von Calcium Ionophor A23187 während der In-vitro-Fertilisation boviner Eizellen auf die präimplantative Embryonalentwicklung. Doktorin der Veterinärmedizin (Dr. med. vet.), Tierärztliche Hochschule Hannover.

Leibfried L., First N.L., 1979. Characterization of bovine follicular oocytes and their ability to mature in vitro. J. Anim. Sci. 48(1): 76-86.

Mahmoud A.M., Depoorter B., 1997. The performance of 10 different methods for the estimation of sperm concentration. Fertil Steril 68(2): 340-345.

Matson P., Tardif S., 2012. A preliminary search for alternatives to albumin as a medium supplement for the culture of human sperm. Reprod Biol 12(3): 329-331.

Naseer Z., Aksoy M., 2014. Cholesterol efflux from sperm: approaches and applications. Turk. J. Vet. Anim. Sci., 38.

Parinaud J., Milhet P., 1998. Human sperm capacitation and in-vitro fertilization in a chemically defined and protein-free medium SMART1. Hum Reprod 13(9): 2579-2582.

Parinaud J., Milhet P., 1999. Use of a medium devoid of any human or animal compound (SMART2) for embryo culture in intracytoplasmic sperm injection. J. Assist. Reprod. Genet. 16(1): 13-16.

Parinaud J., Vieitez G., 1998. Use of a plant enzyme preparation (Coronase) instead of hyaluronidase for cumulus cell removal before intracytoplasmic sperm injection. Hum. Reprod. 13(7): 1933-1935.

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THE PROTECTIVE EFFECT OF L-CARNITINE DURING THE HYPOTHERMIC STORAGE OF BOAR SEMEN

Vladimir BUZAN

Academy of Sciences of Moldova, 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

The effectiveness of livestock farming in great extent depends on the efficiency of animal reproduction. Therefore, researches in this direction acquire theoretical and practical significance. Given the fact that in the practice of reproduction of pigs widely was used the method of artificial insemination of sows' chilled sperm, the purpose of the conducted research was to improve the synthetic mediums through introducing into their composition of the biologically active compounds. As such substance was used L-carnitine. By method of consecutive rows was determined dose-dependent effect of investigated substance included in the base medium on the basis of electrolytes and non-electrolytes. It was found that after dilution of boar sperm the mobility of spermatozoa and their absolute indicator of survival after 12 hours of storage at hypothermic temperatures (16-18˚C) is increased by a statistically significant amount. It is concluded about protective and stimulating activity of L-carnitine at the dilution and storage of boar semen.

Key words*: synthetic mediums, hypothermic storage, sperm, L-carnitine, boar semen.*

INTRODUCTION

The possibility of dynamic growth in livestock production is determined, among other factors, the intensification of reproduction of the herd using artificial insemination. Zoo veterinarian benefits of this biotechnological method of reproduction in comparison with natural reproduction determine its leading role in the technology of production of pigs in farms with different production and economic structure. However, the potential of artificial insemination of pigs is not realized in full. And the reason for this is the fact that the freezing and long-term preservation of boar semen not yet found wide industrial application in connection with cryo technological difficulties and insufficient knowledge of species-specific, morpho–physiological, biochemical and physico–chemical characteristics of reproductive cells of these species (Федина, 2007). In addition, there are significant age, breed and individual differences in cryo sustainability of seed material, and the preparation of sperm for long-term storage also leads to serious disturbances of lipid metabolism, manifested in the change of the content of glycolipids, phospholipids and fatty acids. Progressive method of embryo transfer, due to the complex and costly mediums, tools and equipment also are not used in practice. In this regard, in production conditions generally is accepted the insemination of sows with diluted boar semen stored in hypothermal conditions at 16–18 ºC. However, in this case too more intensively metabolic processes occur. As a result, there is accumulation of toxic products of metabolism and the decrease of the functional parameters of spermatozoa.

Given the fact that under the influence of drugs containing carnitine was noted normalization of acrosomal reaction of spermatozoa (Zhou et al., 2007), was detected a correlation between the concentration of carnitine in sperm and the integrity of the nuclear DNA of the gametes (De Rosa et al., 2005), osmotic resistance of spermatozoa (Yeste et al., 2010), as well as the positive impact of the use of carnitine on the levels of reduced glutathione and 8-hydroxydeoxyguanosine in the testes (Abd-Allah et al., 2009), but the molecular mechanisms of antioxidative action of carnitine

remain unclear until the end (Wang et al., 2010), which served as a prerequisite for its introduction in the composition of the synthetic medium for boar semen.

Based on the above, the purpose of the conducted research was to study the qualitative indicators of reproductive cells of the boar stored after dilution with synthetic medium containing biologically active substance L-carnitine.

MATERIALS AND METHODS

As experimental material used semen of the boars breeders of Landrace breed which contained in the conditions of the breeding enterprise "Moldsuinhibrid", the relevant veterinary requirements. The sperm was received by fractional method using an artificial vagina, the temperature of which was in the range 38-40 °C. For dilution it was used the synthetic medium consisting of glucose, EDTA and sodium citrate. Medium components were dissolved in bidistilled water. In our experiments we used pharmacological 2% L–carnitine and ferric sulfate chemically pure quality. Dilution of semen was performed 1:1 in compliance with the rules of asepsis when working with the experimental material. After that, it was kept in hypothermal conditions at room temperature. The optimal concentration of the test substances was determined by the method of consecutive rows of Milovanov V. K. (Милованов, 1962). Qualitative indicators of the diluted semen were determined using a light microscope "AMPLIVAL" of company Carl Zeiss (Jena) at 200 × magnification. Sperm motility was determined in points on a ten-point grading scale, and the absolute survival rate (ASR) is in conventional units, which is the sum of survival indices multiplied by the number of hours their survival.

Statistical processing of the results of research were conducted using the criterion of Student's t-test.

RESULTS AND DISCUSSIONS

The synthetic mediums for dilution and storage of semen of farm animals at 16–18ºC, as a rule, are not complex. They are designed to maintain osmotic pressure, pH and the viability of spermatozoa. However, the problem of improving of the functional status and increasing of life expectancy of the cells continues to be relevant for practitioners involved in the swine reproduction. Therefore, we consider it expedient to enter into the composition of mediums the components which contribute to homeostasis of metabolic processes. For this purpose, we have studied the protective properties of L-carnitine in the composition of the basic medium for dilution and storage of semen of the boar. The results of conducted researches are presented in table 1.

Table 1

Dose-dependent effect of L-carnitine in the hypothermal storage of boar semen

The experimental variant	Concentration of L-carnitine, mg/ml	Motility of spermatozoa after dilution, points	ASR, c.u.	
			After 12 hours	After 24 hours
1	Control	6.2 ± 0.42	74.7 ± 5.02	132.0 ± 18.00
2	0.02	7.6 ± 0.27	88.8 ± 5.37	165.7 ± 10.73
3	0.04	7.6 ± 0.27*	91.2 ± 3.29*	177.6 ± 6.57
4	0.08	7.6 ± 0.27*	91.2 ± 3.29*	177.7 ± 6.57
5	0.16	7.0 ± 0.35	84.0 ± 4.24	160.8 ± 9.10
6	0.32	6.9 ± 0.27	81.6 ± 4.03	160.8 ± 9.10

*The difference is statistically authentic

The data of table 1 demonstrates that L-carnitine has a dose-dependent effect. Its use in the composition of the medium for dilution of boar semen at a concentration of 0.04-0.08 mg/ml has a positive effect on the functional indices of reproductive cells. In the best experimental variants the motility of spermatozoa after dilution and absolute survival rate after 12 hours of storage of the sperm at 16-18ºC amounted respectively 7.6 ± 0.27 and 91.2 ± 3.29, which indicates an increase of the studied parameters on 22.6 and 22.1% compared to the control variant. L-carnitine (3-Hydroxy-4-(trimethylazaniumyl) butanoate) refers to indispensable substances because they perform the basic role in the transport of fatty acids across the mitochondrial membrane (Спасов et al., 2005). However, L-carnitine is synthesized also in the animal body in the liver and kidneys where through the blood stream is transported to other tissues and organs. Great interest to L-carnitine is due to its role in metabolic processes. Among them we

should mention: transport of long-chain fatty acids into the mitochondrial matrix, where they are included in the process of formation of acetyl coenzyme A; stabilization of the content of the acetyl coenzyme A and the deletion of short-chain fatty acids from mitochondria; regulation of the contents of the CoASH, which is required for detoxification of metabolic products; the maintenance of the optimal ratio of acetyl CoA/CoASH for stimulation of anabolic processes; the maintenance of cell activity through the involvement of L-Carnitine in energy metabolism with the participation of phospholipids (Копылевич, 2005). It is suggested that the increased of mitochondrial energy metabolism may indirectly prevent the formation of free radicals (Abd-Allah et al., 2009; Lombardo et al., 2011). In this regard, it is recommended to use as the most widely utilized antioxidant for regulation of metabolic processes of spermatozoa (De Rosa et al., 2005; Божедемов et al., 2012).

From the analysis of the submitted information it follows that the protective effect of carnitine in the composition of the medium for dilution and hypothermal storage of boar semen, mainly may be due to the regulation of energy metabolism and the detoxification of the products of this process. At the same time, it is obvious that the normalization of the antioxidant characteristics of seminal fluid is a mandatory prerequisite for the recovery of the fertilizing ability of ejaculate, especially in conditions of oxidative stress in hypothermal storage of sperm. The data presented in table 1 prove conclusively that vitamins can perform a protective function, protecting the spermatozoa of the boar from the harmful effects of internal and external factors.

Minerals, along with vitamins and other biologically active substances, are mandatory elements providing cell viability. Therefore, in the next series of experiments it was investigated efficacy of ferric sulfate in hypothermal storage of boar semen and determined its optimal concentration (table 2).

The study results which are presented in table 2 show that the optimal concentration of ferric sulfate in the composition of the medium is 0.6 mg/ml. In this experimental variant, the motility of spermatozoa was 6.9±0.11 points and the absolute survival rate after 12 hours of storage has reached 82.8 ± 1.34 c.u., which respectively is more with 13.4 and 13.1% in comparison with the control variant.

Table 2

The influence of ferric sulfate on the functional indices of boar semen

The experimental variant	Concentration of ferric sulfate, mg/ml	Motility of spermatozoa after dilution, points	ASR, c.u.	
			After 12 hours	After 24 hours
1	Control	6.1 ± 0.17	73.2 ± 1.34	72.0 ± 12.01
2	1.0	6.0 ± 0.01	72.0 ± 0.01	0
3	0.8	6.1 ± 0.27	73.2 ± 6.57	19.2 ± 2.15
4	0.6	6.9 ± 0.11*	82.8 ± 1.34*	96.0 ± 4.24
5	0.4	6.7 ± 0.22	80.4 ± 2.68	96.0 ± 4.24
6	0.2	6.1 ± 0.17	73.2 ± 2.68	86.4 ± 6.57

*The difference is statistically authentic

The positive effect of the use of ferric sulfate may be due to the fact that this element not only maintains the water-salt metabolism, but also participates in the composition of cytochromes in a number of redox reactions (Овчинников, 1987). As a result, may be the inclusion of certain processes that lead to an increase of the functional activity of spermatozoa.

CONCLUSIONS

The researches allow making the following conclusions:

1. For maintenance of the functional status of boar semen after it is received the significant role acquires the regulation of metabolic processes at different technological stages.
2. Improving the quality of boar semen stored at hypothermal conditions can be realized through including in the composition of mediums of L-carnitine and ferric sulfate.
3. For hypothermal storage of boar semen is preferable to use a dense fraction, the volume of which will be increased after the first and repeated dilution.
4. If necessary the prolonged storage of boar sperm after its receipt should be diluted one-for-one with medium containing L-carnitine, and before the actual research of artificial insemination of sows re-diluted one to 0,5 with a similar medium containing ferric sulfate.

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REFERENCES

Abd-Allah A., Helal G., Al-Yahya A. et al., 2009. Pro-inflammatory and oxidative stress pathways which compromise sperm motility and survival may be altered by L-carnitine. Oxid. Med. Cell. Longev., Vol. 2, 73–81.

De Rosa M., Boggia B., Amalfi B. et al., 2005. Correlation between seminal carnitine and functional spermatozoal characteristics in men with semen dysfunction of various origins. Drugs R. D., Vol. 6, 1–9.

Lombardo F., Sansone A., Romanelli F. et al., 2011. The role of antioxidant therapy in the tratment of male inferility: an overview. Asian J. androl., 13, 690-697.

Wang Y., Yang S., Qu C. et al., 2010. L-carnitine: safe and effective for asthenozoospermia. Zhonghua Nan Ke Xue, Vol. 16, 420–422.

Yeste M., Sancho S., Briz M. et al., 2010. A diet supplemented with L-carnitine improves the sperm quality of Pietrain but not of Duroc and Large White boars when photoperiod and temperature increase. Theriogenology, Vol. 73, 577–586.

Zhou X., Liu F., Zhai S., 2007. Effect of L-carnitine and/or L-acetyl-carnitine in nutrition treatment for male infertility: a systematic review. Asia Pac. J. Clin. Nutr., Vol. 16 (1), 383–390.

Божедемов В.А., Виноградов И.В., Липатова Н.А., Спориш Е.А., Рохликов И.М., 2012. Нарушение структуры хроматина Сперматозоидов: клиническое значение, причины, диагностика, лечение (обзор литературы). Проблемы репродукции, том 18 (5), 80-88.

Копылевич В.М., 2005. Витаминоподобные соединения L-карнитин и ацетил- L-карнитин: от биохимических исследований к медицинскому применению. Українский біохімічний журнал., том 77, 25-45.

Милованов В.К., 1962. Биология воспроизведения и искусственное осеменение сельскохозяйственных животных. М.: Сельхозгиз, 696.

Овчинников Ю.А., 1987. Биоорганическая химия. М., Просвещение,. 816.

Спасов А.А., Иежица И.Н., 2005. Стереофармакологические особенности карнитина. Русский физиологический журнал им. М.И. Сеченова, 12, 35-34.

Федина Н.И., 2007. Криозащитное влияние различных антиоксидантов и БАВ при хранение спермы хряков в охлажденном и замороженном состоянии. Автореф. дисс. кандидата биологических наук, 06.02.01. п. Лесные Поляны, Московской обл., 22.

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CAN INFRARED THERMOGRAPHY BE USED TO PREDICT EAR TAGS INFECTIONS IN LAMBS?

Ferda KARAKUŞ[1], Adem DÜZGÜN[2], Murat KARAKUŞ[2], Logman ASLAN[3]

[1]University of Yüzüncü Yıl, Faculty of Agriculture, Department of Animal Sciences, 65080, Van, Turkey, Phone: +90.432.225.10.56, Fax: +90.432.225.11.04, Email: fkarakus@yyu.edu.tr
[2]Directorate of Provincial Food Agriculture and Livestock, Van, Turkey, Phone: +90.432.222.02. 37, Fax: +90.432.222.00.67, Email: ademduzgun@mynet.com, mkarakus21@hotmail.com
[3]University of Yüzüncü Yıl, Faculty of Veterinary Medicine, Department of Surgery, 65080, Van, Turkey, Phone: +90.432.225.11.28, Fax: +90.432.225.11.27, Email: logmanaslan@gmail.com

Corresponding author email: fkarakus@yyu.edu.tr

Abstract

Ear tagging is one of the common husbandry procedures that cause not only pain and stress but also tissue reaction and infection. Reliable and non-invasive tools are needed to determine the stress and/or pain resulting from routine husbandry procedures commonly performed in farms. Thermal imaging is a non-invasive diagnostic method used in veterinary medicine. The aim of the study was to determine the usability of infrared thermography in prediction of infections caused by electronic and visual ear tags in lambs. We hypothesized that reactive temperature increase within the first hour in the ear tissue in response to the ear tags would trigger the formation of infection. The study was carried out on Akkaraman lambs (n=60) reared under rural farm conditions. All lambs at two weeks of age were identified with an electronic ear tag (FDX-B, Allflex) on the left ear and an official plastic ear tag on the right ear. Before tagging, infrared images of the ear region were collected at a consistent distance from the left ear of the animal using an infrared camera (FLIR E50) in the barn. Tag insertion was performed by two practitioners at the same time. An hour after tagging, the thermal measurements of both ears were carried out again with infrared camera. The ears of lambs were individually checked in the week after tagging. The status of ear lesions was monitored until healing (about 8 weeks). Before tagging, the average thermal temperature of the left ear was measured as 16.68˚C. Electronic ear tags caused more problems than official ear tags. Infected ear rate in electronic and official ear tags was 80% and 50% respectively. Significant temperature differences existed between infected and non-infected ears (P<0.05). All ear tags that caused further increase *in reactive* temperature *resulted in an inflammatory reaction. As a result, early detection of inflammation is very crucial in terms of implementation of treatment and animal welfare. Ear lesions caused by ear tags in lambs can be early identified using infrared thermography. The preliminary findings of this study should be supported in subsequent studies.*

Key words*: infrared thermography, ear tags, lamb.*

INTRODUCTION

Ear tagging is one of the identification procedures most commonly performed on livestock for routine on-farm management. Retention rate of ear tags vary from 60-98% depending on the factors such as age at tagging, tag features, healing of the tagging site, species, breeds and environmental conditions. Due to the great variability in losses and external damages, minimum retention rate of 98% recommended by the International Committee for Animal Recording (ICAR) for official identification devices at 1 year after tagging in animals is not fully achieved in many cases (Caja et al., 2004; Carne, 2010).

The ear tags in ewes and lambs were illegible or difficulty legible for reasons such as wear and tear, breakage and fouling. Tag loss rate caused by tearing of the animal's ear were reported to be up to 5% in sheep (Gonzales-Barron et al., 2009). Electronic ear tags are plastic-encapsulated transponders designed to be fixed to the animal's ear using specially designed pliers with the same principle of application as for conventional plastic ear tag (EC, 2006). Losses and damages caused by events such as tissue reaction, infection or ear splitting should also be a reason for the consideration for electronic ear tags similar to conventional ear tags (Carne, 2010).

The measurement and alleviation of fear, pain and stress during routine husbandry procedures

commonly used on farms (e.g. ear-tagging, dehorning, and castration) has crucial importance in terms of animal welfare. Because reliable and non-invasive tools are needed to measure stress or pain, infrared thermography (IRT) can be used as a useful tool for this purpose (Stewart, 2008).

Thermal imaging is a non-invasive diagnostic method used in veterinary medicine. Diseased area can be determined by this method indicating different heat than normal emitted from damaged tissue and organs of animals (Düzgün and Or, 2009). The fact that measurements can be made without touching the animal at close (<1 m) or large distances (>1000 m) and compromising their welfare is the main advantage of IRT in animal research (Church et al., 2009). IRT has been used to determine lameness, injuries, and inflammations (Martins et al., 2013; Renn et al., 2014); to diagnose infectious diseases (Schaefer et al., 2007; Gloster et al., 2011); to detect estrus, ovulation, and male fertility (Scolari, 2010; Menegassi et al., 2014); to control of stress and pain levels for evaluation of animal welfare (Stewart, 2008; Stubsjoen et al., 2009); and to assess thermal comfort (Paim et al., 2012; 2014) in livestock.

The aim of the study was to determine the usability of infrared thermography in prediction of infections caused by electronic and visual ear tags in lambs. The main approach was to measure the thermal responses of tissue to the ear tags. We hypothesized that reactive temperature increase within the first hour in the ear tissue in response to the ear tags would trigger the formation of infection. So that, it would be feasible to determine and follow as early as possible the risk of infected ears from tagging in terms of retention rate of ear tag and animal welfare, as well as time and labor saving.

MATERIALS AND METHODS

The study was carried out on Akkaraman lambs (n=60) reared under rural farm conditions. All lambs at two weeks of age were identified with an electronic ear tag (FDX-B, Allflex) on the left ear and an official plastic ear tag on the right ear. Before tagging, infrared images of the ear region were collected at a consistent distance from the left ear of the animal using an infrared camera (FLIR E50) in the barn. The tags were immersed in a disinfectant before insertion. Tag insertion was performed by two practitioners at the same time. The behavior of the lambs was observed at tagging. An hour after tagging, the thermal measurements of both ears were carried out again with infrared camera. The ears of lambs were individually checked for signs of infection associated with the ear tag in the week after tagging. The status of ear lesions was monitored until healing (about 8 weeks). Statistical analysis was performed using t test.

RESULTS AND DISCUSSIONS

Following ear tagging, all lambs showed characteristic signs of pain or discomfort by bleating, head-shaking and ear-scratching. Leslie et al. (2010) reported that head shakes and ear scratching were the behaviours observed most frequently following the application of ear tag in piglets.

Electronic ear tags caused more problems than official ear tags. Infected ear rate in electronic and official ear tags was 80% and 50% respectively (Figure 1). Signs of infection were observed in the form of swelling of the ear, irritation under the ear tag, inflammation, and discomfort or pain when touched. The severity of ear lesions was monitored until healing. All infected ears healed within 8 weeks of insertion of the ear tag based on lesion severity. Edwards et al. (2001) indicated that the insertion of ear tags resulted in an inflammatory response in ewes and lambs. By the 20th week after inserting the ear tag, all lesions, except those caused by the metal loop tags, were almost completely healed. Carne et al. (2009) reported 3.3% infection and 6.5% tissue reaction rates for electronic ear tags in goat kids, but 90.2% of ears were completely healed at 2 months after tagging. On the other hand, Kowalski et al. (2014) observed only bleeding in one goat during application of the big visual ear tag. It is thought that the problems in ears with e-ET may be caused by the greater weight due to the presence of a transponder.

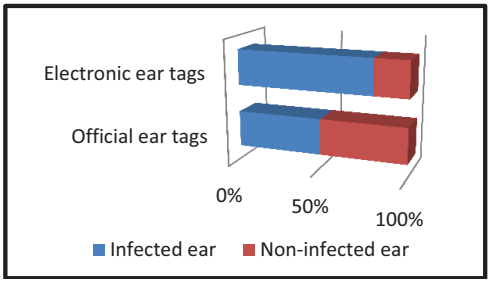

Figure 1. Infected and non-infected ear rate in electronic and official ear tags

Average ear temperatures before tagging and 60 min after insertion of electronic and official ear tags are presented in Table 1. Before tagging, the average thermal temperature of the left ear was measured as 16.68˚C. One hour after insertion of electronic and official ear tags, the average temperature of overall was 24.56˚C and 21.85˚C, respectively.

Table 1. Average ear temperatures before and 60 min after tagging (degree C)

	Before tagging		After tagging			
	n		n	Electronic ear tags	n	Official ear tags
Overall	60	16.68±1.46	60	24.56±4.68 *	60	21.85±5.21 *
Infected ear	48	16.58±1.34	48	25.95±4.05	30	26.18±3.41
Non-infected ear	12	17.09±1.89	12	19.00±2.28	30	17.52±2.22

*: P<0.05

Significant temperature differences existed between infected and non-infected ears. The average temperature of infected ears caused by electronic ear tags was measured as 25.95˚C while the temperature of non-infected ears was 19.00˚C (P<0.05). On the other hand, the average temperature in ears with official tag resulted in an inflammatory reaction was 26.18˚C while the temperature of non-infected ears was 17.52˚C (P<0.05). Temperature increase is a good predictor for the early phase of inflammation development. All ear tags that caused further increase in reactive temperature resulted in an inflammatory reaction.

CONCLUSIONS

Ear tags may result in an inflammatory response due to the wound created when they were inserted into the ear. Ear wounds should be considered in terms of ear tag losses and welfare implications, since re-tagging of an animal result in increased cost and animal stress. Therefore, early detection and treatment of inflammation or ear tissue reaction is economically and strategically advantageous. As a result, infrared thermography as a non-invasive diagnostic tool can be used to identify lambs with inflammations caused by ear tags. However, the preliminary findings of this study should be supported in subsequent studies.

REFERENCES

Caja G., Ghirardi J.J., Hernandez-Jover M., Garin D., 2004. Diversity of animal identification techniques: from ‘fire age’ to ‘electronic age’, ICAR Technical Series, No: 9, 21-39.

Carne S., 2010. Electronic identification of goats: comparison of different types of radio-frequency and visual devices. PhD Thesis, Universitat Autonoma de Barcelona, Bellaterra, Spain, 140.

Carne S., Caja G., Ghirardi J.J., Salama A.A.K., 2009. Long-term performance of visual and electronic identification devices in dairy goats. Journal of Dairy Science, 92: 1500-1511.

Church J.S., Cook N.J., Schaefer A.L., 2009. Recent applications of infrared thermography for animal welfare and veterinary research: everything from chicks to elephants. InfraMation 2009 Proceedings, Vol. 10, 215-224.

Düzgün D., Or M.E., 2009. Termal kameraların tıpta veteriner hekimlikte kullanımı. TÜBAV Bilim Dergisi, 2(4): 468-475.

EC (European Commission), 2006. Technical guidelines for council regulation no.

21/2004 of 17/12/2003. Part 1, in-field aspects: application of identifiers, their reading and recovery, G07-TRVA/TG part 1, 1-18.

Edward D.S., Johnston A.M., Pfeiffer, D.U., 2001. A comparison of commonly used ear tags on the ear damage of sheep. Animal Welfare, 10(2): 141-151.

Gloster J., Ebert K., Gubbins S., Bashiruddin J., Paton D.J., 2011. Normal variation in thermal radiated temperature in cattle: implications for foot-and-mouth disease detection. BMC Veterinary Research, 7(73): 1-10.

Gonzales-Barron U., Butler F., McDonnell K., Ward S., 2009. The end of the identity crisis? Advances in Biometrics Markers for Animal Identification, Irish Veterinary Journal, 62(3): 204-208.

Kowalski L.H., Monteiro A.L.G., Hentz F., Prado O.R., Kulik C.H., Fernandes S.R., da Silva C.J.A. 2014. Electronic and visual identification devices for adult goats reared in semi-intensive system. Revista Brasileira de Zootecnia, 43(2): 100-104.

Leslie E., Hernandez-Jover M., Newman R., Holyoake P., 2010. Assessment of acute pain experienced by piglets from ear tagging, ear notching and intraperitoneal injectable transponders. Applied Animal Behaviour Science, 127: 86-95.

Martins R.F.S., Paim T.P., Cardoso C.A., Dallago B.S.L., Melo C.B., Louvandini H., McManus C., 2013. Mastitis detection in sheep by infrared thermography. Research in Veterinary Science, 94: 722-724.

Menegassi S.R.O., Barcellos J.O.J., Dias E.A., Koetz Jr C., Pereira G.R., Peripolli V., McManus C., Canozzi M.E.A., Lopes F.G. 2014. Scrotal infrared digital thermography as a predictor of seasonal effects on sperm traits in Braford bulls. International Journal of Biometeorology, DOI 10.1007/s00484-014-0847-z.

Paim T.P., Borges B.O., Lima P.M.T., Dallago B.S.L., Louvandini H., McManus C., 2012. Relation between thermographic temperatures of lambs and thermal comfort indices. International Journal of Applied Animal Sciences, 1(4): 108-115.

Paim T.P., Martins R.F.S., Cardoso C., Dallago B., Louvandini H., McManus C., 2014. Thermal comfort index and infrared temperatures for lambs subjected to. Scientia Agricola, 71(5): 345-355.

Renn N., Onyango J., McCormick W., 2014. Digital Infrared Thermal Imaging and manual lameness scoring as a means for lameness detection in cattle. Veterinary Clinical Science, 2(2): 16-23.

Schaefer A.L., Cook N.J., Church J.S., Basarab J., Perry B., Miller C., Tong A.K.W., 2007. The use of infrared thermography as an early indicator of bovine respiratory disease complex in calves. Research in Veterinary Science, 83: 376-384.

Scolari S.C., 2010. Investigation of skin temperature differentials in relation to estrus and ovulation in sows using a thermal infrared scanning technique. Master Thesis, University of Illinois, Urbana, Illinois, 66pp.

Stewart M., 2008. Non-invasive measurement of stress and pain in cattle using infrared thermography. PhD Thesis, Massey University, Palmerston North, New Zealand, 165pp.

Stubsjoen S.M., Flo A.S., Moe R.O., Janczak A.M., Skjerve E., Valle P.S., Zanella A.J., 2009. Exploring non-invasive methods to assess pain in sheep. Physiology & Behavior, 98: 640-648.

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RESEARCHES REGARDING THE HAEMATOLOGICAL PROFILE OF JUVENILE *CYPRINUS CARPIO* VARIETIES

Monica MARIN, Carmen NICOLAE, D. DRĂGOTOIU, Laura URDEŞ, I. RĂDUCUȚĂ, Georgeta DINIȚĂ

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: marin_monica_zoo@yahoo.com

Abstract

The carp is the main aquaculture species which is omnivorous, eating both vegetable and animal food. The aim was to analyze the research undertaken blood parameters at two varieties of carp fry culture, those common carp (Cyprinus carpio var. typica) and mirror carp (Cyprinus carpio var. specularis), which were grown with both natural food (zooplankton and phytoplankton) and with mixed food (natural and supplementary - cereal mixed with soybean meal). The weight of juveniles ranged between 25-45 g, and the blood parameters were determined during two periods, respectively in August and October. The values of blood parameters showed a physiological response similar to both varieties of carp, for similar living conditions. The number of erythrocytes, haemoglobin and the value of hematocrit were higher in summer than autumn, due to metabolic processes more pronounced in summer, when juvenile fish gets feed more intense. Moreover, the blood parameters were influenced by the variation of environmental factors, respectively the water temperature, its decrease causing the lowering of the analyzed blood parameters.

Key words*: carp, blood parameters, food type, water temperature.*

INTRODUCTION

Although carp is a species tolerant to environmental conditions, in fish farms must be ensured a good quality of water supply for the ponds and a right feeding to get proper productions in terms of economic efficiency. In Romania the carp grown in ponds and lakes, in monoculture or polyculture with other species of cyprinids (Chinese carp).

For the breeding in monoculture, it is recommended the use of small ponds (<5 ha), with water alimentation and independent exhaust. Productions are done in cycles of 2-3 summers (1.5-2.5 years) and may be obtained typically 2-3 t/ha, or 5-6 t/ha when using aerators or big flows of fresh water. Feeding is done traditionally with mixtures of cereals, fish meal and meal (soya or sunflower), usually taken in the farm (Parvu et al., 2003). In the last years it has began to be used extruded feed, which allow obtaining high productions, an excellent meat quality and economic efficiency by reducing the production cycle, of labor and other expenses. Common carp ("Romanian") is an omnivorous fish and it needs feed with a protein content of 25-30%, respectively 7-12% fat (Hangan et al., 2008).

The juvenile in the first year of life needs a feed richer in protein (over 35%).

Carp is harvested during the fall period and most of it is sold in the market, fresh or alive, at a weight of about 1.5-2 kg.

The aim was to analyze the research undertaken blood parameters at two varieties of carp fry culture, those common carp (*Cyprinus carpio* var. *typica*) and mirror carp (*Cyprinus carpio* var. *specularis*), which were grown with both natural food (zooplankton and phytoplankton) and with mixed food (natural and supplementary - cereal mixed with soybean meal).

MATERIALS AND METHODS

In order to follow the dynamics of blood parameters for the juvenile culture carp were analyzed two varieties, respectively the common carp (*Cyprinus carpio* var. *typica*) and mirror carp (*Cyprinus carpio* var. *specularis*), which were raised in two variants of feeding, with natural food (zooplankton and phytoplankton) and with mixed food (natural

and supplementary - cereal mixed with soybean meal).

The tested juvenile carp had relatively similar conditions of life: ponds were enclosed, with a surface area of about 2000 m^2; water supply was made from the same source; depths were similar; populating was done simultaneously with alevins coming from the same natural controlled breeding ponds.

The juvenile carp weight ranged between 25 and 45 g, while the blood parameters were determined in the same year, in two periods, respectively summer and fall, for 2 weeks (table 1).

Table 1. Experimental schema

Date of sampling	Carp species	n	Feeding procedure	Objectives
August	Common carp	40	Natural food Mixed food	Determination of blood parameters: - red blood cells number; - hemoglobin; - hematocrit; - eritrocyte index (mean corpuscular volume, mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration); - leukocyte count.
	Mirror carp	40	Natural food Mixed food	
October	Common carp	40	Natural food Mixed food	
	Mirror carp	40	Natural food Mixed food	

From the blood collected from the level of the caudal artery of carp from the experiment, have been determined using an automatic analyzer a number of blood parameters represented by the red blood cells number – RBC ($x10^6$ cells/µl blood), hemoglobin – Hb (g/dl), hematocrit – Ht (%) and the erythrocyte indici: the mean corpuscular volume – MCV (μm^3), the mean corpuscular hemoglobin – MCH (pg), the mean corpuscular hemoglobin concentration – MCHC (g/dl). It has also been determined the leukocyte count – WBC ($x10^3$/ µl blood) from the sampled blood.

Derived parameters were calculated according to the following formulas:

MCV=(Ht×10)/RBC

MCH=Hbx10/RBC

MCHC=(Hb×100)/Ht

The obtained results were subjected to statistical analysis using the Student test.

RESULTS AND DISCUSSIONS

Data regarding the blood profile of juveniles belonging to the common carp and the mirror carp are shown in the tables 2 and 3.

The red blood cells number varied for the blood samples collected in August from the common juvenile carp between 1.32 x 10^6/µl (in case of natural feeding) and 1.58 x 10^6/µl (in the case of mixed feeding). In October, when the water temperature has decreased, there was a decrease in the recorded values, respectively 1.14 x 10^6/µl (in the case of natural feeding) and 1.40 x 10^6/µl (in the case of mixed feeding), the recorded differences being significantly distinct.

For the juvenile mirror carp, it was found the same trend of red blood cells decrease in the number determined by water temperature drop, respectively by the season. Also, there was a slight increase of the recorded values at this variety of carp (about 5%), which demonstrates a better adaptation to environmental conditions.

The hemoglobin content of the blood collected from juvenile carp recorded similar values for the two varieties, ranging between 7.91 and 5.64 g/dl, significant decreases being seen when water temperature drops to 14°C. In these conditions, higher values can be found in the case of mixed feeding.

A similar situation was observed in the case of hematocrit values, which ranged between 35.78 and 30.63%.

One of the determined erythrocyte indices was MCV which recorded the highest value (268 μm^3) for the both varieties in October, at a water temperature of 14°C, in the case of natural feeding.

Table 2. Dynamics of blood parameters determined for the juvenile common carp

Date of sampling	Number of studied samples	Water temperature (°C)	Feeding procedure	RBCx10^6 cells/µl blood	Hb g/dl	Ht %	MCV µm^3	MCH pg	MCHC g/dl	WBC x10^3/ µl blood
August	40	26	Natural food	1.32± 0.04	6.54± 0.65	32.51± 1.25	246.29± 4.22	49.54± 1.87	20.12± 0.95	63.21± 3.17
	40	25	Mixed food	1.58± 0.05	7.78± 0.44	35.34± 0.99	223.67± 5.04	49.24± 3.26	22.01± 1.25	62.76± 4.73
October	40	14	Natural food	1.14± 0.03	5.64± 0.52	30.63± 1.76	268.84± 3.95	49.47± 4.65	18.41± 1.47	62.34± 2.99
	40	13	Mixed food	1.40± 0.04	6.60± 0.31	33.15± 1.65	236.86± 5.36	47.14± 2.17	19.91± 2.05	61.16± 3.06

Table 3. Dynamics of blood parameters determined for the juvenile mirror carp

Date of sampling	Number of studied samples	Water temperature (°C)	Feeding procedure	RBCx10^6 cells/µl blood	Hb g/dl	Ht %	MCV µm^3	MCH pg	MCHC g/dl	WBC x10^3/ µl blood
August	40	24	Natural food	1.40± 0.05	6.77± 0.22	34.48± 1.37	246.28± 5.76	48.36± 2.69	19.63± 2.63	62.36± 4.14
	40	25	Mixed food	1.60± 0.03	7.91± 0.40	35.78± 1.29	223.62± 4.06	49.44± 1.75	22.11± 0.79	63.11± 3.65
October	40	13	Natural food	1.20± 0.04	5.89± 0.36	32.24± 1.07	268.67± 6.12	49.08± 3.49	18.27± 1.48	61.79± 2.89
	40	13	Mixed food	1.46± 0.03	6.90± 0.29	34.57± 1.32	236.78± 4.92	47.26± 2.75	19.96± 2.15	61.39± 3.26

Another determined erythrocyte indicator was MCH, that registered close values, the lowest being during the fall, in the case of mixed feeding (47 pg).

The third determined erythrocyte indicator was MCHC, which recorded higher values in the case of mixed feeding, for both varieties, both during summer, as well as in autumn (22.11, respectively 19.96 g/dl).

In the case of the leukocyte number, the values obtained did not register significant differences depending on the type of feeding, noticing a slight decrease of the values in the case of water temperature decrease (61.16 x10^3/µl blood).

As a consequence of the undertaken research by applying different technological conditions, different values of carp hematological profile were recorded, varying for RBC 0.70-2.50 x 10^6/µl, hemoglobin between 3.00 and 11.00 g/dl, for hematocrit 20.00-45.00%, for MCV 115-368 µm^3, for MCH 18-69 pg, for MCHC 12-35 g/dl (Bocioc et al., 2015; Darvish et al., 2008; Svobodova et al., 2008; Witeska et al., 2010).

CONCLUSIONS

After the undertaken research regarding the determination of the hematologic profile for the

juvenile carp varieties common carp *Cyprinus carpio* var. *typica*) and mirror carp (*Cyprinus carpio* var. *specularis*) have been established the following aspects:

- The values of blood parameters showed a physiological response similar to both varieties of carp, for similar living conditions.
- The number of erythrocytes, haemoglobin and the value of hematocrit were higher in summer than autumn, due to metabolic processes more pronounced in summer, when juvenile fish gets feed more intense.

The blood parameters were influenced by the variation of environmental factors, respectively the water temperature, its decrease causing the lowering of the analyzed blood parameters.

REFERENCES

Bocioc Elena, Cristea V., Patriche N., Grecu Iulia, Antache Alina, Mocanu (Cretu) Mirela, 2015. Hematological profile of the juvenile carp (*Cyprinus carpio*, L. 1758) reared into a recirculating aquaculture system with probiotics supplement. Bulletin UASVM Animal Science and Biotechnologies, 72 (1) : 8-13.

Darvish Bastami K., Haji Moradlou A., Mohamadi Zaragabadi A., Salchi Mir S.V., Shakiba M.M., 2008. Measurement of some haematological characteristics of the wild carp. Comp.Clin. Pathol., 22-24.

Hangan M., Diaconescu C., Vlase G., Serbanescu M., Nicolae C., 2008. A comparative chemical study on the heavy metal content of fish originating from the Sulina branch of the Danube. Veterinary Medicine Romanian Magazine, AGMVR, 18(2) : 199-205.

Parvu Gh., Costea Mihaela, Pirvu M., Nicolae B., 2003. Tratat de nutritia animalelor. Ed. Coral Sanivet, Bucuresti.

Svobodova Z., Kroupova H., Modra H., Flajshans M., Randak T., Savina L.V., Gela D., 2008. Haematological profile of common carp spawners of various breeds. J.Appl.Ichtyiol., 24, 55-59.

Witeska M., Kondera E., Szymanska M., Ostrysz M., 2010. Hematological changes in common carp (*Cyprinus carpio* L.) after short-term lead (Pb) exposure. Polish J. of Environ. Stud., 19(4) : 825-831.

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THE INFLUENCE OF HIGH TEMPERATURE ON THE PROTEIN FRACTIONS OF BULL SEMEN

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

Academy of Sciences of Moldova, 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

Proteins are the most important organic substances which are part of the composition of living cells. Since the specific capabilities of spermatozoa depend on the protein, occurs the necessity of studying the characteristics of changes of the spectrum of the protein fractions of sperm depending on the ambient temperature. In this experiment was studied the effect of temperature of 45°C on the spectrum of protein fractions of the bull semen. From the data of the conducted research it is observed that when sperm of bull was exposed to a temperature of 45°C for 1 minute, has been a trend of increasing the quantity of albumin up to 4.2% and at 10 minutes - up to 3.3% of the total volume. When exposed to a temperature of 45°C for 1 minute the total amount of globulins, typically does not significantly change. The content of α-globulins tends to decrease, while the β- and γ-globulins do not support the changes. After 10 minutes of stress the amount of albumins is also increasing in comparison with the control sample and the quantity of total globulins decreased, especially α - and γ-globulins, while the content of β-globulin is not changed. Thus, the hyperthermic stress of bull semen for 1 minute is reflected only on the quantity of albumin, which has a tendency to increase. The influence of hyperthermic stress for 10 min is manifested by the tendency of growth of the percentage content of albumin and insignificant decrease in the concentration of total globulins, including α-globulin.

Key words*: protein fractions, spermatozoa, high temperature, hyperthermic stress, bull semen.*

INTRODUCTION

Intensification of reproduction of farm animals is impossible without fundamental studies of biochemical changes occurring in seed material in the process of manipulation with it. On the basis of experimental data it is possible to develop the technological methods of stabilizing the functional activity of the reproductive cells and use them for artificial insemination of animals.

Sperm of cattle is a complex system, the basic structural elements of which make up the spermatozoa. Their chemical composition is dominated by organic substances, which determine the viability and functionality.

Among of organic substances included in the composition of living cells, from the viewpoint of structural and biological roles, the most important are the proteins. They form the basis of protoplasm. The specificity of biological processes mainly depends on the composition of proteins with different structure and function. It is known that the reproduction and development of organisms is determined by the properties of conjugated protein - nucleoproteins of spermatozoa and egg cells (Mereuţă, 2010; Исаева et al., 2007).

From a chemical point of view the proteins belong to physiologically very active substances. Protein fractions that may exist in many states are markedly different from each other. Proteins, characterized by a high cooperative subjected to transformation with a relatively small change and limiting manifestation of the maximal cooperatively is the transition between two states. It is known that the chemical activity of proteins determines the physiological properties of cells (Борончук et al., 2003; Фурдуй et al., 2003; Юрченко, 1988).

The purpose of the research, the results of which are presented in this paper, was to study the characteristics of changes of the spectrum of protein fractions of sperm in depending on the ambient temperature, because the specific

opportunities and tolerance of cells, including sperm inevitably depend on proteins.

MATERIALS AND METHODS

The determination of the content of protein fractions was performed through method developed by Oll and Makford with changes of Carpiuc S.A. by the description of Holban D. M. et al. (Голбан et al., 1988).

The principle of the method is based on the properties of phosphate solutions of various concentrations to precipitate proteins. The value of optical density of solutions of different protein fractions was determined using spectrophotometric methods. We used the spectrophotometer SF-26. The color intensity of the solution was determined at a wavelength of 720 nm. The content of the separated fractions are calculated in percent. As the subject of research was used sperm from 5 bulls of Black and White breed at the age of 3 years. In research were used 68 of ejaculate. For stressing of bull semen. as a stress factor was chosen hyperthermia (45°C). Hyperthermic stressing of sperm was performed in a water bath where the temperature was maintained at 45°C. We studied the effects of short-term (1 minute) and long-term stress (10 minutes) at changes in protein fractions of sperm of the bull. To each experimental sample matched control sample (35°C).

Digital materials are statistically processed using the Student's t-test.

RESULTS AND DISCUSSIONS

Proteins which have chemical individuality. responds differently to different stress factors. This experiment consist of the study of influence of hyperthermic stress on the spectrum of protein fractions of bull semen. Obtained results are shown in table 1.

The data of table show that at the effects on spermatozoa of cattle for 1 minute. compared with control. there has been a shift towards increasing of amount of albumin from 1.9 to 4.2%. After 10 minutes of stressing of albumin increased in comparison with the control variant on 13.3% of the total content of protein fractions.

Table 1

The spectrum of the protein fractions of bull semen during of hyperthermal stress (45 °C)

Name of proteins	Content of protein fractions (%)		
	Control	The experimental variant	
		1 min	10 min
Albumins	1.9±0.43%	4.2±1.03%	13.3±1.01%*.**
Globulins	98.1±2.89%	95.8±1.19%	86.7±4.34%
α-globulins	6.6±1.17%	4.1±1.45%	3.3±0.84%*
β-globulins	32.7±4.38%	31.5±0.99%	30.6±4.8%
γ-globulins	58.8±3.13%	60.2±1.15%	52.8±3.38%

* P<0.05 compared with indicators from control sample

** P<0.05 the difference is statistically authentic between the variants of experience.

The amount of globulin at stressing for 1 minute did not undergo any significant changes. The content of α-globulin decreases from 6.6 to 4.1%, while β- and γ-globulin showed no obvious changes. When exposed for 10 minutes the concentration of globulin was decreased from 98.1 to 86.7% compared with the control. Also decreased the level of α-globulins from 6.6 to 3.3%, and γ-globulins from 58.8 to 52.8%, while the concentration of β-globulins has not changed significantly. Statistically authentic changes are observed not only at modification of content of albumin but and α-globulin in particular. Process of its variability , apparently, is aimed at increasing the adaptability of the whole cellular system of gametes to the action of unfavorable factors of hypothermia and, obviously, can be regarded as a manifestation of cell adaptation to the stress effect of cold, and therefore can provide a higher level of survival of gametes.

It should be noted that these changes occur only at long-term hyperthermic exposure, but probably and at short-term. In this regard, in the next series of experiments it was determined the short-term impact of hyperthermic temperatures (45°C) on protein fractions of sperm of the bull. The results of researches are shown in the diagram (fig. 1).

The figure shows that at short-term hyperthermic influence are subjected to insignificant changes the protein fractions of albumins and α-globulins. At a temperature of 45°C the content of the albumin fraction is definitely growing, and the concentration of α-globulin decreases slightly. Other fractions of proteins in experimental conditions have only a tendency to decrease.

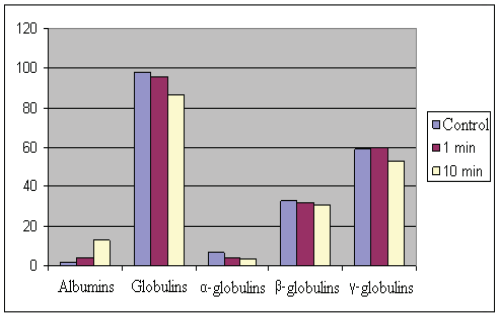

Figure 1. The protein fractions of semen of the bull at hyperthermal stress (45°C)

At short-term hyperthermic stress out of five revealed fractions only the content of albumin is increased but the magnitude of the concentrations of all other fractions is reduced. Because the total protein content of the seed of animals when exposed to high temperatures does not change, it can be explained by the denaturation of proteins and, in particular, these fractions.

It should be noted that the activity of proteins is caused by a sufficient (but not excessive) conformational flexibility. However, this condition is possible only in a limited temperature range, which lies within the optimum temperature of activity of gametes.

Significant temperature changes that occur in the process of conservation should disrupt the optimal ratio of lability and stiffness of cellular structures and thereby cause morphological and functional changes (Наук, 1991).

The reaction of protein macromolecules to the change of temperature are mainly determined by two temperature-dependent specific component included in the entropy of the system multiplied to the absolute temperature: conformational entropy and entropy is determined by hydrophobic interactions. In the field of physiological temperatures their influence is opposite on protein stability that has deep biological sense, since it smoothes the impact of temperature effects on the state of protein macromolecules (Александров et al., 1975).

It should be assumed that gametes must possess a variety of regulatory mechanisms that are, to a certain extent, can compensate for these changes. In the case where environmental factors on the strength and duration, prevail over the capabilities of these mechanisms, conformational mobility of protein molecules can be changed, and then in the cell at different levels of its organization, may occur destructive changes (Борончук et al., 2008).

The temperature change of any biological object can be considered as a powerful stressor effect, which causes a complex set of structural and functional changes (Фурдуй et al., 1992). In cellular systems during freezing don't have time to develop the adaptive change. However, the cooling of the gametes in the area of hypothermic temperatures during equilibration, although accompanied by a sharp decrease of metabolic processes, however, does not stop them completely. This is due to carrying out of adaptive-compensatory reactions.

Given that when the cells are gone from the body some time interval remain viable, can be considered possible to implement in them in this period of self-regulation processes due to the negative feedback aimed to restore the original level of living system as a whole (Юрченко, 1988). The study of adaptive-compensatory reactions of biological objects in the hypothermia is of great importance and is an integral part of the development of the theory of defense mechanisms in biology.

In the case of lack of effectiveness of adaptive-compensatory reactions in cells occur the temperature changes. It should be noted that the most labile cellular structures are membrane (Наук, 1991).

Thus, the hyperthermic stressing of semen of the bull for 1 minute reflects only the tendency towards increasing the percentage content of albumin and decrease of globulin. The outcome of exposure to hyperthermic stress for 10 minutes is manifested by changing upward of the percentage content of albumin, and is reflected downward at concentrations of α-globulin.

CONCLUSIONS

The researches allow making the following conclusions:

1. Changes of the protein spectrum of semen of the bull can be the result of the excess of intensity of the influence of the temperature factor over the adaptive-compensatory reactions of the spermatozoa.

2. Short-term stressing of spermatozoa of the bull does not cause significant changes in the quantitative composition of the investigated proteins.
3. The increased of duration of temperature exposure to ten times accompanied by an increase of content of albumin, while the total amount of globulins and their fractions are not subjected to significant changes.
4. Globular proteins of the sperm of bull are more resistant to the effects of temperature factor compared with their albumin fraction.
5. When creating temperature-protective mediums for sperm of the bull is necessary to consider the ability of their components to stabilize the adaptive-compensatory reactions and the preservation of proteins in particularly their albumin fraction.
6. The increase in the content of albumin fractions after thermal exposure of semen of the bull takes place by reducing the amount of globular proteins.

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REFERENCES

Mereuţă I., 2010. The influences of the factor of hypothermia's action on the spectrum of protein's fractions of bull's semen. In: XIth Middle-European Buiatrics Congress. 5th Symposium of the European College of Bovine Health Management, Brno, 84.

Александров В.Я., 1975. Клетки макромолекулы и температура. Л.: Наука, 330.

Борончук Г.В., Балан И.В., 2008. Структурно-функциональные и биохимические измененитя в биологических системах при криоконсервации. К.: Tipogr. AŞM, 632.

Борончук Г.В., Балан И.В., 2003. Криомембранология. Кишинев: Tipografia Academiei de ştiinţe a Moldovei, 336.

Голбан Д.М., Якуб Т.Г., Донника Г.Г., 1988. Внутренние незаразные болезни. Кишинев: Штиинца, 48.

Исаева В.В., Шукалюк А.И., Ахмадиева А.В., 2007. Стволовые клетки беспозвоночных животных с репродуктивной стратегией, включающей бесполое размножение. В: Биология моря., том. 33 (1), 3–10.

Наук В.А., 1991. Структура и функция спермиев с-х животных при криоконсервации. Кишинев: Штиинца, 200.

Фурдуй Ф.И. et al., 1992. Стресс и адаптация с-х животных в условиях индустриальных технологий. Кишинев: Штиинца, 222.

Фурдуй Ф.И., Павалюк П.П., Чокинэ В.К., 2003. Хронический стресс, диминуация функций и биологическая деградация. В: Buletinul Asciaţiei Medicină Tradiţională din Republica Moldova, 7, 7-11.

Юрченко Т.Н., 1988. Охлаждение и адаптационные процессы в плотных тканях. Достижения и перспективы развития криобиологии и криомедицины. Тезисы Междунар. конф. - Харьков, 96-97.

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THE EFFECT OF EPIDURAL ADMINISTRATION OF FSH IN BOVINE SUPEROVULATION PROTOCOL

Marian OCHEA, Manuela PASCAL, Alexandru ŞONEA, Alin Ion BÎRŢOIU

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, Faculty of Veterinary Medicine of Bucharest, marianochea@yahoo.com, manuelastanescu@hotmail.com, sonea.alexandru@medu.edu.ro, birtoiu_vet@yahoo.com,

Corresponding author e-mail: marianochea@yahoo.com

Abstract
FSH is an important component of superovulations programs in the cow, its administration being made routinely intramusculary, at 12 hours intervals for 4 days. The aim of the study was to evaluate the efficacity of single-dose FSH administration on a mixed route (epidural and intramusculary). 6 cows (Holstein and Montbéliarde) were superovulated according to the following protocol: on day 0 – vaginal application of progesterone spiral (PRID®, CEVA, France); day 7- administration of FSH (Folltropin-V®, Bioniche, Canada) 200 mg epidurally and 200 mg intramusculary; day 9 – administration of 250 mg D- sodium cloprostenol (Prostol®, Syva, Spain) and removal of PRID. Response monitorization was made by ultrasound exams of the ovaries on days 12 (during estrus) and 19 (the day of embrion collection). On average, 14 follicles (minimum 8, maximum 20) and 9 corpora lutea (minimum 6, maximum 12) were identified per donor compared to 15 follicles (min 8 max 22) and 9.5 corpora lutea (7 minimum, maximum 12) per donor in the routine protocols. The results show a very close efficacy to those obtained by classical protocol with daily intramuscular administration of FSH. Due to the epidural administration of FSH, deduction of stres for the donor and of the time of treatment, the method can be used in the protocols of embryotransfer in the cow.

Key words: *FSH, cow, poliovulation, embriotransfer.*

INTRODUCTION

Obtaining calves with high genetical value is a major goal for farm practicioners. Difusion of genetic progress in a bovine population can be accomplished by two strategies: the paternal way, by increasing the intensity of artificial inseminations with semen from a given male and through maternal way, when a female produces more than one calf per year (gemelar gestation). Since gemelar pregnancies are not frequent enough in the bovine population, the only way for maximizing the maternal genetical inheritance remains the embryotransfer.

Over the years, many protocols have been tried in order to obtain as many embryos as possible from a certain donor. Different hormonal products based on seric gonadotrophins or GnRH have been tried, but the most efficient of all proved to be the purified porcine FSH extract.

At the same time, a constant goal was to reduce the stress for females and to simplify the superovulations protocols, in order to collect more embryos from the donor and of better quality.

MATERIALS AND METHODS

Six dairy cows donors (Montbeliarde and Holstein breeds) were selected for embryo collection. Donor sellection was made in accordance with the folllowing phenotypic characteristics: body conformation, milk production, reproductive activity and physico-chemical caractheristics of milk.

Throughout the whole study time, the donor cows were housed in comfortable and well ventilated shelters, with ad libitum access to fresh water and they were fed with optimized nutritional ratios according to their physiological status and production group.

The following products were used: FSH (Folltropin-V®, Bioniche, Canada), a slow release progesterone device manufactured of a silicone elastomer (PRID®, CEVA, France) and D-sodium cloprostenol (Prostol®, Syva, Spain), a synthetic omologue of $PGF_{2\alpha}$.
In order to monitor the ovarian response determined by the superovulaton protocol, we used a linear Tringa ultrasound machine (Esaote®, Olanda).
Treatment was initiated in the 4th day of the estral cycle. The introducing of the progesterone slow releasing intravaginal device (PRID) (fig. 1) was considered day 0 of the superovulation protocol.

Figure 1. Devices and applicators used for intravaginal insertion

On the 7th day, purified FSH obtained from swine pituitary gland (Folltropin-V) (fig. 2) was administered in a mixed manner: 200 mg epidurally and 200 mg intramusculary (Tasdemir et al., 2012).

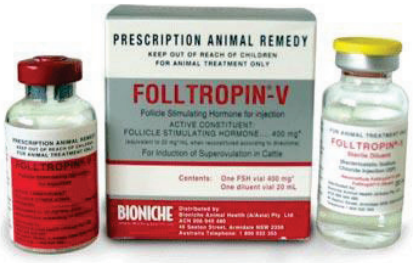

Figure 2. Product Folltropin used in poliovulation protocols

In the 9th day, $PGF_{2\alpha}$ was administered intramusculary and PRID was extracted.

In the 11th day of the treatment protocol, the donors showed signs of heat and they were ineminated with frozen semen straws (first insemination at the begining of the heat, than 2 more inseminations 10 – 12 hours apart) (the AM / PM scheme) (fig. 4).
The ovarian response was monitored through ultrasound examinations starting at 3 days after the administration of $PGF_{2\alpha}$ in order to identify ovarian follicles (Hanzen, 2008; Mapletoft and Hasler, 2014).
The next ultrasound exam was conducted in the day of embryo collection, in order to identify the luteal bodies formed after ovulation.
Classical poliovulation protocols include administration of FSH for 4 days, b.id. This makes the protocol more time consuming and more stressful for the females (Robertson, 2005) (fig. 5).
Statistical analysis was done with SPSS ver. 18 (IBM, USA).

RESULTS AND DISCUSSIONS

Following the ultrasound exam of donors during estrus, a mean of 14 follicles per donor was identified. (fig. 3). There were donors with only 8 preovulatory follicles (1,8 – 2,2 cm diameter). This were mainly older females. In other donors, 20 ovarian follicles were identified. These were young, primipaorus or secundipaorus females, just starting their productive lives.

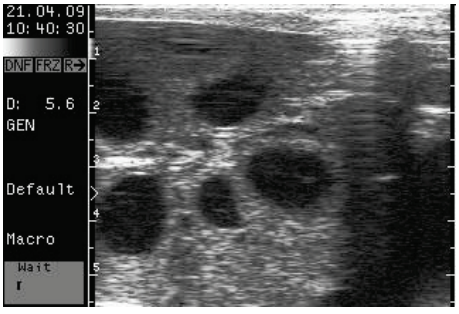

Figure 3. Ultrasound image with superovulated ovary (original)

In the classic protocols with a b.id. administration of FSH for 4 days, a mean of 15 follicles per donor was reported (Bó et al., 2004).
There is not a significant difference ($p > 0,05$) between the results obtained in the study and the classic protocol.
In the day of embryo collection, a mean of 9 luteal bodies (minimum 6 and maximum 12) was seen at the ultrasound exam.
For the classic superovulation protocols, a mean of 9,5 luteal bodies per donor was reported (Bó et al., 2004).
There is no statistical difference ($p > 0,05$) between the mixed way protocol and the classic one.
Due to the unique administration of the FSH in a mixed manner (epidurally and intramusculary) is easier, faster and less stressful for the donors. At the same time, the results regarding the ovarian response are almost similar for the two superovulation protocols.

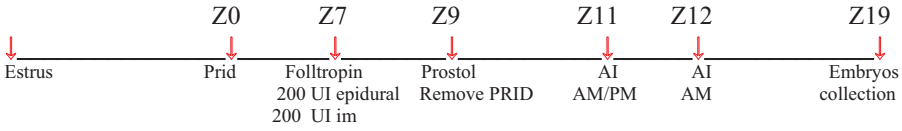

Figure 4. Graphic representation of mixed FSH administration

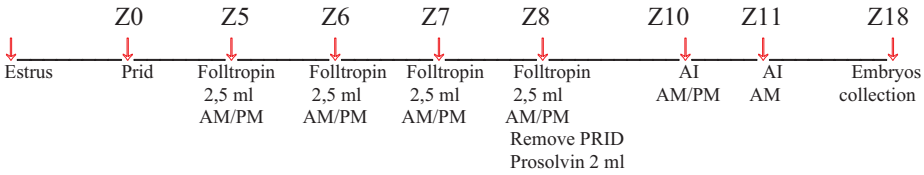

Figure 5. Classic protocol for superovulation (FSH is administered 8 times).

CONCLUSIONS

The results show a very close efficacy for the mixed way administration of FSH (epidurally and intramusculary) to the classical protocol with daily intramuscular administration of FSH. Due to the epidural administration of FSH, the stress over the donor is significantly reduced, the time for treatment is lowered. Thereby, we state that the method can be used in the poliovulation protocols for bovine embryotransfer.

REFERENCES

Ambrose J.D., Drost R.L., Monson R.L., Rutledge J.J., Leibfried-Rutledge M.L., Thatcher M.J., Kassa T., Binelli M., Hansen P.J., Chenoweth P.J., Thatcher W.W., 1999, Efficacy of timed embryo transfer with fresh and frozen in vitro produced embryos to increase pregnancy rates in heat-stressed dairy cattle. J. Dairy Sci, 82: 2369-2376.

Atsushi I., Shin-ichi Sakai, Yuuki Nakamura, Manami Urakawa, Koh Hayama, Kanami Tsuchiya, Hiroshi Fujiwara, Yoshito Aoyagi, 2010 - Administration of peripheral blood mononuclear cells into the uterine horn to improve pregnancy rate following bovine embryo transfer, Animal Reproduction Science 117, 18–23.

Bó G.A., Baruselli P.S, Mapletoft R.J., 2012. Increasing pregnancies following

synchronization of bovine recipients, Anim Reprod, v.9, n.3, p.312-317.
Bó G.A., Moreno D., Cutaia L., Baruselli P.S., Reis E.L., 2004. Hormonal manipulation of the estrous cycle in bovine embryo donors and recipients. Acta Scientiae Veterinariae, 32 (Suppl): 1-22.
Descôteau L., G. Gnemmi, J. Colloton, 2009. Guide pratique d'echographie pour la reproduction des ruminants, Publishing Med'Com, Paris, Chap. 8, 129-149.
Geoffrey A.H., 2001. Arthur's veterinary Reproduction and Obstetrics, (Eighth edition), Publishing Elsevier Limited, 819-831.
Grimard B., Chastant S., Boin E., 2003. Gynécologie bovine - Atlas d'echographie, bases et apllications pratique, Intervet et Ecole Nationale Vétérinaire d'Alfort.
Hanzen Ch., 2008. La production d'embryons in vivo dans l'espèce bovine. http://www.therioruminant.ulg.ac.be/notes/2008 09/R30_Embryons_invivo_2009_PWP.pdf
Lamb G.C., 2011. Embryo transfer: managing donors and recipients, Proceedings, Applied Reproductive Strategies in Beef Cattle August 31 – September 1, Joplin, MO.
Mapletoft R.J,.Hasler J.F., 2014. Embryo transfer 101 with a technical slant N.p.n.d. Web. 3 feb.
Robertson E., 2005. Non surgical embryo transfer, 11-th edition, Harrogate Genetics, International,
S. Buczinski, L. Descôteau, 2009 – Echographie des bovins, Publishing Point Vétérinaire, Pays-Bas, Chap. 6, 110 – 127.
Sales J.N.S., Souza J.C., 2005. Timing of artificial insemination and embryo production in superovulated Holstein cattle, Anim. Reprod., 2 (3) : 183-186.
Saumande J., 1995. La production d'embryons chez les bovins: Quelles voies de recherches pour augmenter l'efficacite des traitements de superovulation? INRA Prod. Anim., 8 (4), 275-283.
Umut Taşdemir, Muharrem Satilmiş, Tahir Karaşahin, Sedat Hamdi Kizil, Mustafa Kaymaz, Kei Imai, 2012. The effect of single epidural plus intramusculer injection of FSH on superovulatory response in Anatolian Black cow, Ankara Üniv Vet Fak Derg, 59, 211-216.

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EFFECT OF LIPOSOMAL PREPARATE WITH SOME ORGANIC TRACE ELEMENTS ON ANTIOXIDANT STATUS AND REPRODUCTIVE ABILITY OF FEMALE RABBITS

Oksana SHTAPENKO[1], Ivan GEVKAN[1], Yevgen DZEN`[2], Svetlana FEDOROVA[1], Yurij SLYVCHYK[1], Vasyl SYRVATKA[1], Iryna MATIUKHA[1]

[1]Laboratory of Reproductive Biotechnology and Animal Breeding,
[2]Laboratory of Metabolism, Institute of Animal Biology of Lviv, 79034, V. Stusa str., 38, Lviv, Ukraine, Phone: +380-32-260-07-95, Fax: + 80-32 - 270-23-89, Email: shtapenko31@gmail.com

Corresponding author email: shtapenko31@gmail.com

Abstract

The paper aimed to investigate the influence of subcutaneous injections of organic microelements in liposomal forms on the performance antioxidant status in blood of female rabbits and their reproduction function during the early stage of pregnancy. Two weeks before fertilization females from experimental group were subcutaneous injected preparations with Zn glutamate, Mn glutamate, Cr methionine, NaSe with vitamins E, A, D in liposomal form. Reproductive organs and blood samples were obtained after hormonal induction and fertilization on 14th day of gestation. Our results showed that supplemental organic trace elements increased the number of implantations and corpus luteum in the ovaries in the experimental group as compared with the control group. The activity of catalase has decreased in the experimental group compared with control group. However, the increasing ceruloplasmin activity in experimental group was noted. Liposomal preparation injection induced the significantly decrease of content oxidatively modified proteins (OMP) in the blood of the experimental group ($p<0.05$; $p<0.01$) compare to that index in control group. Accordingly, TBARS level in experimental group significantly ($p<0.01$) decreased, as compared with the control. The results of our study indicate that supplementation organic microelements in liposomal form before 2 weeks and during fertilization has a positive effect on the reproductive ability of female rabbits to improve the pregnancy, implantation rates. Addition of organic microelements in liposomal form provided increase of antioxidant defense system and lower intensity of peroxidation.

Key words: *organic trace elements, rabbits, reproduction.*

INTRODUCTION

Organic trace minerals make more of the trace mineral bioavailable to the animal than inorganic trace minerals. Numerous studies in cattle, rabbits and poultry have shown improvements in reproductive performance, immune system function and mineral status when complexed organic trace minerals were used. Bioavailability of organic Zn, Cu, Mn and Se relative to inorganic salts has been evaluated in many studies (Pavlata et al., 2012; Cao et al., 2000). Zinc functions as a catalytic, structural and signaling factor in the regulation of a diverse array of cellular pathways involving hundreds of enzymes and proteins. Zinc is an important factor necessary for regulating the meiotic cell cycle and ovulation (Kim A.M., 2011). Many studies have shown that zinc deficiency before conception causes fertility and pregnancy problems, abnormal embryo and fetal development. The study by Tian X. and Diaz F. (2013) noted that zinc deficiency decreases histone and DNA methylation in oocytes. Manganese is an essential element utilized by antioxidants, including superoxidedismutase (MnSOD), and others metalloenzymes that take part in reduction reactions, in multiple physiological processes including reproductive system (Kim S. I., 2012).

Added to that, the combination of trace minerals used in the present study can help assure delivery of the essential trace minerals that can affect reproduction, including implantations rates, embryo development during the early stage of pregnancy.

The objective of this study was to evaluate the effect of subcutaneous injections of organic microelements in liposomal form for 2 weeks prior to mating on implantations and on

antioxidant status in blood of female rabbits during the early stage of pregnancy.

MATERIALS AND METHODS

The study was conducted on female rabbits divided into two groups: experimental and control. Female rabbits experimental group were subcutaneous injection 5 ml liposomal preparation with Zn glutamate (35 µg/kg), Mn glutamate (32 µg/kg), Cr methionine (60 µg/kg), NaSe (20 µg/kg) with vitamins E, A, D two weeks before fertilization.

Artificial insemination with appropriate hormonally treatment was performed in all group of animals. We used 40 IU PMSG (Follimag, Intervet, Holland) for synchronized cycle (was injected 48 h before AI) and 20 µg/doe GnRH (Gonadotropin-releasing hormone) (Fertagil, Intervet, Holland) for induction of ovulation (was injected at the moment of insemination). Rabbits were fertilized intravaginally of 10×10^6 spermatozoa/doe in 0.5 ml tris-citrate diluents. Rabbits were slaughtered on day 14^{th} of gestation. The weight of ovarian and uterine, number of implantations and corpus luteum, the indices of fertilization and pregnancy were determined.

The blood samples were collected for determining antioxidant enzyme activities and levels of lipid peroxides. Activities of antioxidant enzymes such as catalase, ceruloplasmin, and, as well as oxidative stress biomarkers (thiobarbituric acid reactive substances (TBARS) and stable 2.4-dinitrophenyl hydrazine derivates of the oxidative modified carbonyl groups level) were measured.

RESULTS AND DISCUSSIONS

The effects of liposomal preparation on reproductive parameters are summarized in table 1. The mean number of corpus lutea in experimental group and the control group were 11.8±0.74 and 10.8±1.02, respectively. Data analysis showed that the number of corpus lutea in the experimental group increased in compare with the control group.

The injection of liposomal preparation with some organic trace elements showed positive effect on the female in number of implanted embryos. However, the number of resorption in the female rabbits treated with liposomal preparation and control group were in similar level. While the pre- and post-implantation losses were lower in experimental group liposomal-treatment animals than the control (Table 1).

Table 1. Effect of liposome preparation on reproductive ability of female rabbits

Parameters	Control	Experimental
Number of corpora lutea	10.8±1.02	11.8±0.74
Number of implantation sites	9.8±1.52	10.8±0.74
Total Live Fetuses	9.2±1.36	10.2±1.14
Number of resorption sites	0.4±0.24	0.4±0.24
Pre-implantation losses (%)	11.1	10.2
Post-implantation loss (%)	4.2	3.7
Total gestational losses	14.8	13.5

Values are given as mean ± SD for 5 rabbits in each group.

Our results agree with those obtained by Diaz Francisco J. et al., 2014. They used zinc prior to ovulation, and it had marked positive effects on the mice's fertility. The study by Alikwe P.C.N. et al., 2011, showed that dietary supplementation of rabbits with zinc was carried out to determine its effects on reproduction performance and growth rate of rabbits.

We have studied the influence of liposome preparation with some organic trace elements on antioxidant status and lipid peroxidation in the female rabbits during the early stages of pregnancy. The obtained data showed that catalase activity decreased compared with control values (Figure 1). However, the increasing ceruloplasmin activity in experimental group was noted (Figure 2).

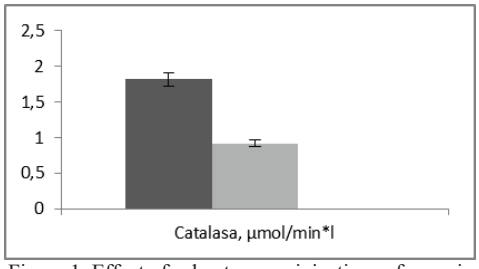

Figure 1. Effect of subcutaneous injections of organic microelements in liposomal form on catalase activity in female rabbits

Values are expressed as mean ± SD for 5 rabbits in each group.

Catalase is one of the important antioxidant enzymes regulating the levels of intracellular hydrogen peroxide and hydroxyl radical. It is known that the trace elements as Zn, Mn, Cu, Se are involved in the metabolic activities via metalloenzymes (Cu-Zn SOD, Mn SOD, Catalase, GSH-Px, etc.), which are essential for the antioxidant protection of cells (Ozturk-Urek R. et al., 2001).

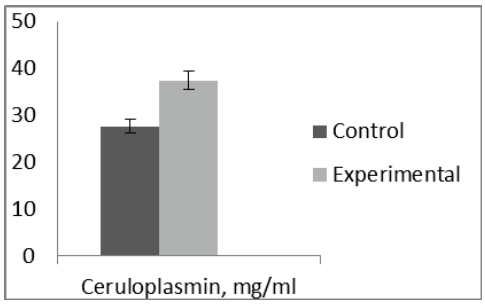

Figure 2. Effect of subcutaneous injections of organic microelements in liposomal form on ceruloplasmin activity in female rabbits.
Values are expressed as mean ± SD for 5 rabbits in each group.

In our study the decreasing of catalase activity in experimental female rabbits which might be having been due to depletion or inhibition of the enzyme was found. As a result of decreasing of catalase activity production of free radicals increased during early gestation period, but serum ceruloplasmin activity in experimental group increased as compared to the control group.

Figure 3 show the effect of administration of liposomal preparation with some organic trace elements on serum lipid peroxidation and oxidative modification of proteins. Thiobarbituric acid reactive species (TBARS) level significantly decreased ($p<0.01$) up 1.76±0.06, when compared with 2.64±0.06 in the control value (Figure 3). The observed decrease of TBARS concentration in co-treated group is agreement with investigations Oshiro M., 2001, and can be explained by the enhanced activities of SOD. In many papers it have described the correlation between plasma concentration of the level of trace elements and SOD activity of erythrocytes, because copper, zinc and magnesium are the main components of SOD that plays a vital role as an antioxidant and protects from oxidative stress.

Intensification of free radicals oxidation leads to oxidative modification of proteins (OMP), destruction of nucleic acids, sugars, and causes to structural and metabolic damages in the cells. Initiation of OMP is the most dangerous link in the cell damages, which leads to cytoplasmic enzymes and membrane ion pumps inactivation with gradual initiation different mechanisms of cell apoptosis.

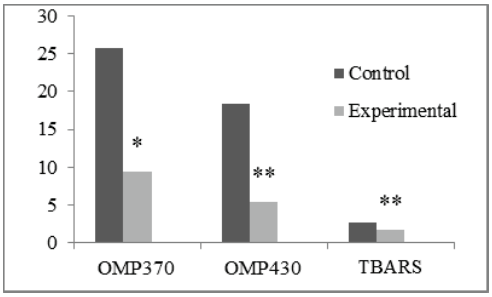

Figure 3. Effect of subcutaneous injections of organic microelements in liposomal form on OMP content and TBARS level in female rabbits

Oxidatively modified proteins (OMP) content, measured by quantity of carbonyl oxidation (aldehyde derivates, OMP_{370}; ketonic derivates, OMP_{430}) in the blood of females rabbits from control (non-treatment) and liposomal preparation injected experimental groups.

Values are expressed as mean ± SD for 5 rabbits in each group. Figures in parenthesis are differences relative to control Significantly different from control * - ($p<0.05$); ** - ($p<0.01$).

However, destruction of proteins is a more reliable marker of oxidative damages in tissues then the products of lipid peroxidation, because derivatives of OMP are more stable. The oxidatively modified proteins content in the serum of female rabbits experimental group, measured as carbonyl oxidation levels, are shown in Figure 3. Liposomal preparation injection induced the decrease of carbonyl oxidation level (aldehyde derivatives) in the blood of the experimental group ($p<0.05$) compare to that index in control group. Similarly, the ketogenic derivatives of oxidatively modified proteins level experimental animals was significantly lower ($p<0.01$) than in control group.

CONCLUSIONS

The results of this study indicate that subcutaneous injection organic forms of trace elements in liposomal preparation 2 weeks before fertilization improves female rabbits' reproductive performance and is efficacious in enhancing implantation rates and also promotes the normalization of oxidation-antioxidant balance during pregnancy. The activity of antioxidant enzymes was significantly decreased by supplementation of liposomal preparation indicating improvement in the antioxidant activity and decrease oxidative stress to female rabbits during early state of gestation.

REFERENCES

Alikwe P.C.N., Ojezeh T.I., Olagboye S.A., 2011. Effects of zinc supplement on rabbits performance and growth rate. J. of Agriculture and Social Research (JASR), Vol. 11(2):46-50.

Cao J., Henry P.R., Guo R., Holwerda R.A., Toth J.P., Littell R.C., Miles R.D., Ammerman C.B., 2000. Chemical characteristics and relative bioavailability of supplemental organic zinc sources for poultry and ruminants. Journal Animal Science, 78:2039-2054.

Diaz F.J., Tian X., Anthony K., Neuberger T., 2014. Preconception zinc deficiency disrupts postimplantation fetal and placental development in mice. Biol. Reprod., 90(4):83.

Kim A.M, Bernhardt M.L, Kong B.Y, Ahn R.W, Vogt S, Woodruff T.K, O'Halloran T.V., 2011. Zinc sparks are triggered by fertilization and facilitate cell cycle resumption in mammalian eggs. ACS Chem. Biol, 6:716-723.

Kim S.I., Jang Y.S., Han S.H., Choi M.J., Go E.H., Cheon Y.P., Lee J.S., Lee S.H., 2012. Effect of Manganese Exposure on the Reproductive Organs in Immature Female Rats. Devel. and Reprod., Vol.16(4):295-300.

Nazifi S., Saeb M., Abangah E., Karimi T., 2008. Studies on the relationship between thyroid hormones and some trace elements in the blood serum of Iranian fat-tailed sheep. Vet. Arhiv, 78 (2):159-165.

Oshiro M., Mimura S, Hayakawa M., Watanabe K., 2001. Plasma and erythrocyte levels of trace elements and related antioxidant enzyme activities in low-birthweight infants during the early postnatal period. Acta Paediatrica, Vol. 90 (11):1283–1287.

Ozturk-Urek R., Bozkaya L.A, Tarhan L., 2001. The effects of some antioxidant vitamin- and trace element supplemented diets on activities of SOD, CAT, GSH-Px and LPO levels in chicken tissues. Cell Biochem. Funct., 19:125-132.

Pavlata L., Mišurova L., Pechova A., Dvořak R., 2012. Comparison of organic and inorganic forms of selenium in the mother and kid relationship in goats. Czech J. Anim. Sci., 57(8):361–369.

Rukgauer M, Neugebauer R.J, Plecko T., 2001. The relation between selenium, zinc and cooper concentration and the trace element dependent antioxidative status. J. Trace Elem. Med. Biol., 15:73-78.

Tian X., Diaz F., 2013. Acute dietary zinc deficiency before conception compromises oocyte epigenetic programming and disrupts embryonic development. Dev. Biol., 376(1):51-61.

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RESEARCH ABOUT PRODUCTIVE AND ECONOMIC PARAMETERS OF RSC ACTIVE POPULATION FROM THE BUCHAREST AREA OF MILK SUPPLY

Dana BARBA, Gabriela-Lucica MARGARIT

University of Agronomic Sciences and Veterinary Medicine of Bucharest, 59 Mărăşti Blvd, District 1, 011464, Bucharest, Romania, Faculty of Biotechnologies,
Phone: +40 (21) 318 22 66, Fax: +40 (21) 318 28 88,
E-mail: danutzabarba@gmail.com; gabriela.margarit@biotehnologii.usamv.ro

Corresponding author e-mail: danutzabarba@gmail.com

Abstract

This paper explores breed characteristics of the Romanian Spotted Cattle, as far as the parameters of milk production and economic ones from Pantelimon, Mogosoaia and Afumati farms. For the purpose of determining total duration of lactation, milk production on total and normal lactation, the proportion of protein on total and normal lactation, the proportion of fat on total and normal lactation have been calculated using the statistics indicators: average, the variation, standard deviation, standard error of the average and the coefficient of variability. The researches carried out showed that the highest total duration of lactation has been obtained by Romanian Spotted Cattle on the Afumati farm (334.68 ± 1.82 days) with approximately 2.5% higher than lowest total duration of lactation (326.46 ± 1.87 days) at the Mogosoaia farm. The best average production of milk has been obtained at the Pantelimon farm 8092.63 ± 35.53 kg, top with 6,45% than that of Afumati farm and 17.85% than milk production obtained in Mogosoaia farm. Regard to the results obtained on average quantity of milk on normal lactation, showed that again the Pantelimon farm have been reach out the best results. Regarding at the percentage of fat, it showed that the best results have been obtained in the Mogosoaia farm 3.9 ± 0.004% followed by Pantelimon farm 3.75 ± 0.003 % and Afumati farm with 3.7 ± 0.004 %. About the developments in the milk quantity, of the percentage of protein and a percentage and quantity of fat in relation to lactation, it is found that biological material from the farms named above has a good precocity in the direction of milk production.

Key words*: breed, cattle, farm, percentage of protein/normal lactation, percentage of fat/normal lactation.*

INTRODUCTION

Cattle were in the economy in general and agriculture in particular, an important socio-economic features that result from their main function, food function, as it provides 96% of the world production of milk, 30% of the world production of meat and over 90% of the world production of skins and directly participate in the growth, development and health insurance mankind (Georgescu, 1990; Georgescu, 1993; Pantazi, 2000).

This paper studies the characteristics of the Romanian Spotted Cattle breed, in terms of milk production and economic parameters in Pantelimon, Mogosoaia and Afumati farms.

MATERIALS AND METHODS

Milk production is the main objective pursued in milk production operation of the three farms analyzed.

For the purpose of determining total duration of lactation, milk production on total and normal lactation, the proportion of protein on total and normal lactation, the proportion of fat on total and normal lactation have been calculated using the statistics indicators: average, the variation, standard deviation, standard error of the average and the coefficient of variability (Bockisch et al., 1999; Calin et al., 1999).

Total lactation duration is the time elapsed from the time of birth and at weaning cows (Banica, 1995; Bordeianu, 1991).

RESULTS AND DISCUSSIONS

Data on total lactation length in relation to the sequence of lactations and average populations analyzed are described in tables 1, 2 and 3:

Table 1. Total lactation length in relation to the sequence of lactations of cows in the Pantelimon farm

Nr.crt	Specification	n	x± s_x	s	v%
1	First lactation	75	324.17±3.44	98.97	30.53
2	Second lactation	55	336.13±3.49	83.90	24.96
3	Third lactation	48	330.17±4.32	88.02	26.66
4	Forth lactation	24	336.22±5.63	94.41	28.08
5	Fifth lactation	16	333.15±7.54	101.79	30.53
6	Sixth lactation	12	318.23±10.57	110.87	34.84
Average		**-**	**329.68±1.90**	**94.72**	**28.73**

Table 2. Total lactation length in relation to the sequence of lactations of cows in the Mogosoaia farm

Nr.crt	Specification	n	x± s_x	s	v%
1	First lactation	124	320.12±3.44	97.26	30.38
2	Second lactation	84	333.84±4.49	84.65	25.36
3	Third Lactation	61	328.27±4.84	84.12	25.63
4	Forth Lactation	46	334.27±5.62	92.28	27.61
5	Fifth Lactation	22	325.05±6.64	98.68	30.36
6	Sixth Lactation	18	317.21±8.56	101.25	31.92
Average		**-**	**326.46±1.87**	**92.08**	**28.21**

Table.3. Total lactation length in relation to the sequence of lactations of cows in the Afumati farm

Nr. crt	Specification	n	x± s_x	s	v%
1	First lactation	155	325.32±3.21	96.54	29.68
2	Second lactation	126	341.86±3.68	89.62	26.22
3	Third lactation	71	340.42±4.73	84.95	24.95
4	Forth lactation	50	345.36±5.52	96.52	28.07
5	Fifth lactation	25	329.18±7.84	101.24	27.95
6	Sixth lactation	14	325.94±8.26	95.68	29.36
Average		**-**	**334.68±1.82**	**98.26**	**29.36**

Following research revealed that the highest total duration of lactation was achieved by herd on the Afumati farm, about 2.5% higher than the lowest total duration of lactation found in Mogosoaia farm.

The amount of milk is expressing in total lactation, normal lactation, in maturity equivalent and productive life.

The best average milk production was achieved in Pantelimon farm (8092.63 ± 35.53 kg), top with 490.18kg (ie 6.45%) than production in Afumati farm with 1225.87 kg (ie 17.85%) than the milk production achieved in Mogosoaia farm.

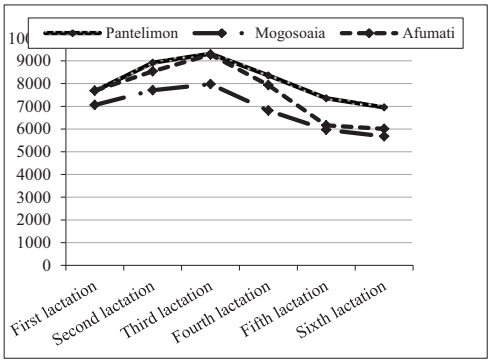

Figure 1.The variation of milk production / total lactation in lactating dairy cows succession from farms Pantelimon, Afumati and Mogosoaia (kg milk / total lactation)

The RSC cows achieved an average amount of milk / normal-lactation at 7178.37 ± 165.65 kg in Pantelimon farm, 6485.33 ± 142.34 kg in Afumati farm and 5641.16 ± 34.28 kg in Mogosoaia farm.

The milk production/normal lactation in Pantelimon farm is greater by approx. 10.75% dairying/normal lactation than Afumati farm and with approx. 27.24% than milk production/lactation normal in Mogosoaia farm and place the Pantelimon farm on top of the pyramid farm improvement, as performances are unique.

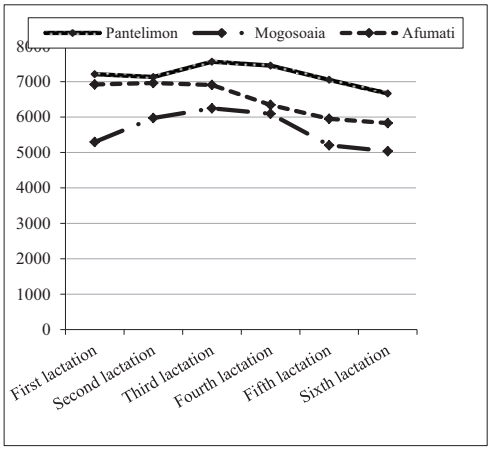

Figure 2.The variation of milk production / normal lactation in lactating dairy cows' succession from farms Pantelimon, Afumati and Mogosoaia (kg milk / normal lactation)

Fat percentage (among other parameters - milk protein, including casein, especially k-casein, somatic cell count, total plate count, etc.) was and is an important parameter that defines milk quality reflecting particularly in butter yield obtained after processing it.

After analyzing the data obtained from the three farms located in the study, the greater percentage of fat on total lactation was achieved in Mogosoaia farm (3.9 ± 0.004%), followed by Pantelimon farm with 3.75 ± 0.003% and Afumati farm with 3.7 ± 0.004%.

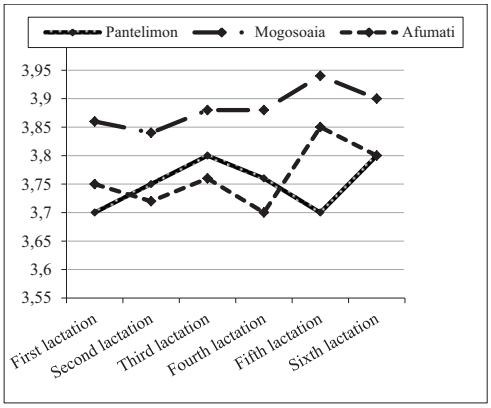

Figure 3.The variation of fat percentage / total lactation in lactating dairy cows succession from farms Pantelimon, Afumati and Mogosoaia

The highest average percentage of fat on normal lactation was found at Mogosoaia farm (3.88 ± 0.005%) with a lower value of the coefficient of variation 5.56% which shows a good homogeneity of biological material from this farm. Compared to the same indicator found in other farms located in the study, Mogosoaia farm fat percentage is superior to the one found on the Afumati farm (cca 2.37%) and 3.19% from Pantelimon farm.

The amount of fat is a basic criterion in the selection of dairy cows because it offers synthetically information about both the quantity and quality of milk. Data analysis showed that the greatest amount of fat / total lactation was obtained at Pantelimon farm 305.55 ± 1.39 kg, recording a high variability (23.32%).

The pure fat is superior to other elite farms of cows in the country, but is lower than that achieved by other strains Holstein-Friesian (by 46% compared to the American Holstein, by 34% compared with Israeli Frieze, by 16-18% from European strains Danish, Dutch, German, Swedish and Italian Frieze).

In other farms located in the study, the average amount of fat / total lactation ranged between 266.67 ± 1.42 kg at Mogosoaia farm and 285.47 ± 1.46 kg at Afumati farm.

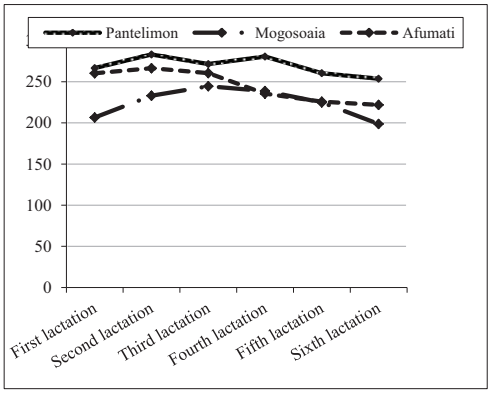

Figure 4.The variation of fat quantity (kg) / normal lactation in lactating dairy cows succession from farms Pantelimon, Afumati and Mogosoaia

The amount of fat on normal lactation in Pantelimon farm was 269.34 ± 4.45 kg, Afumati farm to 245.09 ± 4.52 kg, and the smallest amount of fat obtained was at Mogosoaia farm (224.4 ± 3.37 kg).

The cow populations breed and improved from farm Pantelimon is a plus version; it is

not situated in the amount of fat made by RSC, which are active populations subject to improvement of performance from animal husbandry countries.

CONCLUSIONS

From research done we have a number of useful conclusions on the state of knowledge of RSC breed improvement in our country:

- amount of milk varied in relation to the total duration of lactation, the cows in the herd analyzed varied between 334 days to Afumati farm, 329 days Pantelimon farm to and 326 days Mogosoaia farm. The data regarding the real total lactation milk production shown a balance obtained between 8029 kg at Pantelimon farm and 6866 kg at Mogosoaia farm and on normal lactation milk yields varied between 7187 kg milk at Pantelimon farm and 5641 kg milk at Mogosoaia farm;
- the evolution of the amount of milk in relation to the lactation certify a good early in lactation milk production of biological material from the analyzed farms, which produces 37 to 46.5% more than the average RSC active population in the country;
- milk fat content / total lactation from the population analyzed cows varied within relatively close: in Mogosoaia farm was obtained 3.9 ± 0.004%, in Pantelimon farm 3.75 ± 0.003 and in Afumati farm 3.7% ± 0.004%; the fat percentage in normal lactation varied between 3.76% at Pantelimon farm and 3.88% at Mogosoaia farm;
- the total amount of fat on total lactation varies between 266 kg in Mogosoaia farm and 305 kg to Pantelimon farm; the total amount of fat on normal lactation gets the most amount of fat in Pantelimon farm 269.34 ± 4.45 kg fat / normal lactation, in Afumati farm were obtained 245.09 ± 4.52 kg fat / normal lactation and lowest amount of fat was obtained in Mogosoaia farm 224.4 ± 3.37 kg (with about 20.02% lower than Pantelimonfarm and approx. 9.22% lower than in Afumati farm). Both fat percentage and the amount of pure fat are much lower than those achieved in Western European countries, ie 1-18% and 91-199%.

REFERENCES

Banica T., 1995. Studii de fezabilitate pentru fermele de vaci. Lucr. Stiintifice, I.C.P.C.B. Balotesti, vol. 15.

Bockisch F.J., Reusch S., 1999. Evolution of dairy cows in loose housing systems with deep litter and/or different surface of walking areas as basis to improve the design of walking and lying areas, Institut fur Landtechnik der TU Munchen-Weihensteephan.

Bordeianu C., 1991. Caile de sporire a productiei de lapte , Ed. Agro-silvica, Bucharest

Calin I., Vidu Livia, 1999. Conveerul verde - o tehnologie eficienta de hranire a vacilor de lapte. Rev. Crescatorului de taurine, no.6, Bucharest.

Georgescu Gh. et al., 1990. Tehnologia cresterii bovinelor, Ed. Didactica si Pedagogica, Bucuresti.

Georgescu Gh., 1993. Strategia cresterii vacilor de lapte in economia de piata a Romaniei. Rev. de Med. Vet. si crestereaanimalelor, no.6-7, Bucharest.

Pantazi D., 2000. Cercetari privind performantele productive si reproductive la rasa BNR din Moldova. Teza de doctorat, U.S.A.Iasi.

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STUDY ON THE TECHNOLOGY USED FOR ADAPTING THE BEES TO THE PEDOCLIMATE CONDITIONS OF ROMANIA

Gabriel Dan BIRLOIU, Ștefan DIACONESCU, Carmen Georgeta NICOLAE

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: birloiu_gabriel_dan@yahoo.com

Abstract

The paper presents the study done on the frame model technology used for adapting the bees the pedoclimate conditions of Romania. It is based on the observation done on four models, two widely used ones (Dadant and Layenes) and two models than provide conditions closer to the ones the bee hive experiences in nature (Warre and Dellon). Considering the current weather conditions and taking into account that bee family should have a strong development in the spring time to acquire a large quantity of bee forage in the first major harvesting of the year (harvesting canola), we concluded that the pattern of the hive most used in Romania (Dadant hive) no longer meets the requirements. To better understand the development of bee colonies in natural conditions, we began to study swarms of bees who have found shelter in hollow trees in the wilderness. By studding a swarm of bees that form a family in natural conditions (in a hollow tree and without any human intervention), we can see that they prefer a round enclosure and stat building combs from the top down. As bees begin to bring in nectar reserves, honeycomb building will continue so that the queen cans oviposition, while food reserves will be stored in the top of the hive. Bees prefer not to overcome the honeycombs with honey, and concentrate in the center where the area is more easily heated. Best results were observed in hives with an inner diameter of 25-35 cm and a height of 70-120 cm. If we take into account that the winter mat has a diameter of 26 to 30 cm, we can see that these families enclosures in the winter conditions were entirely occupied by the wintering ball and that the displacement occurred only vertically. Higher temperatures were observed above the wintering ball so than honey is kept a higher temperature, and thus keep its liquid state and be ready for consumption by the bees. If the temperature drops below a certain level and the bees only have food reserves in one side of the ball, they cannot move left or right to consume the honey and starve, even though they have food reserves in the hive. The conclusion reached after the research is that the Layens frame with the internal dimensions of 310x370 mm and a total area of 11.47 dm^2 best suits this purpose, but the technology of the Layens frame model can be adapted to the pedoclimate conditions of Romania.

Key words*: bees, frame, hive, pedoclimate, Romania.*

INTRODUCTION

Apiculture in Romania is a remarkable success due to the favorable pedoclimatic conditions and the valuable traits of the Romanian bee (*Apis mellifera carpatica*). The most used bee in apiculture in Romania is a cross between the local bee (*Apis mellifera carpatica*) with Italian species (*Apis mellifera ligustica*), Carniolan bee (*Apis mellifera carnica*) and other hybrid species from Banat and Wallachian plain (Bura et al., 2005; Marghitas, 2005). This species has successfully adapted to the climate changes of the past years, but the apiculture technology must also adapt. In the current climatic condition and considering the fact that the bee family must grow intensely in the spring for it to have great numbers of working bees in time for the first harvest (rapeseed harvest), we have come to the conclusion that the beehive model most used in Romania (the Dadant beehive) is no longer adequate. For all those passionate about apiculture, the beehive has always been a special topic, always improving it for better results. Due to the new concepts of ecologic apiculture and the need to have a working beehive full by the time of the first harvest near the end of April (Iordache et al., 2008), we have tried adapting the beehive model and technology to maximize the results.

The goal of this paper is compare four beehive models: two of the most used in Romania (Dadant and Layens) (Bura, 1996) and two that provide closer conditions to ones found in nature (Warre and Delon), to modify and adapt the beehive and apiculture technology to the current pedoclimatic conditions.

To better understand the development of a bee family in natural condition, we began studying bee families that found shelter in tree hollows

in the wild. If we study a bee family in these natural conditions, we will observe that they prefer a round enclosure, where they will begin constructing honeycombs from the bottom up (Volcinschi, 1988). As bees begin to bring into the hive nectar supplies, the honeycomb will continue to grow so that the queen can lay the eggs, while the future supplies will be stored in the upper part of the comb structure. Thus it appears that bees prefer not to cross over the full honeycombs, in order to protect them. It also seems that the round enclosure is easier to adapt into a hive.

If we are to follow the development of the winter cluster we will notice that as the outer temperature drops, the bees form the cluster right under the full honeycombs, proffering the empty cells beneath the supplies to better anchor onto the combs. The best results were with bee families within enclosures of 25 to 35 cm diameter and 70-120 cm in height. Considering that the winter cluster is 26 to 30 cm in diameter, we can observe that these families occupied the entire diameter of the enclosure and the movement occurred only on the vertical plane. Higher temperatures were kept above the winter cluster and thus the honey was kept at a good temperature to ensure it's liquid state, prime for bee consumption. If the temperature drops too much and the bees have the food supplies on the sides of the winter cluster, they cannot move left or right to consume the honey, and even if they are able to reach that point, the honey is at a low temperature and requires an even greater effort to warm up and liquefy. Thus, the bees can starve to death with honey supplies in the hive.

MATERIALS AND METHODS

The research was conducted on our own 50 beehives and of those of Mr. Mierlesteanu Valentin, situated in Tamadau, Calarasi County. Thee hives are stationary and receive the same care in the same timeframe.

Knowing the major influence of the bee queen quality on the development of the bee families, only queens obtained by double transfusion, of the same age, from the same original bee family were used

The power of the bee family was determined by weighting at certain times: during the winter preparations, and the end of the winter, at the beginning of the harvest and at the beginning of the summer

The determination of the hatchlings and honey quantities was made by measuring the surface occupied at certain times: 1 dm^2 of honeycomb contains on one side 175gr of honey or 400 worker bee cells

The number of beehives used was twelve, three for each model, with the following characteristics:

a) The Dadant hive (Photo 1) (systematic hive), invented by Ch. Dadant, American beekeeper of French nationality, who also invented the wax sheet of the frame (Nicolaescu, 1928). The innovation was the frame that suits centrifuging very well.

Photo 1. Vertical Dadant hives during spring harvest

The hive's sizes are:

- hive body – 446 mm X 370 mm X 306 mm (interior size);
- harvest magazine – 446 mm X 370 mm X153 mm (interior size);
- hive bottom – 586 mm X 410 mm;
- inner cover – 466 mm X 390 mm (multiple parts);
- outer cover – 486 mm X 390 mm X 150 mm (interior size).

The hive is built out of fir boards 2 cm thick and can use one or two harvest magazines (Antonescu, 1979).

The frames are from basswood and have interior size of 414mm x 272mm with a surface of 11.26 dm^2, with frames used in the harvest magazine at half that height. The hive has a volume of 0.05 m^3, a surface of 112.6 dm^2 on the 10 frames inside and 56.3 dm^2 honeycomb surface inside a magazine harvest.

b) The Layens (Photo 2) hive was designed and built by M. de Layens, and was an adaptation from the hive of Abbot Voirnot, to improve the winter conditions.
The hive's sizes are:
- hive body – 342 mm X 333 mm X 404 mm (interior size);
- harvest magazine – 342 mm X 333 mm X 404 mm (interior size);
- hive bottom – 482 mm X 373 mm;
- inner cover – 362 mm X 343 mm (multiple parts);
- outer cover – 382 mm X 373 mm X 150 mm (interior size).

The hive is built out of fir boards 2 cm thick.
The frames are from basswood and have interior size of 310mm X 370mm (Gustav, 1972) with a surface of 11.47 dm^2. The hive has a volume of 50.28 cm^3, a surface of 103.23 dm^2 on the 9 frames inside.

Photo 2. Layens hives during spring harvesting

c) The Warre hive (the popular hive) with fixed frames. Was designed by Abbot Emile Warre (Antonescu, 1966), initially built only with fixed frames, but wanting to centrifuge the frames and control the swarming, the mobile frames model was built.
The hive's sizes are:
- module height – 208 mm (interior size);
- module length – 296 mm (interior size);
- module width – 296 mm (interior size).

The frames are from basswood and have interior size of 264mm X 174mm with a surface of 4.59 dm^2.
In the upper side of the modules, on opposite walls, channels must be made to support the frames. These 8 frames are 296 mm X 25 mm X 18 mm. There is a 12 mm space where the bee can pass (between the frames and the walls). The particularity of this hive is that the bee can built the honeycombs without a wax sheet and the cell will have a distance between two opposite sides of 4.9 mm (the natural cell size built by bees) instead of 5.4 mm in the Dadant frame. With this size reduction, an increased resistance to the parasite Varroa Destructor (named in 2000 by Anderson and Trueman) was noticed, by the mechanism called "VSH". The hive is formed by a bottom, 3 modules and a cover. During the winter only 2 modules will be used, a third to be added beneath following the coming of spring. This system ensures that the interventions on the hive are reduced, thus cutting down the beekeeping time.

Photo 3. Hives during spring harvesting (original)

d) The Delon hive, designed by beekeeper Roger Delon (1919-2007), introduced the concept of Stable Climate Hive. He built a hive with a 300 mm x 300 mm base and a 215 mm height, with a volume of 0.019m^3 on each module. He also invented the Alpine frame, from a V shaped body and a metallic wire support. This type of hive can stack up to 5 modules and due to this fact it has an upper bee entrance to facilitate bee movement and vertical currents inside the hive during the summer.
The 8 frames are built from basswood and stainless 8 mm wire, with the interior size of 272 mm x 173 mm and a surface of 4.7 dm^2.
The following procedures were followed:
- The work done over the year on the four hive systems were identical to highlight the proposed parameters for the experiment;
- In the preparations for the winter, an equalization of the families was made (Hristea, 1976), by adding bees the classic

way (smell uniformization by adding flavoured tea with water and sugar 1:1);
- Protection of the hives was done according to the technology required for each hive type (adding thermo isolation diaphragms where needed);
- Widening the hive was made progressively, following each hive type instructions;
- The stimulation feedings were done during the winter with powdered sugar cakes and honey (Louveaux, 1987) (25% honey and 75% powdered sugar), and the beginning of the spring with powdered sugar cakes, honey and inactive yeast. (25% honey, 62.5% powdered sugar and 12.5% yeast);
- The treatments against Varroa were done with varachet, according to the treatment scheme: two in October-November (7 days apart) and two after the black locust (Istratie, 2010);
- The experiment was conducted over the course of one year (October 2013 – October 2014).

RESULTS AND DISCUSSIONS

To highlight the quantity evolution of the bee population, the research done on the four hive models took place over one year, starting in the period before the winter (October 2013) and ending with the last days of summer (September 2014). The results are presented in 1-5 tables, and concern the average bee quantities obtained using the same hive model. The standard quantity used was 2.2kg bees per family. Because the variation in bee mortality is greater in the winter even within the same hive model, we used the same quantity in all the hives so that de deviation could be kept to a minimum.

Table 1. Quantity evolution of bee families kept in Dadant hives in the winter

Month	Hive no 1			Hive no 2			Hive no 3			Mortality rate	
	Bee weight kg	Mortality		Bee weight kg	Mortality		Bee weight kg	Mortality		Kg	%
		Kg	%		Kg	%		Kg	%		
Oct	2.20	0	0	2.20	0	0	2.20	0	0	0	0
Mar	1.90	0.30	13.70	1.84	0.36	16.30	1.75	0.45	20.40	0.37	16.8

Table 2. Quantity evolution of bee families kept in Layens hives in the winter

Month	Hive no 1			Hive no 2			Hive no 3			Mortality rate	
	Bee weight kg	Mortality		Bee weight kg	Mortality		Bee weight kg	Mortality		Kg	%
		Kg	%		Kg	%		Kg	%		
Oct	2.20	0	0	2.20	0	0	2.20	0	0	0	0
Mar	2.09	0.11	5.20	2.11	0.09	4.20	2.07	0.13	6.20	0.11	5

Table 3. Quantity evolution of bee families kept in Warre hives in the winter

Month	Hive no 1			Hive no 2			Hive no 3			Mortality rate	
	Bee weight kg	Mortality		Bee weight kg	Mortality		Bee weight kg	Mortality		Kg	%
		Kg	%		Kg	%		Kg	%		
Oct	2.20	0	0	2.20	0	0	2.20	0	0	0	0
Mar	2.10	0.10	4.70	2.11	0.09	4.20	2.13	0.07	3.20	0.08	3.63

Table 4. Quantity evolution of bee families kept in Delon hives in the winter

Month	Hive no 1			Hive no 2			Hive no 3			Mortality rate	
	Bee weight kg	Mortality		Bee weight kg	Mortality		Bee weight kg	Mortality		Kg	%
		Kg	%		Kg	%		Kg	%		
Oct	2.20	0	0	2.20	0	0	2.20	0	0	0	0
Mar	2	0.20	10	2.14	0.06	2.80	2.12	0.08	3.70	0.11	5

Table 5. Quantity evolution comparison of bee families kept in the four hive models in the winter

Month	Hive Dadant			Hive Layens			Hive Warre			Hive Delon		
	Bee weight kg	Mortality		Bee weight kg	Mortality		Bee weight kg	Mortality		Bee weight kg	Mortality	
		kg	%		kg	%		kg	%		kg	%
Oct	2.20	0	0	2.20	0	0	2.20	0	0	2.20	0	0
Mar	1.80	0.40	16.80	2.10	0.10	5	2.10	0.10	3.63	2.08	0.12	5

If we follow the quantity evolution of the bee families, we notice that the Dadant hive model has suffered the biggest loss in the winter (16.8% average), the Layens and Delon hives have the same loss (5% average), while the Warre hive has the best percentage (3.63% average), thus the fewest losses.

Brood is an important factor in the development of bee families. The main factors that influence this are:

- Exterior temperature;
- Queen quality;
- Bee family power;
- Food supplies in nature and inside the hive;
- Hive model and quality.

To follow the development of the families we measured the brood honeycombs at different dates, 21 days apart, according to the development cycle of the working bee from egg to adult.

Table 6. Bee family growth dynamics between February 2014 and April 2014 in the four hive models

Hive model	Bee weight (Kg)	February 15 (dm^2)	March 7 (dm^2)	March 28 (dm^2)	April 18 (dm^2)	Brood (dm^2)	Brood (cell no)
Dadant 1	1.90	6.87	8.50	14.25	22.50	52.12	20 848
Dadant 2	1.84	5.62	7.50	13	22	48.12	19 248
Dadant 3	1.75	6.12	8	14	22.20	50.32	20 128
Dadant average	1.83	6.20	8	13.75	22.23	50.18	20072
Layens 1	2.09	9.10	12.50	18	29	68.60	27 440
Layens 2	2.11	10	12.80	18.20	30	71	28 400
Layens 3	2.07	9.80	12.80	18.20	30	70.80	28 320
Layens average	2.09	9.63	12.70	18.13	29.66	70.13	28 052
Warre 1	2.10	10.20	13	18.80	30	72	28 800
Warre 2	2.11	11	12.80	19	31	73.80	29 520
Warre 3	2.13	10.90	12	19	30	71.90	28 760
Warre average	2.11	10.70	12.60	18.93	30.33	72.56	29 026
Delon 1	2	10.20	13.50	19	31	73.70	29 480
Delon 2	2.14	10.80	14	20	32	76.80	30 720
Delon 3	2.12	11.50	14.80	21	32	79.30	31 720
Delon average	2.08	10.83	14.1	20	31.66	76.60	30 640

From the analysis of Table 6 we can observe that compared to the Dadant hive, the growth is bigger with:

- 39.75% inside the Layens hive;
- 44.60 % inside the Warre hive;
- 52.65 % inside the Delon hive.

This is due to the fact that the Dadant hive has a $0.050m^3$ and thus is very difficult to warm up, affecting the growth in the cold periods of spring. The best growth is in the Delon hive, with its narrow frame that allows the winter cluster to fill the entire space, preserving warmth and putting less stress on the bees.

Also to be noted is the fact that the brood frames are situated in the upper side of the hive where the conditions are best for development (in this model, the winter must be passed using 2 modules).

Compared to the Layens hive, the Delon hive had a 9.22% bigger growth, starting from identical conditions. The Delon had better results than the Warre hive also, with a 5.56% increase, leading us to the conclusion that it is the best hive model for the spring growth interval.

Another element to be considered is the honey production. This is different from hive to hive, being influenced by the following factors:

- Queen quality and egg-laying capacity;

- Brood quantity in the hive at the harvest time;
- Exterior temperature;
- Hive model used;
- Number of working bees;
- Distance to main harvest;
- Wind direction and speed;
- Rapeseed harvest began on 19th April 2014, and the honey extraction was made on 11th May 2014.

Looking at table 7 we can make some observations.

In the Dadant hives, the bee loss is the lowest during harvesting (7.9%), even though it is the hive model with the smallest bee quantity (2.91 kg average at the beginning). In the Delon hive, the bee loss is the highest (12.87%), even though it is the hive model with the biggest bee quantity (4.04 kg average at the beginning); The Layens hive has an almost equal loss with the Warre hive (11.5%).

Table 7. Honey production and bee weight evolution in the four hive models during rapeseed harvest 19th April 2014 to 11th May 2014

Hive model	Bee family weight		Bee quantity difference		Honey production	
	Before harvest (kg) 19.04.2014	After harvest (kg) 11.05.2014	kg	%	kg	kg of honey per kg of bees
Dadant 1	3.03	2.80	0.23	7.59	7.50	2.47
Dadant 2	2.84	2.62	0.22	7.74	7	2.46
Dadant 3	2.88	2.62	0.26	9.02	7.20	2.5
Dadant average	2.91	2.68	0.23	7.90	7.23	2.48
Layens 1	3.78	3.38	0.40	10.58	10	2.64
Layens 2	3.89	3.40	0.49	12.50	10.50	2.69
Layens 3	3.86	3.40	0.46	11.91	10.50	2.72
Layens average	3.84	3.39	0.45	11.71	10.33	2.69
Warre 1	3.93	3.50	0.43	10.94	10.80	2.74
Warre 2	3.89	3.44	0.45	11.56	10.50	2.69
Warre 3	3.93	3.46	0.47	11.95	11	2.79
Warre average	3.91	3.46	0.45	11.50	10.76	2.75
Delon 1	3.94	3.50	0.44	11.16	11	2.79
Delon 2	4.14	3.56	0.58	14	11.40	2.75
Delon 3	4.06	3.50	0.56	13.79	11	2.70
Delon average	4.04	3.52	0.52	12.87	11.13	2.75

We can conclude that if the bee family is strong, the work is taking its toll during harvest and bee losses are greater.

The honey production in the Delon hives (11.13 kg average) puts it in the lead; with 53.94% more than the Dadant hives (7.23 kg average). The difference between the Delon hive and other models is that it has an upper bee entrance that allows a better circulation and time saving with transporting nectar inside the hive. The Delon production is 3.43% larger compared to the Warre and 7.74% larger compared to the Layens.

Concerning the transformation of nectar to honey, Delon and Warre take the first place with 2.75 kg honey per kg of bees, followed by Layens with 2.69 kg honey per kg of bees and Dadant with 2.48 kg honey per kg of bees.

Acacia harvest due to adverse weather conditions could not be performed. The sunflower harvests a home in the 3rd of July 2014, while the honey extraction took place in the 5th of August 2014.

Looking at table 8 we can make some observations. In the Dadant hives, the bee loss is the highest during harvesting (31.16%), even though it is the hive model with the smallest bee quantity (3.53 kg average at the beginning).

In the Warre hive, the bee loss is the lowest (20.44%), even though it is the hive model with the second biggest bee quantity (4.06 kg average at the beginning). The Layens hive has a biggest loss (21.53%) than the Delon hive (20.44%).

Table 8. Honey production and bee weight evolution in the four hive models during sunflower harvest 3rd July 2014 to 5th August 2014

Hive model	Bee family weight		Bee quantity difference		Honey production	
	Before harvest (kg) 03.07.2014	After harvest (kg) 05.08.2014	kg	%	kg	kg of honey per kg of bees
Dadant 1	3.60	2.40	1.20	33.30	12.00	3.33
Dadant 2	3.50	2.40	1.10	31.42	12.00	3.42
Dadant 3	3.50	2.50	1.00	28.57	11.70	3.34
Dadant average	3.53	2.43	1.10	31.16	11.90	3.37
Layens 1	4.00	3.10	0.90	22.50	14.00	3.50
Layens 2	3.80	3.00	0.80	21.05	13.50	3.55
Layens 3	3.90	3.10	0.80	20.51	13.70	3.51
Layens average	3.90	3.06	0.84	21.53	13.73	3.52
Warre 1	3.90	3.10	0.80	20.51	13.00	3.33
Warre 2	4.20	3.50	0.70	16.66	13.20	3.14
Warre 3	4.10	3.10	1.00	24.39	13.10	3.19
Warre average	4.06	3.23	0.83	20.44	13.10	3.22
Delon 1	4.10	3.20	0.90	21.95	14.10	3.43
Delon 2	4.30	3.50	0.80	18.60	13.50	3.13
Delon 3	4.30	3.30	1.00	23.25	14.00	3.25
Delon average	4.23	3.33	0.90	21.27	13.86	3.27

We can conclude that due to the low temperatures during the night and the high air humidity, the stress is greater on the bees inside the Dadant hives due to the large volume.

The honey production in the Delon hives (13.86 kg average) puts it in the lead, with 16.4% more than the Dadant hives (11.9 kg average).

The Delon production almost the same with Layens hives (13.73 kg average) and Warre hives (13.1 kg average). The difference between the Dadant hive and the other models is that the frame surface and lengths is larger and thus the bees work more and spend more energy. The Delon production is 5.8% larger than Warre's and 0.94% larger than Layens.

Concerning the transformation of nectar to honey, Layens hives take the first place with 3.52 kg honey per kg of bees, followed by Dadant with 3.37 kg honey per kg of bees, Delon with 3.27 kg honey per kg of bees and Warre with 3.22 kg honey per kg of bees.

CONCLUSIONS

After analyzing all the date, we have reached a number of conclusions.

The bee family quantity evolution during the winter is very different depending on the hive model used. In Layens and Warre hives, the mortality is at 5.2%, in Delon hives at 5.7% and in Dadant hives at 20.3%. The explanation in the fact that Layens hives, Warre and Delon has the same internal dimensions of the ball size of the wintering area and therefore losses are small, while Dadant hives have grater inner dimension 100-150 mm than the ball it does not form on the center of the frame so that, the bees will fall laterally for food and bee losses are high.

There are great differences in the bee family growth dynamic in the spring. In Layens hives there is 39.75% more brood than in Dadant hives, in Warre there is 44.60 % more brood and in Delon there is 52.65% more brood than in Dadant hives. Because of the rate of 100-150mm higher, Dadant hives updrafts form internal cooling leading to the family nest of bees and queen laying eggs correlates with the ability to heat the family nest. On Delon hives, Alpine frame is reduced to the use of free space inside the hive, the nest is very well protected and the capacity of the queen is the best.

The Dadant hives have the smallest bee loss rate during rapeseed harvest (7.9%), Delon has the biggest bee loss rate (12.87%), Layens (11.71%) is close to Warre (11.5%). Bee loss is correlated with the strength of the bee family. The Dadant hives where bee families are still growing, the quantity of bee forage is lower, resulting lower losses, many of them being nurse bees.

Concerning rapeseed harvest, the nectar to honey transformation rate is the best in Warre and Delon with 2.75 kg of honey per kg of bees, followed by Layens with 2.69 kg of honey per kg of bees and Dadant with 2.48 kg of honey per kg of bees. The quantity of the nectar bought to the hive is directly proportional to the strength of the bee family.

During sunflower harvest, when the difference between day and night temperatures is big, the Dadant hives suffer the greatest loss (31.16%), Warre the smallest one (20.44%), Layens (21.53%) nearly the same as Delon (21.27%). Being the last great harvest of the year, forcing the bees gathering nectar and quantity bought in to the hive is relatively equal, but in Dadant hives, bees wear out quickly being forced to work harder at night to maintain the indoor temperature.

Concerning sunflower harvest, the nectar to honey transformation rate is best in Layens hives with 3.52 kg of honey per kg of bees, followed by Dadant with 3.37 kg of honey per kg of bees, Delon with 3.27 kg of honey per kg of bees and Warre with 3.22 kg of honey per kg of bees. The parameter shows no large differences between the four models of hives, but we can see that Dadant hives are not good enough at this respect.

It is clear that in the current pedoclimatic conditions of Romania the use of the Dadant hive model is no longer justifiable, considering that it has the largest mortality rate during winter, the slowest growth during spring, the smallest nectar to honey rate during the first harvest, the largest mortality rate during summer and a low nectar to honey rate during spring.

Layens, Delon and Warre hive models are showing differences between the parameters taken into account, but each beekeeper, depending on the purpose it pursues in beekeeping can choose one of the other models.

The overall conclusion is that the Layens frame with the interior size of 310 mm x 370 mm and a total surface of 11.47 dm^2 is best fitted for winter beekeeping and good growth in the spring. The technology must be adapted by adding a second module under the main one in the spring and by using the upper bee entrance during the summer. It remains however to solve the problem of obtaining flower honeys because of the large dimension of the frame.

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REFERENCES

Antonescu C., 1966. Ingrijirea familiilor de albine, Biblioteca Apicultorului.

Antonescu C., 1979. Albinele si noi, Editura I.I.T.E.A. Apimondia, Bucuresti.

Bura M., Patruica S., Bura V. A., 2005. Tehnologie Apicola, Ed. Solness, Timisoara.

Bura M.,1996. Cresterea intensive a albinelor, Editura Helicon.

Gustav-Adolf Oeser, 1972. Der Bien Und Du, VEB Deutscher Landwirtschaftsverlang Berlin.

Hristea L. C., 1976. Stuparitul Nou, Editura I.I.T.E.A. Apimondia, Bucuresti.

Iordache P., Rosca I., Cismaru M., 2008. Plantele melifere de foarte mare si mare pondere economic-apicola, Ed. Lumea Apicola, Bucuresti.

Istratie D., 2010. Calitate si Securitate Ambientala, Buletinul AGIR nr. 2-3/2010 aprilie-septembrie, pag. 50 - 64, Timisoara.

Louveaux J., 1987. Albinele si cresterea lor, Editura I.I.T.E.A. Apimondia, Bucuresti.

Marghitas A. L., 2005. Albinele si produsele lor, Editura Ceres, Bucuresti.

Nicolaescu N., 1928. Calauza Stuparului, Editura Casei Scoalelor, Bucuresti.

Volcinschi T., 1988. Ceara, Ed. I.P. Filaret, Bucuresti.

*** Asociatia Crescatorilor de Albine din Republica Socialista Romania, 1986 – Manualul Apicultorului, Editia VI-a, Editura I.I.T.E.A. Apimondia, Bucuresti.

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STUDY ON THE MAINTENANCE OF BEE FAMILIES INTO VERTICAL HIVES ON DADANT AND LAYENS FRAMES

Gabriel Dan BIRLOIU, Ștefan DIACONESCU, Carmen Georgeta NICOLAE

University of Agronomic Sciences and Veterinary Medicine of Bucharest, 59 Mărăști Blvd, District 1, 011464, Bucharest, Romania, Phone: +4021.318.25.64, Fax: + 4021.318.25.67

Corresponding author email: birloiu_gabriel_dan@yahoo.com

Abstract

The paper presents the study done on the frame model technology and its effects on productivity. The productivity of a bee family is correlated with the hive's productive capacity, the micro-climate conditions and the volume available for the optimal growth of the bee family. For finding solutions that limit the negative influence that a certain hive model has on a bee family, the paper aims to compare the two main frame technologies used in apiculture. The study was made using numerically equal bee families, kept in the same conditions and under the same preventive treatments, with the purpose of determining their productive capacity and development patterns.

Key words*: bees, frame, hive, Dadant, Layens.*

INTRODUCTION

From the ancient times because of the favorable podeclimatic conditions of our country, there has been a big concern about beekeeping. Thus, the first document appears in the writings of the Greek historian Herodot (484 – 425 b.C.) which says that "in the south of Danubis the earth is inhabited by bees".

The type of bee exploited at present is a cross between the local bee (*Apis mellifica carpatica*) with qeens of Italian species (*Apis mellifica lingustica*), Carniolan bee (*Apis mellifica carnica*) and other hybrid species from Banat and Wallachian plain (Bura et al., 2005). Besides improving the quality of the biological material always existed a concern for discovering new effective methods of raising bees. The production capacity of the bee families is correlated with several factors: the productive potential of the queens, resistance to diseases and pests, the characteristics of the hive, the hive ability, the mining technology applied, the apiary honey production (Nicolaescu, 1928; Antonescu, 1966; Bura, 1996). Depending on the goal you are pursuing every beekeeper along the time created many models of hives . Between all the systematic hives created, only two models have managed to establish itself until today: Dadant and Layens (Hristea, 1976).

The aim of the paper is to highlight the effectiveness of the technology maintenance compared bee hives in two models, to find solutions that can limit the negative influence of climatic factors on the bee and beekeeping production.

MATERIAL AND METHODS

To highlight the influence that both models have on the biology of the bee hives and beekeeping production , researches have been conducted in own apiary on a number of six hives, three vertical Dadant model and three Layens vertical model in September 2012 - September 2013. For results to be closer to reality the bee families that were populated hives were brought to the same initial weight (2.5 kg) weight ensure normal development in the two models of hives during winter. Bees were applied to the same treatments performed in the same period and were stimulated with the same amount of food.

BIOLOGICAL MATERIAL

Knowing the major influence on the development of quality queen bee family were used queens obtained by double tapping, having the same age, from the same bee family.

Power bee family was determined by weighing at certain period of time: in preparation for the winter period" at the end of winter, early harvest of rape, early summer".

Determine the amount of sapling and honey was made by measuring the area occupied by

them from time to time so: 1 dm^2 comb on one side contains 175 g or 400 cells empower honey bee working.

AUXILIARY MATERIALS

The lot of hives subject experiment consists of six hives (three of each type), with the following characteristics:

A. Dadant hive was invented by Ch. Dadant, American beekeeper with French nationality, who invented and related wax leaf frame. Innovation in beekeeping was brought by frame which is very well suited to spin. In the first phase was invented longue 18 frames then create a frame due to the need for storing smaller harvest hive appeared vertical (systematic hive) with three models of frames: Frame ½ , ¾ frame and frame 1/1 (Antonescu, 1979; Manualul Apicultorului, 1986).

The hive dimensions are:

- Nest body - 446 mm x 370 mm x 306 mm (internal dimensions);
- Store harvest - 446 mm x 370 mm mm x 153 (internal rates);
- The bottom of the hive - 586 mm x 410 mm
- Plateau - 466 mm x 390 mm (made up of several parts);
- Cover - 486 mm x 390 mm x 150 mm (internal dimensions).

The hive is made of fir plank thickness of 2 cm and can be used as appropriate with one or two stores harvest. Stores crop used in this study are those of ½. The frames are made of linden and 414 mm x 272 mm have internal dimensions with an area of 11.26 dm^2, with frames for harvest shop with a height reduced by half. Hive has a volume of 0.050 m^3 (body nest), an area of 112.6 dm^2, 10 frames per nest and 56.3 dm^2 surface combs shop harvest.

B. Layens hive was designed and built by M. de Layens and it has been adapted by the model of Voirnot abbot's hive , improving conditions for wintering and work easier for beginners in beekeeping. At first horizontal version was built with 16 frames after that, because naturally bee hive inside vertical movement was built and vertical version with new frames.

In this study it was used a modified version of the hive Layens using ten frames as well (instead of the new hive has Layens as original).

Layens hive has the following dimensions:

- Body 342 mm x 370 mm hive x 404 mm (internal dimensions);
- Store the harvest 342 mm x 370 mm x 404 mm (internal dimensions);
- Hive bottom 482 mm x 390 mm;
- Plateau 362 mm x 343 mm (made up of several parts);
- Cover 382 mm x 150 mm x 373 mm (internal dimensions).

The hive is made of fire wood 2 cm thick. The frames are made of linden and 310 mm x 370 mm (Gustav, 1972), have internal dimensions, with an area of 11.47 dm^2. Hive has a volume of 0.051 m^3 and 114.7 dm^2 the frames nest.

PROCEDURE

Working technology used was as follows:

- The work done over the year in the two hive systems were identical pointing out by determining the parameters proposed for the experiment;
- In preparation for wintering hives has been performed and equalize weight of the bee families(LOUVEUX., 1987), by adding bees using classical methods (uniform smell by adding flavoring tea made with water and sugar 1: 1);
- Protecting the nest was made according to each model technology hive (aperture adding insulation);
- Expanding the nest was made progressively as the technology for each hive model;
- Stimulating feeding winter cakes were made from powdered sugar and honey (25% and 75% powdered sugar honey) and in early spring with powdered sugar cakes, honey and inactivated yeast (25% honey, 62.5% powdered sugar and inactivated yeast 12.5%);
- Against Varroa treatments were performed with varachet, as planned: two in October (at a distance of seven days) and two spring after picking acacia (Istratie, 2010);
- The experiment was conducted over a period of one year (September 2012 - September 2013).

RESULTS AND DISCUSSIONS

To highlight the quantitative evolution of the population of bees in winter, comparative research conducted on two models of hives that were conducted over the period September 2012 to early March 2013 and are presented in Table 3. The amount of bee used in the experiment was 2.5 kg bee \ family. Because

variability in mortality of bees in winter is high even when using the same model of the hive, in Table 3 we worked with the average amount of bee hive within each model for the deviation to be as small as possible.

Table 1. Quantity evolution of bee families kept in Dadant hives in the winter: September 2012- March 2013

Month	Hive no 1			Hive no 2			Hive no 3			Mortality rate	
	Bee weight kg	Mortality		Bee weight kg	Mortality		Bee weight kg	Mortality		Kg	%
		Kg	%		Kg	%		Kg	%		
Sept.	2.5	0	0	2.5	0	0	2.5	0	0	0	0
March	2	0.500	20	2.15	0.35	14	1.95	0.55	22	0.46	18.4

Table 2. Quantity evolution of bee families kept in Laynes hives in the winter: September 2012- March 2013

Month	Hive no 1			Hive no 2			Hive no 3			Mortality rate	
	Bee weight kg	Mortality		Bee weight kg	Mortality		Bee weight kg	Mortality		Kg	%
		Kg	%		Kg	%		Kg	%		
Sept.	2.5	0	0	2.5	0	0	2.5	0	0	0	0
March	2.3	0.20	8	2.35	0.15	6	2.30	0.20	8	0.19	7.6

Table 3. Comparison of quantitative average evolution of bee families, maintained in two models of hives during the winter in September 2012 - March 2013

Month	Dadant hive			Layens hive		
	Bee weight kg	Mortality		Bee weight kg	Mortality	
		Kg	%		Kg	%
September	2.5	0	0	2.5	0	0
March	2.04	0.46	18.4	2.32	0.18	7.2

If we follow the quantity evolution of the bee families, we notice that the Dadant hive model has suffered the biggest loss in the winter (18.4 % average) and the Layens (7.2% average), while the thus the fewest losses (Figure 1).

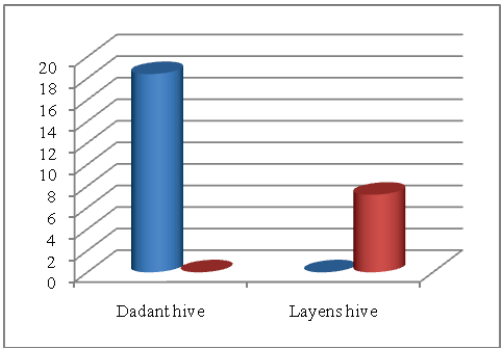

Figure 1. Mortality of bee families maintained in both models of hives during the winter: September 2013- March 2013 (%)

This is possible because, Dadant hives (even if the family was isolated with a diaphragm in a total of seven frames) is hardly heated enclosure frames having the largest share of horizontal skein may not fully occupy space in the hive and thus in the remaining unoccupied, the low temperature condensation occurs.

The space inside of the Layens hive during the winter (seven frames isolated diaphragms) is almost equal to the Dadant hive (0.0357 m3) because of the largest space that is occupied by the vertical frames, the family occupies the entire premises and so the conditions of wintering are close to the natural ones, so the bee family has the optimal conditions for wintering.

A basic indicator in the growth and development of bee families is the presence of the brood. The main factors that influence this indicator are:

- Outdoor temperature;
- Queen quality;
- The quality of the hive model used;
- The power of bee family;
- Reserves of food from nature and from inside the hive.

To pursue further development of bee families was done to measure surface brood combs at

different dates at a period of about 21 days, period corresponding to a working bee development cycle from egg stage to adult.
To understand better the dynamics of development of bee family in one year research was split into three periods: the development during spring, summer development during and development during the autumn.
Development during spring begins with the first background check and ends at the first big harvest of the year. The data obtained are presented in Table 4.

Table 4. The growth dinamics of bee families in the two hive models in spring: March 2013 – May 2013

Hive model	Bee weight (Kg)	March 7 (dm^2)	March 28 (dm^2)	April 18 (dm^2)	May 9 (dm^2)	Brood (dm^2)	Brood (cell no.)
Dadant 1	2	8	13.80	21	50	92.80	37120
Dadant 2	2.15	8.50	14	21.50	50	94	37600
Dadant 3	1.950	8	13.50	21	48	90.50	36200
Dadant average	2.03	8.16	13.76	21.16	49.3	92.43	36972
Layens 1	2.30	12.30	18	29	67.10	126.40	50560
Layens 2	2.35	12.50	19	30	68	129.50	51800
Layens 3	2.30	12	17.50	28.50	67	125	50000
Layens average	2.31	12.26	18.16	29.16	67.36	126.96	50784

The hive used has a large influence on egg-laying queen as shown in Table 4 the bees maintained in the Layens are with 37.35 % higher than those maintained in Dadant hive, the development period of the year before the first big harvest (Figure 2, Figure 3).

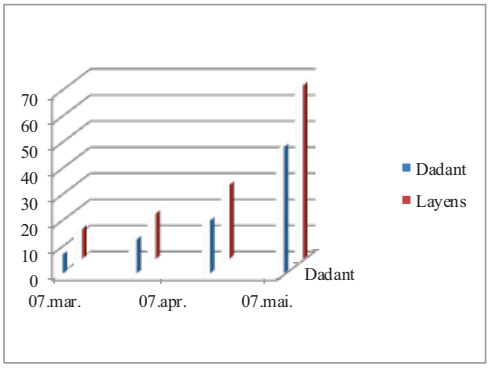

Figure 2. The dynamic development of bee families in the two hive models during spring March 2013 - May 2013 (dm^2 brood cells)

This difference is even greater in early March when the bees in hives Layens had maintained with 50.24 % higher than the Dadant hives.
The fact that the height of the frame is longer than the length makes the volume of the family nest of bees to occupy full frames, bee family development occurs vertically and temperature inside the hive is much easier to control.

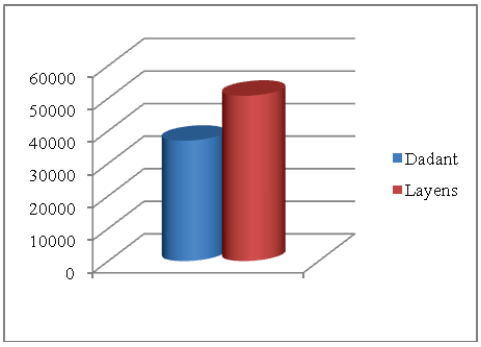

Figure 3. The dynamic development of bee families, expressed as average in the two hive models during the spring, March 2013 - May 2013 (brood cells no.)

For the second phase of development of the bee family research started after completion of the first harvest and was completed with the completion of the last great harvest of the year (sunflower harvesting). The data obtained are presented in Table 5.
The analysis of the obtained data we can see that during this period in the Dadant hive, the lay-eggs queen decreases by 41% while the hives Layens decreases only 34.13% (Figure 4).
We can conclude saying that summer frame Layens is superior to the summer frame Dadant for the growth of the bee families inside the hive, the heat is concentrated at the top (which has a height of 404 mm) and this makes the bees work less for conditioning the

nest. It should be noted that during this period Dadant hives had attached one harvest shop.
For the third growth period for the bee family researches begun after the last great harvest of the year and were completed late in September before performing treatments to destroy the Varroa Destructor parasite. The data obtained are presented in Table 6.

Table 5. The dynamic development of bee families in the two hive models in summer May 2013 - July 2013

Hive model	Bee weight (Kg)	May 30 (dm^2)	June 20 (dm^2)	July 10 (dm^2)	July 30 (dm^2)	Brood (dm^2)	Brood (cell no.)
Dadant 1	3.39	74	78	62	42	256	102 400
Dadant 2	3.60	76	81	65	45	267	106 800
Dadant 3	3.30	72	77	62	44	255	102 000
Dadant hive average	3.43	74	78.66	63	43.66	259,33	103 732
Layens 1	4.56	90	100	82	62	334	133 600
Layens 2	4.68	95	98	80	60	333	133 200
Layens 3	4.52	89.8	94	78	59	320.8	128 320
Layens hive average	4.58	91.60	97.33	80	60.33	329.26	131 704

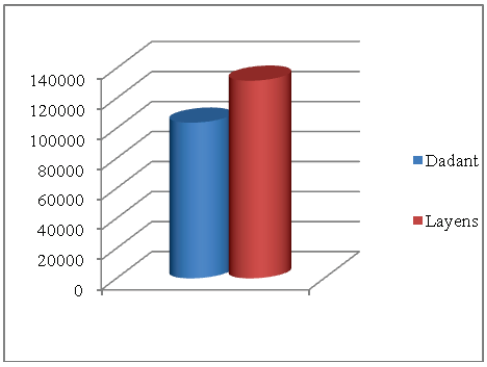

Figure 4. Bee family growth dimanics in both hive models between May 2013 – July 2013 (dm^2 brood cells)

Making a review of the data obtained we can observed that during this period the Dadant hives , queen drops 66.22 % as hives Layens decreases 65.04 %. It can be concluded as the autumn Layens frame is superior to Dadant frame for the growth of bee families.
If we analyze the amount of brood is submitted after the date of August 20, 2013 we notice that Layens hives it is 17.996 and in Dadant hives the average is 9464. Considering that the queen bees and brood submitted by the end of October - early November we conclude that Layens hives for winter will be enough but Dadant hives will need to make unification of hives to pass safely over the winter.

Analyzing the dynamics of bee family in terms of the amount deposit of brood (Figure 5) we can see that Layens is superior to the Dadant hive throughout the year.

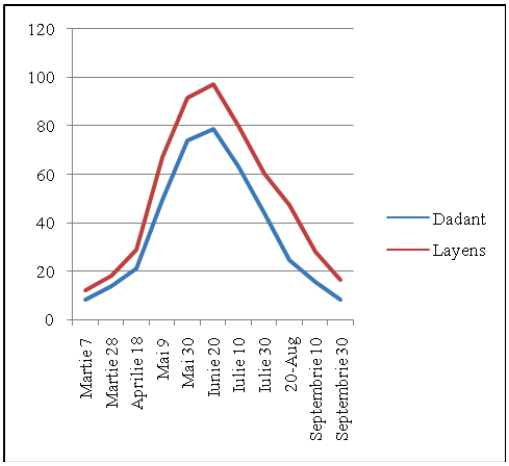

Figure 5. Bee family growth dynamics in both hive models during all the year 2013

The floral honey results from the processing of floral nectar honey bee brought by it in the hive (Iordache et al., 2008). The quantity of honey needed by the bee colonies during a year is very difficult to quantify, it varies depending on several factors: the strength of the bee family, the amount of eggs and brood in the hive, microclimate conditions that

ensure their hive model, outdoor temperature and humidity (Marghitas, 2005).

To draw a conclusion about the potential production per hive model, we analyzed the parameter "quantity of extracted honey" and quantity of bee families that conducted us to this parameter. Rapeseed harvest began in May 9 data and lasted until 20 May 2013. The data obtained are presented in Table 7.

Table 6. Bee family growth dynamics in both hive models during the autumn period August 2013 – September 2013

Hive model	Bee weight (Kg)	August 20 (dm^2)	September 10 (dm^2)	September 30 (dm^2)	Brood (dm^2)	Brood (cell no.)	Bee weight (Kg)
Dadant 1	2.7	27	16	9	52	20800	2.7
Dadant 2	2.5	24	15	8	47	18800	2.5
Dadant 3	2.4	23	15	8	46	18400	2.4
Dadant hive average	2.53	24.66	15.33	8.33	48.33	19333	2.53
Layens 1	3.2	47	29	16	92	36 800	3.2
Layens 2	3.3	48	28	17	93	37 200	3.3
Layens 3	3.2	48	28	17	93	37 200	3.2
Layens hive average	3.23	47.66	28.33	16.66	92.66	37066.66	3.23

Table 7. Honey production and bee weight evolution in both of hive models during the rapeseed harvest 9th May 2013 to 20th May 2013

Hive model	Weight of bee family		Differences between the weight of the bees		The honey production	
	Before harvest (kg) 9.05.2013	After harvest (kg) 20.05.2013	(kg)	(%)	(kg)	(kg honey/kg bee)
Dadant 1	3.39	3	0.39	11.5	7	2.06
Dadant 2	3.60	3.1	0.5	13.88	7.5	2.08
Dadant 3	3.30	3	0.3	9.09	7	2.12
Average	3.43	3.03	0.4	11.66	7.16	2.08
Layens 1	4.56	3.8	0.76	16.66	10.5	2.3
Layens 2	4.68	3.7	0.98	20.94	12	2.56
Layens 3	4.52	3.8	0.72	15.92	11	2.43
Average	4.58	3.76	0.82	17.9	11.16	2.43

According to Table 7 at the rapeseed harvest the quantity of harvested honey is 55.86 % higher in Layens hives to Dandant hives but also the bee loss is 6.24 % higher (Figure 6).

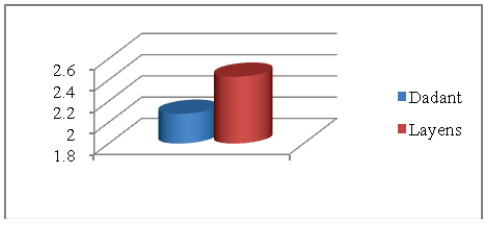

Figure 6. The efficiency of transforming the nectar in honey bee at bee families maintained in Dadant and Layens hives (kg honey / bee kg) at rape harvest

We conclude that stronger families during spring collect more honey bee but also the bee loss family is higher. The honey production is higher at bee families from Layens hives that collect 2.43 kg / kg to bee families from Dadant hives that collect with 2.08 kg / kg bee.

Looking at table 8 we can make some observations. The honey production is 32.81% higher at Laynes hives than Dadant hives after sunflower harvest, but also the bee loss in higher with 1.41% at Laynes hives. The honey production is 6.07% honey kg/ bee kg higher at Laynes hives than Dadant hives with 5.42 honey kg/bee kg (Figure 7).

Table 8. Honey production and bee weight evolution in both hive models during sunflower harvest 24th June 2013 to 30th July 2013

Hive model	Weight of the bee family		Difference between bee weight		Honey production	
	Before harvest (kg) 24.06.2013	After harvest (kg) 30.07.2013	(kg)	(%)	(kg)	(kg honey/kg bee)
Dadant 1	4.1	2.7	1.4	34.1	22	5.36
Dadant 2	3.9	2.5	1.4	35.89	21	5.38
Dadant 3	3.9	2.4	1.5	38.46	21	5.38
Average	3.93	2.53	1.43	36.38	21.33	5.42
Layens 1	4.8	3.2	1.6	33.33	28	5.83
Layens 2	4.9	3.2	1.7	34.69	29	5.91
Layens 3	4.8	3.2	1.6	33.33	28	5.83
Average	4.66	3.23	1.63	34.97	28.33	6.07

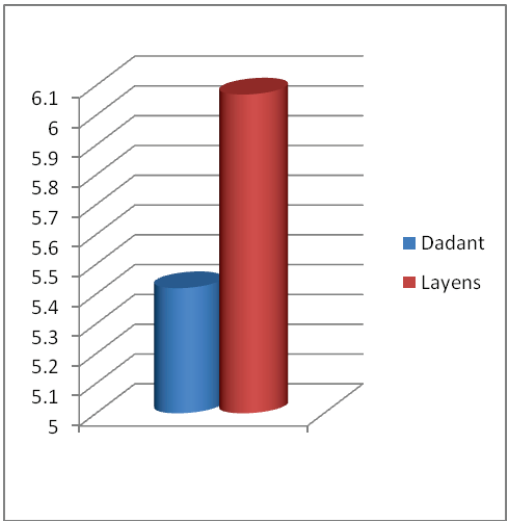

Figure 7. The efficiency of transforming the nectar in honey bee at the bee families maintained in Dadant and Layens hives (kg honey / bee kg) at sunflower harvest

CONCLUSIONS

After analyzing the evolution of bee families and quantity of honey collected we can conclude:

- The study undertaken aimed at comparing the behavior of bee colonies increased in two types of hives (Dedant and Layens), widespread in beekeeping practice. The study case was carried out in the south of the country, in the author's own apiary.
- Have been targeted a number of technical and biological parameters (power bee family, the ability to produce juvenile, survival during winter, the amount of honey harvested etc.).
- In winter, bees from Layens hives losses are lower than the 11.2% Dadant hives. Inside Layens hives, the bee fully occupy the space, wintering mat is formed in the middle frame and the bee will only move vertically constantly having food over the ball of winter. This system helps to decrease the mortality during the winter.
- During the winter, bee losses in Layens hives are lower than the losses in Dadant hives with 11,2%.Inside Layens hives, the bee fully occupy the space, wintering mat is formed in the middle frame and the bee will only move vertically constantly having food over the ball of winter. This system helps to decrease the mortality during the winter.
- During the spring, the bee families of Layens hives have showed a stronger growth, in March the bee queens layed with 50,24% more eggs than the bees from Dadant hives. The lack of vertical currents in Layens hives, have determined the temperature maintenance and the bee queens lays more eggs.
- The bees growth in summer from Layens hives is 6.87% higher than Dadant hives of bees. In the summer, the queen of the hive Layens, sustained over a longer time lay ceiling , with less need for conditioning bee hive.
- During the autumn, bees in Layens hives is higher by 3.88% from Dadant hives of bees lay eggs and brood amount obtained, allow us to get through the winter without unify hives. Also with plentiful bee, bees growth will cover a longer period in the late autumn, which will ensure less waste bee in the spring and will influence the growth of earlier brood start.

- The quantity of honey obtained and extracted from rapessed harvest is higher in Layens hives than Dadant hives, with 55.16%. With a stronger development in the early part of the spring, the Layens hives have a larger amount of bee for the first harvest of the year.Honey extracted from sunflower harvest is higher in Layens hives than Dadant hives with 32.81%.

- The research carried out in the mentioned conditions (plains) shows the superiority of the Layens hives to Dadant hives.

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REFERENCES

Antonescu C., 1966. Ingrijirea familiilor de albine, Biblioteca Apicultorului.

Antonescu C., 1979. Albinele si noi, Editura I.I.T.E.A. Apimondia, Bucuresti.

Bura M., 1996. Cresterea intensive a albinelor, Editura Helicon.

Bura M., Patruica S., Bura V. A., 2005. Tehnologie Apicola, Editura Solness, Timisoara.

Gustav-Adolf Oeser, 1972. Der Bien Und Du, VEB Deutscher Landwirtschaftsverlang Berlin.

Hristea L. C., 1976. Stuparitul Nou, Editura I.I.T.E.A. Apimondia, Bucuresti.

Iordache P., Rosca I., Cismaru M., 2008. Plantele melifere de foarte mare si mare pondere economic-apicola, Editura Lumea Apicola, Bucuresti.

Istratie D., 2010. Calitate si Securitate Ambientala, Buletinul AGIR nr. 2-3/2010 aprilie-septembrie, pag. 50 - 64, Timisoara.

Marghitas A. L., 2005. Albinele si produsele lor, Editura Ceres, Bucuresti.

Nicolaescu N., 1928. Calauza Stuparului, Ed. Casei Scoalelor, Bucuresti.

*** Asociatia Crescatorilor de Albine din Republica Socialista Romania, 1986 – Manualul Apicultorului, Editia VI-a, Editura I.I.T.E.A. Apimondia, Bucuresti.

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EVALUATION OF TWO GRAZING SYSTEMS APPLIED ON ARTIFICIAL PASTURES IN THE WEST MEDITERRANEAN REGION OF TURKEY

Yalçın BOZKURT[1], Mevlut TURK[2], Sebahattin ALBAYRAK[2], Stepan VARBAN[1]

[1]University of Süleyman Demirel, Faculty of Agriculture, Department of Animal Science, Isparta, Turkey, Phone: 00 90 246 85 31, E-mail: yalcinbozkurt@sdu.edu.tr; stephanvarban@sdu.edu.tr
[2]University of Süleyman Demirel, Faculty of Agriculture, Department of Field Crops, Isparta, Turkey, E-mail: mevlutturk@sdu.edu.tr; sebahattinalbayrak@sdu.edu.tr

Corresponding author email: yalcinbozkurt@sdu.edu.tr

Abstract

In this study, it was aimed to evaluate two grazing systems for the performance of beef cattle grazing on artificially established pastures under the West Mediterranean climate conditions. For this purpose, an experiment was conducted at university farm in Isparta province located in the west Mediterranean region of Turkey in 2012 and lasted for 70 days. A total of 20 Holstein breed beef cattle with an average of 6 months old were assigned equally to two grazing pastures which were composed of Medicago sativa L. (20%) + Bromus inermis L. (40%) + Agropyron cristatum L. (30%) + Poterium sanguisorba (10%). Two pasture areas with a 3 ha in size were established artificially next to each other and designed as one with zero grazing (ZG) and the other one with rotational grazing, using electrical fencing system (RG) to determine the grazing performance of beef cattle. Biomass available for grazing was also monitored. It was found that there were no effects of grazing types on the performance of the animals. The total weight gains of the animals were 66 and 69 kg for ZG and RG respectively at the end of the experiment. Similarly, there were also no statistical significant differences in daily live weight gains (DLWG) of the animals. DLWGs were 0.954 and 0.996 kg for ZG and RG respectively. Consequently, both type of grazing systems can be recommended for beef cattle production in the region. However, it should be taken into consideration that there was a tendency for the animals perform better in rotational grazing system on artificially established pastures in the West Mediterranean climate conditions.

Key words*: Holstein, Artificial Grassland, Performance, Beef Production, Mediterranean.*

INTRODUCTION

In developing countries, where there is a much smaller scale of farming practices divided mainly into smaller farms, meat is produced primarily as a by-product of dairy production and the cattle are mainly dual purpose for milk and beef. For the last decade, beef producers in Turkey have been facing a big challenge in meeting the great demand for red meat consumption of the population along with its rapid growth rate and due to the lack of roughage and insufficient natural grasslands. Therefore, beef production systems using artificial grasslands have gained a big interest due to its low investment and efficient management applications (Ecevit, 1999).

Beef production constitutes an important sector of the agricultural industry of many countries. The type of beef industry which develops in any country depends largely on climatic conditions and land types (Allen and Kilkenny, 1984).

One of the ways to resolve the lack of roughage was to establish artificial pastures. Artificial pasture establishment increased in recent years in Turkey. The commonly used species in establishing artificial pasture in Turkey is crested wheatgrass, smooth bromegrass and alfalfa (Acar et al., 2011). Flora, stage of maturity, soil composition, climate, altitude and other managerial factors affect the physical and chemical properties of grassland (Church, 1991; Holmes, 1994; McDonald et al., 1995).

Beef cattle production systems ranges from the beef cow herds that typically graze on pastureland or graze the remaining residue on the land after grain harvest to growing and finishing young cattle in feedlots. The feedlot-housing systems used in beef cattle production typically varies by climate and can range from open earthen lots with very little shelter to open shed and lot or an enclosed confinement building (Pastoor et al., 2012).

In literature there are many studies in favour of different grazing systems for improving

performance of grazing animals. However, the best grazing system can change according the situations. Producers always search for the most effective grazing system and that utilize grazing livestock are continually faced with the need to develop, implement, monitor and evaluate their grazing systems. It is important to have effective and efficient grazing systems for profitable cattle and sheep productions.
Therefore, in this study it was aimed to evaluate two different grazing systems (zero grazing) for the performance of grazing beef cattle on artificial pastures.

MATERIALS AND METHODS

Experimental Location

This research was conducted in 2012 in Isparta Province (37°45′N, 30°33′E, elevation 1035 m) located in the Mediterranean region of Turkey. During the experimental year, total precipitation as a long-term average was 450 mm. Average temperature was 12.1ºC.

Animals

The experiment was set up at Süleyman Demirel University Research Farm and lasted for 70 days in 2012. It was involved a total of 20 Holstein beef cattle with an average 6 months old and divided into two grazing groups in this experiment with an initial weight of 230 and 240 kg for Rotational Grazing (RG) and Zero Grazing (ZG) experiments respectively.

Animal and Pasture Management

Animals were initially weighed at the beginning of the experiments and were randomly divided according to their weights into two grazing groups. Each group was weighed and monitored on a fortnightly basis, using electronic weighing scale (True-Test2000 SmartUnit). The animals had free access to water throughout the experimental period.
For the establishment of artificial grazing land, 3 ha pasture land was chosen adjacent to the university farm and cultivated in March 2010 with a botanical composition of *Medicago sativa* L. (20%) + *Bromus inermis* L. (40%) + *Agropyron cristatum* L. (30%) + *Poterium sanguisorba* (10%).

The chemical composition changes in pastures were monitored in order to determine the nutritious properties of grasses. The grass samples were collected by using $1m^2$ quadrates fortnightly from May to August. The fresh biomass (FB) yield, dry matter (DM) yield, crude protein (CP), acid detergent fibre (ADF) and neutral detergent fibre (NDF) contents were determined as well.

Statistical Analysis

The data were subjected to the statistical analysis by performing General Linear Model (GLM) procedure using Minitab.16 statistical software programme and in the statistical model, initial weight and age were taken as covariates to eliminate the weight and age differences at the start of the experiment.

$$Y_{ijk} = \mu + \alpha_i + \beta_j + \varepsilon_{ijk}$$

where *Yijk* is the *ijk* th observation of animal weight,

μ is the overall mean,

αi is the effect of treatments,

βj is the effect of initial weight and,

εijk is the residual effect or random error associated with the individual animal

RESULTS AND DISCUSSIONS

The least-squares means and standard errors for liveweights for grazing systems are shown in Table 1.
As it is presented in Table 1, final weights of the animals in 2012 were 306 and 299 kg; the average total weight gains 66 and 69 kg and finally daily liveweight gains of 0.954 and 0.996 kg respectively. Similarly, in respect to performance of animals in grazing systems, the final weights were 306 and 299 kg for ZG and RG respectively. The average total weight gains 66 and 69 kg and finally daily live weight gains of 0.954 and 0.996 kg respectively.
There were no significant (P >0.05) differences between grazing systems in terms of Final Weights (FW), Total Weight Gains (TWG) and Daily Liveweight Gains (DLWG). However, the animals in RG tended to perform better than the cattle in ZG in all parameters observed.

Table 1. Overall performance comparisons of animals by grazing system types*

Grazing System	N	IW(kg)	s.e.	FW(kg)	s.e.	TWG(kg)	s.e.	DLWG(kg)	s.e.
ZG	10	240	19.7	306	16.7	66	4.93	0.954	0.071
RG	10	230	17.7	299	15.6	69	5.44	0.996	0.078

IW= Initial weight, FW= Final weight, TWG= Total weight gain, DLWG= Daily liveweight gain
* The means with the same superscripts presented in the table are not statistically significant (P >0.05).

There were also no statistical differences in chemical compositions of grasses in both pastures.

In the literature, there are similar or contradictory results obtained to the findings of this study. In contrast to the finding of this study, Bozkurt and Kaya (2011) reported that rotational grazing resulted in greater weight gains than set stocking to achieve maximum cattle performance. However, their study was carried out at a high altitude on hilly rangeland conditions.

The results of the study conducted by Bozkurt and Kaya (2011) confirmed that rotational grazing has shown superiority over set-stocking grazing on high mountain ranges in many studies (Howery et al., 2000). It was also pointed out by Poland et al. (2004) that using a rotational grazing system improved animal performance with increased stocking rate, calf average daily gain and calf gain per acre and resulted in an improved financial status for the operation. However, in this study there was no superiority of any grazing systems over each other. Vendramini and Sollanberger (2007) reported that no single grazing management system is suitable for all forage systems in all environments. Because of the possibility of greater forage production and pasture persistence, rotational grazing has potential to improve animal production on beef cattle operations in many grazing conditions. The results of this research can be considered to be consistent with the statement that rotational grazing increased performance of the animas although there was no statistical difference in the performance of animals in both grazing systems in this study (Hensler et al., 2007).

Pointed out that rotational grazing provides continual ground cover and high quality, good-yielding forage for the livestock and as a result better animal performance In contrast, animals in a set stocking grazing system are left in a single, undivided pasture for weeks or months, often yielding overgrazed, sparse pastures with low persistence (Broomer and Moore, 2000; Teague and Dowhower, 2003). Bozkurt and Kaya (2011) concluded that rotational grazing using electrical fencing system can substantially improve grazing performance of beef cattle on hilly rangeland conditions.

Performance potential varies greatly between different breeds of cattle and different production systems. While there are certainly differences between performance of animals in growth rate, the liveweight gain which can be achieved from a given area of grass or quantity of feed is similar for most breeds of animals, provided that animal is fed and managed according its own environment (Wilkinson, 1985; Bozkurt and Ap Dewi, 1996; Bozkurt, 2012).

The results of these comparisons, including those reported in literature are not necessarily applicable outside the countries where such experiments were carried out due to the differences in factors such as production systems, slaughter weights and climate etc.

CONCLUSIONS

Consequently, both type of grazing systems can be recommended for beef cattle production in the region. However, it should be taken into consideration that there was a tendency for the animals perform better in rotational grazing system on artificially established pastures in the West Mediterranean climate conditions.

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REFERENCES

Acar R., Demiryürek M., Okur M., Bitgi S., 2011. An investigation of artificial pasture establishment under dryland conditions. African Journal of Biotechnology, 10(5):764-769.

Allen D., Kilkenny B., 1984. Planned beef production, Collins, London.

Bozkurt Y., 2012. Seasonal performance of different breeds of feedlot beef cattle grown under the mediterranean conditions. Bulgarian J. Agric. Sci., 18(3):443-445.

Bozkurt Y., Ap dewi I., 1996. Effect of breed type, sex, birth year and season of birth and their interactions on liveweight change in beef cattle. Selcuk Univ. J. Agric. Fac., 10(13):125-140.

Bozkurt Y., Kaya I., 2011. Effect of two different grazing systems on the performance of beef cattle grazing on hilly rangeland conditions, Journal of Applied Animal Research, 39(2):94-96.

Brummer, E.C. and Moore, K.J. 2000. Persistence of perennial cool-season grass and legume cultivars under continuous grazing by beef cattle. Agronomy J., 92: 466-471.

Church D.C., 1991. Roughages, livestock feeds and feeding, Simon and Schuster, New Jersey, 56-68.

Ecevit F., 1999. Açıkta sığır besisi paneli, Süleyman Demirel Üniversitesi Ziraat Fakültesi, Isparta, 3-6.

Hensler A.L., Barker D.J., Sulc R.M., Loerch S.C., Owens L.B., 2007. Comparison of management intensive grazing and continuous grazing in beef cattle pasture. Proceedings of American Forage and Grassland Conference, 16, 48-51, June 24-26, State College, Pennsylvania.

Holmes W., 1994. Grass: its production and utilization, The British Grassland Society, Blackwell Scientific Publications, London,

Howery L.D., Sprinkle J.E., Bowns J.E. 2000. A summary of livestock grazing systems used on rangelands in the Western United States and Canada, Arizona Cooperative Extension Bulletin, AZ1136.

McDonald P., Edwards R.A., Greenhalgh J.F.D., Morgan C.A., 1995. Grass and forage crops, animal nutrition, England Longman Scientific and Technical, 434-444.

Minitab, 2006. Statistical Software, Bortland Inc., version 16.

Pastoor J. W., Loy D.D., Trenkle A., Lawrence J.D., 2012. Comparing fed cattle performance in open lot and bedded confinement feedlot facilities, The Professional Animal Scientist, 28(4):410-416.

Poland C., Walker J., Patterson T., 2004. Economic comparison of grazing systems for the northern great plains. Annual Report, Beef Section, Dickinson Research Extension Center 1089 State Avenue Dickinson, ND 58601.

Teague W.R., Dowhower S.L., 2003. Patch dynamics under rotational and continuous grazing management in large, heterogeneous paddocks. J. Arid Environ., 53(2): 211-229.

Vendramini J., Sollenberger L., 2007. Impact of grazing methods on forage and cattle production, Publication no: SS-AGR-133.

Wilkinson J.M., 1985. Beef production from silage and other conserved forage. Longman Group Limited, London and New York, 128.

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INFLUENCE OF SOME ORGANIC COORDINATION COMPOUNDS CONTAINING COBALT AND BISMUTH ON DEVELOPMENT MORPHO-PRODUCTIVE CHARACTERS OF THE BEE FAMILIES

Valentina CEBOTARI[1], Ion BUZU[1], Ion TODERAŞ[1], Aurelian GULEA[2], Olga POSTOLACHI[1], Valeriu TODERICI[1], Olesea GLIGA[1]

[1]Institute of Zoology of Academy of Sciences of Moldova (ASM) , MD 2028, Chişinău, Str. Academiei, 1, Republic of Moldova, Phone: +373.22.73.98.09, Fax: + 373.22.73.98.09, Email: izoolasm@yahoo.com
[2]University of State of Moldova, str. A. Mateevici, 60, MD-2009, Chişinău, Republic of Moldova, Phone: +373 22 577787, Fax: +373 22 244248, Email: usm.international@yahoo.com

Coresponding author email: valentinaceb@yahoo.com

Abstract

The aim of the research was to evaluate and test the influence of organic coordination compounds containing rare microelements, on the vital functions and on the development of the morpho-character of Apis mellifera bee colonies. Several experiments where conducted and was tested in feeding bees in spring (April 1 to 21) a period with poor harvest in nature, a nutritious blend of 50% sugar syrup supplement enriched with bioactive organic compound heteronuclear coordinative sulfate [tris-thiosemicarbazide cobalt (III)] [1, 2-diaminociclohexantetraacetat bismuth (III)] hexahydrate - [Co (tios) 3], [Bi (CDTA)] $SO_4 \cdot 6H_2O$ in aqueous solution with concentration of 1 mg% (hereinafter referred to as "compound + Co + Bi), which was mixed with sugar syrup in a ratio of 2: 100, and was administered directly into the food of bees and bee families where feed with the nutrient in amount of 100 ... 130 ml for every frame with bees, every 2 days for a period of three weeks. Bee colonies were formed into three lots, each lot formed from 16 families, of which: group I - control, which were fed only with sugar syrup 50%, group II - prototype, which where feed with sugar syrup by adding nutritional supplement enriched with patented "Apispir + Fe + It" (MD 477 Z 2012.09.30) and group III - experimental, which received sugar syrup enriched with supplement "compound + Co + Bi". The research results have shown that using energizing nutritional supplements enriched with biologically active substances of organic compounds coordination in bee feeding in times when there is a poor harvest in nature, helps stimulate vital functions of bee families and increase their productivity: the prolificity of queen - with 5.2 to 9.7% (P <0.001); the amount of capped brood - with 5.2 to 9.7% (P <0.001); family strenght- by 2.5 to 9.7% (P <0.1s P <0.001); disease resistance - by 2.4 to 5.0% (P <0.001); brood viability - with 1.2 to 2.2% (P <0,01s (P <0.001); the amount of accumulated bee bread in brood chamber- by 15.0 to 23.3% (P <0.001), an increased amount of wax combs - by 21.4 to 39.3% (P <0.001) and the amount of honey accumulated in the colony - with 13.9 to 25.4% (P <0.01 and P <0.001). The beneficial effect of feeding bees with biologically active nutritional supplements Apispir + Fe + + Co + Se and Bi compound denotes the fact that in the spring (March-April), a period poor in harvesting, in the area studied, in nature there is a shortage of biologically active substances, including rare microelements.

Key words: *testing, supplements, rare microelements, feeding, bees.*

INTRODUCTION

Many biological research (Istriteanu et al., 2002; Van Strallen, 1994; Войнар, 1960; Игнатьев et al., 2006; Ковальский, 1971; Колосова, 1968; Ноздрюхина, 1977; Тома et al., 1980) performed both in our country and abroad, have proved that that trace elements play an important role in the metabolism of biotic environments both plants and animals. From the numerous number of trace elements, Co is one of the most well studied in the animals and the humans, which can not be said about Bi.

In warm-blooded animal organisms, Co fulfills various functions: synthesis of blood elements, enzymes, vitamin B12 (cyanocobalamin), to boost protein metabolism and assimilation of vitamins A, E, C, activation of some enzymes and antibiotics, inhibition of some pathogenic micro-organisms (Колосова, 1968; Яковлев, 1972). It is assumed that such functions could have cobalt in the insect body through the hem lymph.

The inclusion of cobalt chloride (CoCl2) in bees feed, the researcher Яковлев А.С. 1972 (Яковлев, 1972) obtained an increase of working bees longevity by 5 days compared with controls. Based on this research, the author inferred conclusion that Co strengthens the defence functions of bee's body.

However, a number of other researchers (Hernandes et al., 1985; Somlyay, 1983; Кирилюк, 2006) studied the existence of these trace elements in the biosphere components in terms of heavy metals as environmental pollutants, if their concentration exceeds the maximum allowable. After the particularities of migration in the biosphere, the researcher Кирилюк, 2006 classifies trace Co and Bi in aqueous cations category respectively poorly circulating (low intensity) and less circulating (insufficient), noting their impact, beneficial or harmful on living organisms depends on the concentration and form of their existence in nature and the accessibility of the active circulating forms. For example, bismuth from nature is assimilated by plants very difficult. So, the concentration of the trace element in the flower nectar and pollen is very low. Therefore, we can ascertain, rather, an insufficiency of these rare micronutrients in the nature, but extremely important for living organisms, including bees, than heavy metal pollution. In the spring, after wintering colony of bees, the bee's body there is a deficiency of bioactive nutrients, especially micronutrients, which have a catalytic role in physiological processes of vital activity of bees, fulfilling multiple functions in the bees body at the cellular level, entering into the composition of enzymes and hormones with decisive role in metabolism. Insufficient of active biological substances, especially trace elements, leading to the weakening of resistance to diseases of bee families and decrease their productivity (Колосова, 1968).

The main natural sources of supply the body with micronutrients are bees nectar and pollen collected from melliferious plants. According to scientific information in honey and pollen are more than 30 micro-macro, including trace elements. Among them, cobalt and bismuth are found in very small quantities, from 10 to 14 mg%, but their role in the metabolism of substances in living organisms is enormous.

Given that the spring (poor harvesting period in kind), most beekeepers feeds bee families with sugar syrup, which constituted more than half of the necessary trace elements are missing, then identify sources of available trace elements to enrich ration nutritional supplement of bees families becomes an actual problem.

Are known processes for the feeding of bee families sources with trace elements, in particular in salt form of cobalt $CoCl_2$ at a dose of 8-25 mg per litre of sugar syrup (Яковлев, 1972), but the efficiency of these methods is very low, because digestion and assimilation of this salt in the body of bioactive substances are very weak.

For these reasons, researchers of the Institute of Zoology of the ASM, together with those from Moldova State University, undertook a series of studies focused on the identification of available sources of biologically active substances to feed bees, including trace elements, obtained using coordination of organic compounds (Patent MD 475, 476, 477 Z 2012; Cebotari et al., 2012; Cebotari et al., 2013; Cebotari et al., 2013) According to the information of Заозерский (1965), coordination complexes compounds plays an enormous role in the processes of vital activity of living organisms. Such substances extremely important as regards biological, such as haemoglobin and chlorophyll are into complex compounds. It was observed that a number of rare elements which are found in animal and plant tissues, enter into the coordination complex compounds. Metals linked in complex are part of the enzymes, in particular to those oxidative.

In this context, the aim of the research was to assess the influence of some coordination organic compounds containing rare trace elements, on the vital functions and development morph productive character of *Apis mellifera* bee families.

MATERIALS AND METHODS

The researches were conducted on families of bees *Apis mellifera carpathian* grown at experimental apiary of Zoological Institute of the Academy of Sciences. Apiary is located at stationary in a clearing of the forest, near its edge. The main melliferious sources in this area

are white acacia, linden and spontaneous flora inclusive yellow melilot.

In special, experiments was tested in bees feeding in spring (April 1 to 21) from poor harvesting in nature, a nutrient mixture of 50% sugar syrup supplement enriched with bioactive supplement from hetero nuclear organic coordination compounds *sulphate [tris-thiosemicarbazide of cobalt (III)] [1, 2-diaminociclohexantetraacetat of bismuth (III)] hex hydrate - [Co (tios) 3], [Bi (CDTA)]* $SO_4 \cdot 6H_2O$ in aqueous solution to the concentration of 1 mg% (hereinafter referred to as "Compound + Co + Bi), which was mixed with sugar syrup in a ratio of 2: 100, administered directly into bees food, and bee families feeding with nutrient mixture was carried out at 100 ... 130 ml of mixture at every frames interval populated with bees, every 2 days for three weeks.

To estimate the efficiency of the process of bees feeding with up-nominated nutritional supplement, experiments were conducted comparative testing it on bee colonies formed in three batches, each batch by 16 families in each group, which batch I - control, which: bees were fed only 50% sugar syrup only, batch II - prototype, the bees which received in food sugar syrup enriched with nutritional supplement patented "Apispir + Fe + Se" (MD 477 Z 2012.09.30) obtaining from *Spirulina platensis* biomass grown in the presence of organic coordination compounds *selenite Fe (III) chloride hex hydrate* $FeSeO_3 \cdot 6H_2O$ and batch III - the actual experiment, the bees which received the sugar syrup enriched with food supplement *"Compound + Co + Bi"*.

After spring stimulation to the 45 days of the experiment start and first collected (70 days after the start of the experiment), at the bee families were evaluated the following morpho - productive characters: queen´s prolificacy, brood´s capacity, family strength, resistance to disease, brood´s viability, increased amount of wax combs in the nest, the quantity of honey and bee bread accumulated in the nest.

Determining the level of development bee families morpho-productive character were carried, out according to the methodology developed by us (Cebotari, 2010) for livestock norm concerning bee families assessment, growth and certification of beekeeping genitor material certification, approved by Government Decision no. 306 of 28.04.2011 (OJ no. 78-81 of 13.05.2011, art. 366) (Livestock norm, 2011). The data obtained in experience, was statistically processed using computer software "STATISTICA - 6" and was evaluated their certainty, according to variation biometric statistics, by the methods of Плохинский (1969).

RESULTS AND DISCUSSIONS

The test results in bees feeding, of nutritional supplements enriched with aqueous solutions of coordination organic compounds containing rare trace elements, demonstrated that they (supplements) had a beneficial action in general, on vital activity of bee colonies and growing, especially, their productivity (Table 1).

Mentioned that in the 45 days after the beginning of the experiment, the bee families of experimental groups who received in feed nutritional supplements enriched with coordination organic compounds containing rare microelements with both „*Apispir + Fe + Se*", and the *"Compound + Co + Bi",* had only a rising trend compared with controls batch the level of character and morpho- productive feature, within the limits 1.7 - 4.0% (P>0.1). Given the fact that the coefficient of certainty of these differences was recorded as zero the threshold level of probability theory of forecasts contest without error after Student (Плохинский, 1969) meaning beneficial effect at this stage (45 days after the start of the experiment), gives only a tendency character. At the same time, the period of 70 days from the start of the experiment (see Table first collected), beneficial influence of biologically active substances of organic coordination compounds supplement *"Apispir + Fe + Se",* as well the appendix "*Compound + Co + Bi"* has become significant, and the certainty coefficient of difference between of bee families value morpho-productive characters from experimental batches and control batch when reached the highest certainty threshold according to the probability theory of forecasts contest without error.

Table 1. The test results in bees feeding (first harvest) with nutrient supplements, enriched with active organic coordination compounds, containing rare microelements.

The experimental batch and bioactive element	The number of families in batch, N	The average value of character **M ± m**	The difference from the control batch		The coefficient of certainty, t_d
			d	%	
Queens prolificacy, eggs/24 hour					
Batch I (control)	16	1590 ± 20	-	-	-
Batch II (*Apispir+Fe+Se*)	16	1673 ± 14	+ 83	5.2	3.4**
Batch III (*Compound+Co+Bi*)	16	1745 ± 14	+ 155	9.7	6.3***
Quantity of capped brood, hundred cells					
Batch I (control)	16	190.8 ± 2.4	-	-	-
Batch II (*Apispir+Fe+Se*)	16	200.7 ± 1.6	+ 9.9	5.2	3.4**
Batch III (*Compound+Co+Bi*)	16	209.4 ± 1.6	+ 18.6	9.7	6.4***
Family strength, kg					
Batch I (control)	16	3.20 ± 0.02	-	-	-
Batch II (*Apispir+Fe+Se*)	16	3.28 ± 0.04	+ 0.08	2.5	1.7
Batch III (*Compound+Co+Bi*)	16	3.51 ± 0.05	+ 0.31	9.7	5.8***
Resistance to disease, %					
Batch I (control)	16	88,4 ± 0,4	-	-	-
Batch II (*Apispir+Fe+Se*)	16	90,5 ± 0,3	+ 2,1	2,4	4,2***
Batch III (*Compound+Co+Bi*)	16	92,8 ± 0,3	+ 4,4	5,0	8,8***
Broods viability, %					
Batch I (control)	16	89.3 ± 0.3	-	-	-
Batch II (*Apispir+Fe+Se*)	16	90.4 ± 0.3	+1.1	1.2	2.6**
Batch III (*Compound+Co+Bi*)	16	91.3 ± 0.4	+2.0	2.2	4.0***
Quantity of bread, hundreds of cells					
Batch I (control)	16	90.5 ± 1.8	-	-	-
Batch II (*Apispir+Fe+Se*)	16	104.1 ± 1.5	+ 13.6	15.0	5.8***
Batch III (*Compound+Co+Bi*)	16	111.6 ± 2.1	+ 21.1	23.3	7.6***
Quantity of wax, kg					
Batch I (control)	16	0.28 ± 0.01	-	-	-
Batch II (*Apispir+Fe+Se*)	16	0.34 ± 0.01	+ 0.06	21.4	4.3***
Batch III (*Compound+Co+Bi*)	16	0.39 ± 0.01	+ 0.11	39.3	7.8***
Quantity of honey, kg					
Batch I (control)	16	11.62 ± 0.40	-	-	-
Batch II (*Apispir+Fe+Se*)	16	13.24 ± 0.40	+ 1.62	13.9	2.9**
Batch III (*Compound+Co+Bi*)	16	14.57 ± 0.38	+ 2.95	25.4	5.3***

Notice: * P < 0.05; ** P < 0.01; *** P < 0.001.

So, the **queens prolificacy** from bee families, being identical in all batches at the beginning of the experience, then increased significantly at the first harvest, compared with control batch, in the experimental batches, whose families have received the feed supplement enriched with both *Apispir + Fe + Se*, and *Compound + Co + Bi.* the coordinating organic compound. The most pronounced increase queens prolificacy was recorded in batch III, bees were fed during spring (April) with energy nutritional supplements, enriched with organic coordination compounds which contained rare microelements *Compound+Co+Bi*. The Queens of bee families from this batch exceeded certainly after prolificacy, their congeners from the control group - with 155 eggs or 9.7% (td=6.3; P<0.001). Be mentioned, that the difference, compared with the control batch, the queen prolificacy from bee families in the batch III, who received in feed nutritional supplement enriched with the coordinating organic compound containing rare microelements (Co, Bi) was 1.9 times higher, than the queen bee families in batch II, who received feed supplement enriched with *Apispir + Fe + Se* (P<0.001).

The quantity of capped brood, as determined by the queen prolificacy, as also positively influenced by nutritional supplements enriched with both biomass extract *Apispir + Fe + Se,* as well the coordinating organic compound containing rare trace elements such as *Compound + Co + Bi.* Thus, the bee families from experimental batches significantly exceeded after this character in the first harvest their congener from the control batch. The highest increase in the amount of capped brood,

compared with controls, was registered at the bee families from the batch III, who received in feed nutritional supplement enriched with the coordinating organic compound containing rare microelements *Compound + Co + Bi*. The difference in increasing amount of brood capped in the bee families from this batch compared with the control batch was 18.6 hundred cells, or 9.7%. This difference is certain, with the highest certainty threshold of the probability theory of forecasts without error after Student (td= 6.4; P<0.001).

Thus, the strange of bee families, being identical in all groups at the beginning of the experience, then, at the first harvest, significantly increased compared with controls, in experimental lots, whose families received in feed nutritional supplements enriched, both the biomass extract *Apispir + Fe + Se,* and with the coordinating organic compound containing rare microelements (Co, Bi). The most pronounced increase of the strange of bee families was recorded in group III; the bees were fed during spring (April) with energy nutritional supplement enriched with coordination compounds *Compound + Co + Bi*. The Bee families from this lot exceeded with certainty, after power, their congeners from the control group - with 0.31 kg or 9.7% (td=5.8; P<0.001). Be mentioned, that the difference compared with the control group, the power from group III bee families, who received in feed nutritional supplement enriched with the coordinating organic compound containing rare microelements (Co, Bi), was about 3.9 times higher, than in bee families from batch II, who received feed supplement enriched with *Apispir + Fe + Se.*

Resistance to diseases of bee families, as a biological trait determined hereditary, can be influenced, at the same time, by external factors, among which, the most important is nutrition. The experiment results showed that at the first harvest, the bee families from experimental batches had a higher resistance, compared with controls batch. As a high resistance to disease was recorded in the bee families from the batch III, who received in feed nutritional supplement enriched with organic coordination compounds which contained rare microelements *Compound + Co + Bi*. After the value of this character, the bee families from this experimental group exceeded their congener from the control batch with 4.4 percentage points or 5.0% (td= 8.8; P<0.001). Given the fact, that biological variability of this character is very narrow, significance of this difference (small at first glance, the absolute size) is quite high and corresponds to a high certainty threshold of the probability theory forecasts contest without error.

The more obvious, this increased of development morpho-productive characters of bee families from experimental batches can be seen in the histogram (see figure). From the diagram it can be seen that all the morpho-productive characters of the bee families from experimental lots, shown in brown and yellow colours pillars is significantly higher than the control batch.

The broods viability, such as disease resistance, the bee families from bees whose experimental lots received feed supplements enriched with biologically active substances, was significantly higher compared with batch controls. The highest viability of brood was recorded in the bee families from the batch III, who received in feed nutritional supplement enriched with organic coordination compounds, *Compound + Co + Bi.* After this important biological character, the bee families from the batch III significantly exceeded their contemporaries from the control group with 2.0 or 2.2%. Because brood viability character has a narrow variability (as disease resistance), this small difference at first sight is certainly the highest threshold (td=4.0; P<0.001), according to forecasts contest without error probability theory.

The quantity of bee bread accumulated in nest was also positively influenced by nutritional supplements enriched, both biomass extract *Apispir + Fe + Se*, and with the coordinative organic coordination compounds rare microelements such as Co and Bi. Thus, the bee families from experimental lots significantly exceeded by the level of this character, at the first harvest, their congener from the control group with 13.6-21.1 hundred cells, or 15.0-23.3% (P <0.001). The highest increase amount of bee bread accumulated in the nest, compared with controls, was registered that the bee families from the batch III, who received in feed nutritional supplement enriched with the

coordinative organic compound containing rare microelements *Compound + Co + Bi.* The difference in increasing amount of brood capped in the bee families from this batch, compared to with the control batch is 21.1 hundred cells or 23.3%. This difference is certain with the highest certainty threshold of probability forecasts without error (td=7.6; P<0.001).

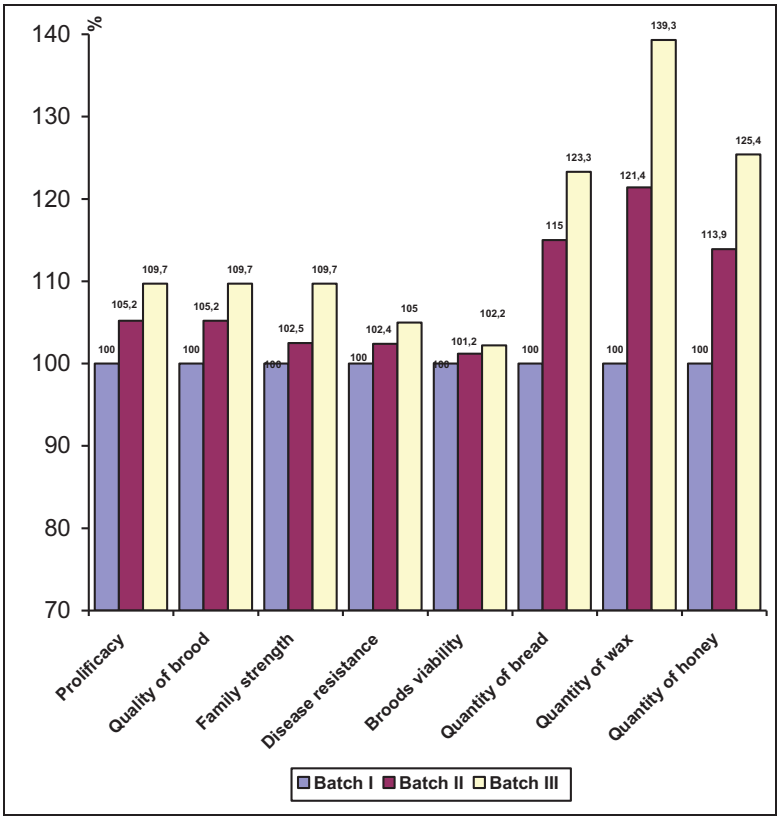

Figure 1. The level of morpho- productive characters of the bee families, compared with control batch.

The quantity of wax increased on the combs in the nest was equally influenced positively by nutritional supplements enriched with both biomass extract *Apispir + Fe + Se,* and with the organic coordination compounds containing microelements such as Co and Bi.

Thus, the bee families from experimental lots significantly exceeded by the level of this character, at the first harvest, their congener from the control batch with 0.06-0.11 kg or 21.4-39.3% (P<0.001). The highest increase of wax quantity in the nest, compared with controls, was registered to the bee families from the batch III, who received in feed nutritional supplement enriched with the organic coordination compounds containing rare microelements (Co + Bi). The difference in increase, compared to controls, of wax quantity accumulated in the nest bee families from this lot, was 0.11 kg, or 39.3%. This difference is certain with the highest certainty threshold of the probability forecasts without error (td=7.8; P<0.001).

The quantity of honey accumulated in the nest, is the most important morpho-productive character, was also positively influenced by the nutritional supplements, enriched with the extract of biomass as *Apispir + Fe + Se,* and with the organic coordination compounds containing rare microelements such as Co and Bi.

Thus, the bee families from experimental lots II and III significantly exceeded level of production of this character at the first harvest, their congener from the control group with 13.9-25.4% (P<0.01 and P<0.001). Of these two

groups, the largest increase in the amount of honey accumulated in the nest, compared with controls, was registered at the bee families from batch III, who received in feed nutritional supplement enriched with organic coordination compounds containing rare microelements *Compound + Co + Bi*. The difference in growth of the amount of honey accumulated in the nest bee families from this batch, compared with controls, is 2.95 kg or 25.4%. This difference is certain, with the highest certainty threshold of the probability theory of forecasts without error after Stiudent (td=5, 3; P< 0,001).

Generalizing the results of research development level of morpho-productive character of bee families can conclude, that biologically active substances from organic coordination compounds mentioned above, certainly contributes to the activation of family reproductive functions (queens prolificacy, quantity of capped brood, family strength), strengthening immunity of body insect (increasing resistance to disease and brood viability) and substantially increasing as a whole productivity of bee families. Beneficial influence of coordinative organic compounds on the vital functions of bee families is explained by us, not only by the action of rare microelements, and through the complex action of all biologically active substances from their complicated molecular structure with their structural ties very close and stable, including, of the complexes ions of the ligands and metal ions of the radicals with modified valence and increased penetration properties of cell membranes of living tissues of the bee body.

CONCLUSIONS

Using bees in feed during periods of poor harvest in nature, of energizing nutritional supplements, enriched with biologically active organic compounds of organic coordination compounds hetero nuclear sulphate [tris-thiosemicarbazide of cobalt (III)] [1, 2 – diaminociclohexan tetraacetat of bismuth (III)] hex hydrate - [Co (tios) 3] [Bi (CDTA)] $SO_4 \cdot 6H_2O$, contributes to stimulating the vital functions of bee families and increase to their productivity: the queen prolificacy - 9.7%; the amount of capped brood - 9.7%; family strength - with 9.7%; resistance to disease - 5.0%; the brood viability - with 2.2%; the quantity of bees bread accumulated in the nest – with 23.3%; increased amount of wax combs - by 39.3% the honey quantity honey gathered in the nest - by 25.4%.

The beneficial effect of the feeding bees of biologically active nutritional supplements Apispir + Fe + Co + Se and Bi compound indicates, that in the spring (March-April) poor harvesting in the studied area, in nature there is a shortage of biologically active substances, including rare microelements.

The deficit of biologically active substances in bees ration can be completed by synthesizing new organic coordinative compounds by new generation, highly effective in stimulating vital activity processes of *Apis mellifera* bee families.

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REFERENCES

Patent MD 475 Z 2012.09.30

Patent MD 476 Z 2012.09.30

Patent MD 477 Z 2012.09.30

Cebotari Valentina, Buzu I., 2010. Zootechnical norms regarding the honeybee colonies evaluation, breeding and certification of genetic material in beekeeping. Contemporary Science Association. Proceedings of the 1st International Animal Health Science Conference: The Beekeeping Conference. Addleton Academic Publishers, Library of Congress Control Number, New York, 26-30.

Cebotari Valentina, Toderaş I., Buzu I. , 2012. The use of biologically active substances for strengthening of resistance to discases of honeybee colonies *Apis mellifera.* Simpozion Ştiinţific Internaţional „Zootehnia modernă, factor al dezvoltării durabile". Universitatea de Ştiinţe Agricole şi Medicină Veterinară din Iaşi. Facultatea de Zootehnie. Lucrări Ştiinţifice, Seria Zootehnie, Editura „Ion Ionescu de la Brad", România, Iaşi, 57, 39-43.

Cebotari Valentina, Toderaş I., Buzu I., Rudic V., 2013 The role of „*Apispir+Zn*" biostimulator in increasing of productivity of *Apis mellifera* bee colonies. University of Agricultural Sciences and Veterinary Medicine Iasi. Scientific Papers. Series Animal Science, ISSN-L 1454-7368, Iaşi, 59 (18):103-107.

Cebotari Valentina, Toderaş I., Buzu I., Rudic V., 2013. Use of chrome trace for vital activities functions

stimulation of *Apis mellifera* bee colonies. International Conference of University of Agronomic Sciences and Veterinary Medicine of Bucharest. Faculty of Animal Science. Scientific papers, Series D Animal Science, ISSN-L 2285-5750, Editura „Ceres", Romania, Bucharest, LVI, 73-77.

Hernandes L.M. et al., 1985. Presence and biomagnetification of organochloride poluants and heavy metals of Donana National Park (Spain), 1982-1983. J. Environ. Sci. Health, 20 (6):633-650.

Istriteanu D., Dumitru M., 2002. Monitoring of the evolution of soil gvality from the power plants influence area. Advances and prospects of ecological chemistry. Conference proceeding. The second Int. Conf. on Ecological Chemistry. Chişinău, Ed. „Ştiinţa", 66-71.

Normă zootehnică privind bonitarea familiilor de albine, creşterea şi certificarea materialului genitor apicol, aprobată prin Hotărârea Guvernului nr. 306 din 28.04.2011 (M.O. nr. 78-81 din 13.05.2011, art. 366).

Somlyay J., Varnagy L., Fancsi T. , 1983. Monitoring of pesticide and heavy metals residues in tiscue of roe, red dur, and wild boar in westwrn and southern parts of Hungary. In: Erkrankung. Zootiere. Verhandlungsber. 25 Int. Symp. Wien. Berlin, 423-428.

Van Straalen N.M. , 1994. Biodiversity of ecotoxicological responses in animals. Netherlands. J. Of Zoology, 44 (1-2):112-134.

Войнар А.И., 1960. Биологическая роль микроэлементов в организме животных и человека. Изд. «Высшая школа», Москва, 544.

Заозерский И.Н. и др., 1965. Неорганическая химия. Изд. «Высшая школа», Москва, 495.

Игнатьев В.Н., 1969. Содержание микроэлементов в основных кормах Молдавской ССР. Труды МНИИЖиВ, т. 4, Кишинев, 17-29.

Кирилюк В.П., 2006. Микроэлементы в компонентах биосферы Молдовы. Ed. «Pontos", Chişinău, 155.

Ковальский В.В., 1971. Изменчивость обмена веществ у животных, вызываемая естественными химическими факторами среды. Ж. «Вестник с.-х. науки», №1, 11-28.

Колосова А.М., 1968. Эндемические болезни животных. Изд. «Колос», Москва, 288.

Ноздрюхина Л.П. Биологическая роль микроэлементов в организме животных и человека. Изд. «Наука», Москва, 1977, 184.

Плохинский Н.А., 1969. Руководство по биометрии для зоотехников. Изд. «Колос», Москва, 256.

Тома С.И., Рябинович И.З., Великсар С.Г., 1980. Микроэлементы и урожай. Изд. «Штиинца», Кишинев, 172.

Яковлев А.С., 1972. Итоги исследований по выявлению стимулирующих подкормок на семьи пчел. Труды НИИ пчеловодства. Изд. «Московский рабочий», Москва, вып. 7, 87-101.

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STUDY ON THE CHARACTERISTICS OF THE AUTOCHTHONOUS GOAT BREEDS EXPLOITED IN THE FARM OF S.C. AGROFAM HOLDING FETEŞTI

Rodica CHETROIU, Ion CĂLIN

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, Email: rodigeo7@yahoo.com, icalin63@yahoo.com

Corresponding author email: rodigeo7@yahoo.com

Abstract

The paper studies the characteristics of the goats' breeds Alba de Banat and Carpatina, in terms of fur aspect, exterior particularities, assessing the body development level and body indices determination. For determining the goats' body development level, the somato-metrical method was used, in which animal body measurements were made, at females inlactations1, 2and 3 and at males of ages1, 2 and 3 years and, based on these, the following statistical indicators were calculated: average, variance, standard deviation, standard error of the average and coefficient of variation. The results of body measurements performed at Alba de Banat show that the superior line of the animal body describes an upward trend from the anterior train to the back train, medium size (67.467 ± 0.886 cm for females and males 72.278 ± 0.932 cm) and back train more developed than the anterior (for females, the height at croup 68.500 ± 0.753 cm, for males 73.278 ± 0.651 cm). At Carpatina breed, the superior line of the animal body also describes an upward trend from the anterior train to back train, medium size (67.033 ± 1.033 cm for females and 71.833 ± 1.070 cm for males) and back train more developed than the anterior train (for females, the height at croup 68.033 ± 0.902 cm, 72.167 ± 1.054 cm for males). The values calculated of body indices (lateral body format index, transverse body format index, skeleton index, massiveness index) show that the animals fit in the morpho-productive type of milk. Regarding the characteristics of goats breeds exploited in the farm of S.C. AGROFAM HOLDING FETEŞTI, the research results has shown that they fit within the breed's standard.

Key words*: breeds, goats, index, measurements, size.*

INTRODUCTION

The goats' raising had in the recent period a remarkable development, both in terms of herds and of productions level. Thus, according to FAO statistics, in 2013, worldwide there were over 1,005.6 millions heads. In Romania, raising goats shows an upward trend in the structure of animal production; following the global evolution manifested, in our country, in the past 10 years, was an upward trend of the total goats herds, from 558,000 heads in 2000, reaching to 1,265,676 heads in 2013, so an increase of 126% (http://faostat3.fao.org/browse/Q/QA/E).

MATERIALS AND METHODS

The research was conducted on the two autochthonous breeds of goats, Alba de Banat and Carpatina, exploited in the Farm 1 Ovidiu, from Borcea commune, Calarasi County, a farm owned by S.C. AGROFAM HOLDING S.R.L. Thus, Alba de Banat breed represents 50.4% (340 females and 9 bucks) of the total number of goats of the farm and Carpatina breed 43% (302 females and 8 bucks).

The fur colours and exterior particularities were evaluated outside in daylight conditions.

For determining the goats' body development degree, the somato-metrical method was used, in which animal body measurements were made, their position being placed forced. The measurements were performed at females in lactations 1, 2 and 3 and at males of ages 1, 2 and 3 years, with zoometer, tape measure and Wilkens compass.

There were performed 10 different body measurements: size (height at withers), croup height, trunk length, chest width, chest perimeter, chest depth, croup length, croup width at ischia, croup width at ilium, whistle perimeter for which were calculated the following statistical indicators: average, variance, standard deviation,

standard error of the average and coefficient of variation, by using the available application from Microsoft Excel program.

RESULTS AND DISCUSSIONS

ALBA DE BANAT BREED

The fur colour and exterior particularities

The fur colour of the goats from Alba de Banat breed in the farm under study is white, with some particularities: uniform white, white with reddish and white with brown aroused out as a result of a crossing the pure breed with getters from other breeds. In Table 1, there are displayed these characteristics.

Table 1. Fur colour particularities at the goats of Alba de Banat breed

Colour	Particularity	No. heads	%
White	uniform	442	87,7
	with reddish	27	5,4
	with brown	35	6,9
Total white colour		504	100,0

Assessments of body development at the goats of Alba de Banat breed

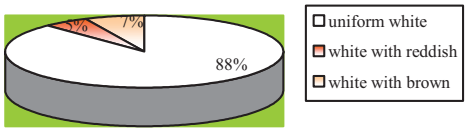

Figure 1 – The fur colour and its particularities at Alba de Banat breed

Regarding the exterior particularities, following the evaluation, it was found that there are some differences between animals regarding presence or absence, shape or size of the horns and ears, udder shape, its degree of development, the presence of formations called earrings, or goatee. Among these particularities, some are presented in Table 2.

Table 2. Morphological particularities at Alba de Banat goats

Morphological particularities	No. heads	%
Goats with horns	294	58.37
Goats with goatee	451	89.46
Goats with earrings	427	84.8
Earless goats	42	8.29
Total	504	100

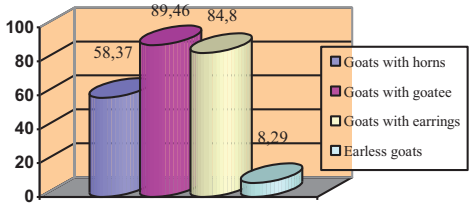

Figure 2. Alba de Banat breed – morphological particularities

The results show that almost 90% of the Alba de Banat goats herd presents a goatee and at nearly 85% are present the earrings. Regarding the absence of ears, this feature is found in only 8.29% of the herd.

Table 3. The main body dimensions at Alba de Banat breed (cm)

Specification	*Males*			*Females*		
	Average ± standard error of the average	V%	% of size	Average ± standard error of the average	V%	% of size
Height at withers (size)	72,278 ± 0,932	3,869	100	67,467 ± 0,886	5,084	100
Croup height	73,278 ± 0,651	2,667	101,4	68,500 ± 0,753	4,256	101,5
Trunk length	73,333 ± 1,083	4,432	101,5	68,933 ± 0,862	4,841	102,2
Chest depth	35,944 ± 1,232	10,280	49,7	32,500 ± 0,535	6,37	48,2
Chest width	25,333 ± 0,882	10,444	35	21,800 ± 0,942	16,738	32,3
Croup width	18,611 ± 0,865	13,945	25,7	16,333 ± 0,326	7,733	24,2
Chest perimeter	92,167 ± 2,705	8,806	127,5	87,567 ± 1,724	7,627	129,8
Whistle perimeter	10,278 ± 0,409	11,944	14,2	9,067 ± 0,182	7,762	13,4

The body measurement results show that the top line of the animals body describes an upward trend from the anterior train to the back train, medium size (67.47 cm for females and 72.28 cm for males) and the back train more developed than the anterior train (the croup height at females 68.50 cm, and at males 73.28 cm). The values obtained are

mostly similar to those reported by other authors for this breed (Călin, 2004; Taftă, 2002).
The animals have long shapes and small widths, the body format being dolicomorph, having a relatively harmonious conformation, fine, thin and elongated head, medium ears, in a slight bent position, thin neck, medium sized horns, held back, with divergent direction, laterally flattened, elongated trunk, pear-shaped, narrow back, sloping croup, relatively narrow chest, developed abdomen, long and thin limbs, but powerful, with normal position (Fig. 1).
The udder is generally medium or well developed, globular or pear-shaped, with good fixation, supple and smooth skin and well-developed nipples, suitable for mechanical milking.

Photo 1. Alba de Banat goats, from the farm in study
Source: Photo made by author

Determining the body indexes

In order to assess the proportionality and the development harmony of the different body regions or parts, as well as of the productive capabilities assessment, based on the body measurements performed, there have been calculated the body indexes, which are the ratio between two dimensions morpho-physiological correlated. They are presented in Table 4.

Table 4. The main body indexes at Alba de Banat goats (%)

Index	Males	Females
Lateral body format index	102,0	101,63
Transverse body format index	35,74	34,78
Skeleton index	11,15	10,39
Massiveness index	127,52	129,79

The lateral body format index values, expressed by the trunk length value reported to height at withers and the transverse body format index, obtained by reporting the chest width to the size, show that the animals fall into the morpho-productive type of milk.
Also, the skeleton index, with a value greater than 10%, indicates a population with well-developed bones, belonging to the morpho-productive type of milk. The massiveness index shows that these animals have a body development characteristic to the milk production type, with a relatively low massiveness.

CARPATINA BREED

The fur colour and exterior particularities

The predominant colours of the fur at the goats of Carpathian breed under study are brown and black (29.9% and 20.9%), but there are animals which have the following colours and particularities: black with white, reddish, grey, dark grey with black, grey with brown, brown with black (Table 5).

Table 5. Colours and colour particularities at Carpatina breed

Colour	Particularity	No. heads	%
Black	uniform	121	20,9
	with white	52	9,0
Total black colour		173	29,9
Reddish	uniform	97	16,8
Grey	uniform	43	7,4
Dark grey	with black	52	9,0
	with brown	27	4,7
Total dark grey colour		79	13,6
Brown	uniform	173	29,9
	with black	14	2,4
Total brown		187	32,3
Total		579	100

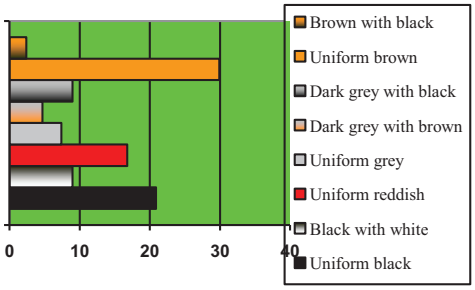

Figure 3. Carpatina breed – the fur colours and colour particularities

Regarding some particularities of exterior, following the assessment, it was found that there are certain differences between animals, which are presented in Table 6.

Table 6. Morphological particularities at Carpatina goats

Morphological particularities	No. heads	%
Goats with horns	401	69,25
Goats with goatee	529	91,47
Goats with earrings	410	70,83
Earless goats	25	4,37
Total	579	100

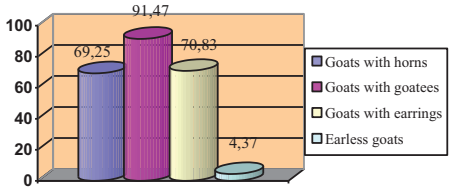

Figure 4. Carpatina breed – morphological particularities

The results show that 91.47% of Carpatina goats have goatee and at a number of approximately 70% are present earrings and horns. Regarding the absence of ears, this feature is found in only 4.37% of the herd. Between the two breeds, there are horned goats with 18.6% more at Carpatina than Alba de Banat and with 2.2% more goats with goatee, but with 16.5% fewer goats with earrings and with 47% fewer earless goats at Carpatina compared to Alba de Banat.

Assessments of body development at the goats of Carpatina breed

The body measurement results show that the top line of the animals body describes an upward trend from the anterior train to the back train, medium size (67.03 cm for females and 71.83 cm for males) and the back train more developed than the anterior train (the croup height at females 68.03 cm, and at males 72.17 cm). For this breed, also, the values are similar with those from other authors (Călin, 2004; Taftă, 2002). In Table 7 are presented these data.

Table 7. The main body dimensions at Carpatina breed (cm)

Specification	***Males***			***Females***		
	Average ± standard error of the average	V%		Average ± standard error of the average	V%	
Height at withers (size)	71,833 ± 1,070	3,648	100	67,033 ± 1,033	5,970	100
Croup height	72,167 ± 1,054	3,578	100,5	68,033 ± 0,902	5,133	101,5
Trunk length	72,167 ± 1,295	4,396	100,5	67,533 ± 0,983	5,635	100,7
Chest depth	35,583 ± 1,344	9,253	49,5	32,333 ± 0,410	4,913	48,2
Chest width	24,833 ± 1,014	10,000	34,6	21,233 ± 0,649	11,837	31,7
Croup width	18,750 ± 0,588	7,683	26,1	16,400 ± 0,219	11,380	24,5
Chest perimeter	91,833 ± 3,683	9,823	127,8	83,333 ± 1,560	7,252	124,3
Whistle perimeter	10,083 ± 0,539	13,088	14,0	9,267 ± 0,153	6,406	13,8

The animals of this breed have also long forms and small widths, dolicomorph body format, conformation relatively smooth, thin and fine head, medium ears, medium-sized horns, more developed at males, longish trunk, narrow back, sloping croup, relatively tight chest, thin limbs (Fig. 2). The udder is generally medium or well developed, globular or pear-shaped, with smooth skin, nipples generally well developed, most suitable for mechanical milking.

Photo 2 – Carpatina goats, from the farm in study
Source: Photo made by author

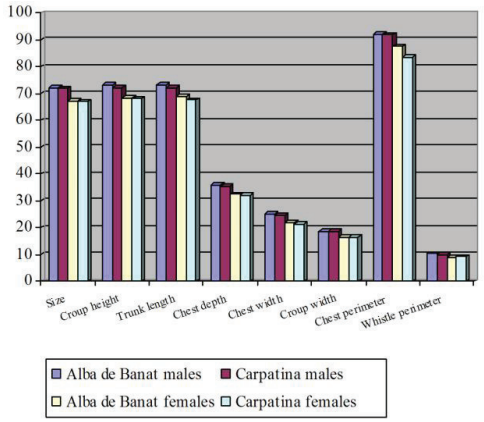

Figure 5. Comparison between the two breeds regarding the main body dimensions

Comparing the body dimensions of the two breeds, it appears that they are superior at Alba de Banat breed compared to Carpatina and at males compared to females.

Determining the body indexes

Based on the body measurements performed, there have been calculated the body indexes, which are presented in Table 8:

Table 8. The main body indexes at Carpatina goats (%)

Index	Males	Females
Lateral body format index	101,39	100,75
Transverse body format index	35,04	34,80
Skeleton index	10,98	10,76
Massiveness index	127,84	124,32

The lateral body format index values, as well as of the transverse body format index show that the animals of this breed fall also into the morpho-productive type of milk.

Also, the skeleton index, with a value greater than 10%, indicates a population with well-developed bones, belonging to the morpho-productive type of milk. Also, the skeleton index, of more than 10%, indicates a population with well developed bones, belonging to the milk production type. The massiveness index shows that these animals have a development characteristic to the milk production type, with a relatively low massiveness.

CONCLUSIONS

Regarding the characteristics of the goat breeds exploited in the farm of S.C. AGROFAM HOLDING FETEŞTI (Alba de Banat and Carpatina), the research results show that they fall within the breed standard. The fur colour of the goats from Alba de Banat in the farm under study is white, with some peculiarities: uniform white (87.7%), white with reddish and white with brown. The predominant colours of the fur at the goat of Carpatina breed in the farm under study are brown and black (29.9% and 20.9% respectively), but there are animals who have the following colours and particularities: black with white, reddish, grey, dark grey with black, dark grey with brown, brown with black.

The body measurements results performed at Alba de Banat breed show that the top line of the animal body describes an upward trend from the anterior train to back train, the medium size (67.47 cm at females and 72.28 cm at males) and the back train more developed than the anterior train (at females, the croup height 68.50 cm, at males 73.28 cm). The values obtained are mostly similar to those reported by other authors for this breed.

At Carpatina breed, the top line of the body of animals also describes an upward trend from the anterior train to back train, the medium size (67.03 cm at females and 71.83 cm at males) and the back train more developed than the anterior (at females, the croup height 68.03 cm, at males 72.17 cm). For this breed also, the values are similar to those reported by other authors. The values calculated of the body indexes show that the animals fall in the morpho-productive type of milk.

REFERENCES

Călin I., 2004. Technology of rising sheep and goats, Universităţii ”Lucian Blaga” Publishing, Sibiu.

Sandu Gh., 1995. Experimental models in livestock breeding, Coral Sanivet Publishing, Bucharest.

Taftă V., 2002. Production and reproduction of goats, Ceres Publishing, Bucharest.

Taftă V., 2008. Rising sheep and goats, Ceres Publishing, Bucharest.

FAOSTAT, http://faostat3.fao.org/browse/Q/QA/E

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COMPARATIVE ASPECTS ON THE WEIGHT GAIN OF THE KIDS OF ALBA DE BANAT AND CARPATINA BREEDS

Rodica CHETROIU, Ion CĂLIN

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, Email: rodigeo7@yahoo.com, icalin63@yahoo.com

Corresponding author email: rodigeo7@yahoo.com

Abstract

The growing process of kids from birth to adulthood is not a uniform process, but has a stage character, in the sense of achievement of more intense or slower growth in different periods. The assessment of growing process of the goat's youth was performed on kids of Alba de Banat and Carpatina breeds, at S.C. Agrofam Holding Feteşti, where weighing were made at different stages, namely at birth, at age of 28 days, at weaning (45 days) and at 6 months. The research results show that the weight gain of kids is superior at Alba de Banat compared to Carpatina; also, the males recorded a higher increase in weight than the females in both breeds, and the kids resulting from single births, on total period from birth until 6 months, have a higher growth than those from twin births. The weight gain curve is upward in the period of 28 days – weaning, compared to 0-28 days; in the period after weaning, until 6 months of age, the weight gain decreased at all categories, this may be due to the stress of weaning, transition to the exclusively foddered nutrition, fodders quality compared to nutrients of the maternal milk etc.

Key words*: kids, weight, weight gain, youth.*

INTRODUCTION

In all the countries, due to the accelerated increase in the number of human population, it practices most efficiently the exploitation of animal resources, applying more efficient technologies for animal breeding. In this context, the goat rising has known recently a remarkable development, both globally and in Romania. The effectiveness of a goat farm is subject also, among other things, of obtaining a weight gain of kids raised, in a short time.

MATERIALS AND METHODS

The assessment of growing process of the goat's youth was performed on kids of Alba de Banat and Carpatina breeds, raised in semi-intensive system, at S.C. Agrofam Holding Feteşti, where the weighing were made at different stages, namely at birth, at age of 28 days, at weaning (45 days) and at 6 months.

RESULTS AND DISCUSSIONS

The main factors influencing the intensity of growth and kid's development are the body weight at birth, the sucked milk quantity and the care and maintenance conditions. In Table 1 are given the weights of kids of Carpatina and Alba de Banat breeds in the farm under study, at different ages.

Table 1. The average weight of the kids, on breeds, at different ages (kg/head)

Breed	Weight at birth		Weight at 28 days		Weight at weaning		Weight at 6 months	
	Average ± standard error of the average	V%	Average ± standard error of the average	V%	Average ± standard error of the average	V%	Average ± standard error of the average	V%
Alba de Banat	2.961 ± 0.117	16.791	6.550 ± 0.173	11.210	8.889 ± 0.174	8.311	24.128 ± 0.268	4.708
Carpatina	2.710 ± 0.099	16.411	6.170 ± 0.087	6.290	8.460 ± 0.096	5.079	23.070 ± 0.211	4.088

From the table above, it appears that the average weight of kids from Alba de Banat breed is superior to those of Carpatina breed, at all the weighing moments, as follows: average weight at birth is higher by 9.26%, weight at 28 days with 6.2%, weaning weight by 5.1% and weight at 6 months by 4.6%.

The research results are within the range of values reported by other authors for these species (Tafta, 2008). In terms of the kids' weight in the two breeds, by gender, this is shown in Figure 1.

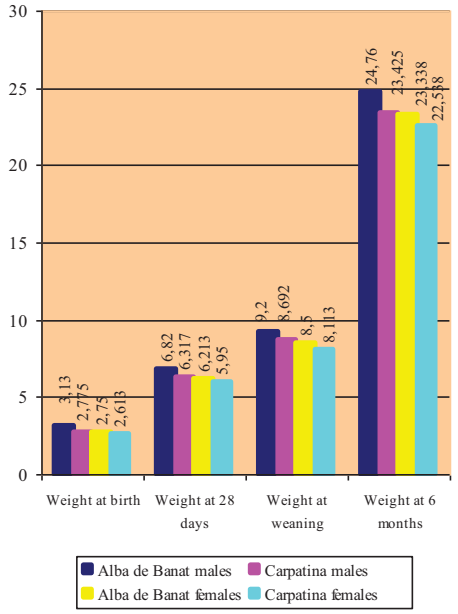

Figure 1. Evolution of the life weight at kids of Alba de Banat and Carpatina, by gender (kg)

The above data show that, by gender, the weight of males is higher than of females in both breeds. Thus, at Alba de Banat breed, the males weigh 13.8% more than the females at birth, with 9.8% more at the age of 28 days, with 8.2% at weaning and with 6.1% more at the age of 6 months.

At Carpatina breed, the males weigh 6.2% more than the females at birth, with 6.2% more at the age of 28 days, with 7.1% more at weaning and with 3.9% more at the age of 6 months.

Between the two breeds, the males of Alba de Banat weigh more than those of Carpatina breed, as follows: at birth with 12.8%, at the age of 28 days with 8%, at weaning with 5.8% and at the age of 6 months with 5.7%. Also, the females of Alba de Banat have a higher weight than the Carpatina breed: at birth with 5.2%, at the age of 28 days with 4.4%, at weaning with 4.8% and at 6 months with 3.5%.

Regarding the of weight gain of the kids from the two breeds, at different ages, it is shown in Table 2.

The results in this table show that average daily gain of kids of Alba de Banat breed is superior to the Carpatina breed kids, in all periods of growth, as follows: from birth to 28 days by 4.9%, from 28 days to weaning by 3%, from weaning at 6 months by 4.8% and from birth to 6 months by 4.5%. The largest increase in weight is recorded during 28 days - weaning, and the smallest increase, from weaning to 6 months.

Table 2. Average daily gain (ADG) of the kids from the two breeds, at different ages (kg/day)

Breed	ADG birth – 28 days		ADG 28 days - weaning		ADG weaning – 6 months		ADG birth – 6 months	
	Average ± standard error of the average	V%	Average ± standard error of the average	V%	Average ± standard error of the average	V%	Average ± standard error of the average	V%
Alba de Banat	0.128 ± 0.003	9.552	0.138 ± 0.003	8.314	0.110 ± 0.001	4.457	0.116 ± 0.001	3.916
Carpatina	0.122 ± 0.002	6.839	0.134 ± 0.003	9.938	0.105 ± 0.001	2.983	0.111 ± 0.001	2.625

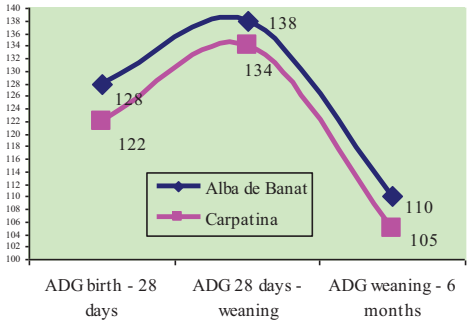

Figure 2. Average daily gain curves at Alba de Banat and Carpatina kids (g/day)

In the Figures 3 and 4, are shown the average daily gain curves, on gender, at the two breeds.

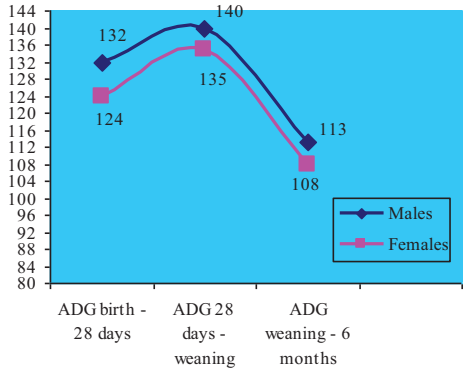

Figure 3. Average daily gain at kids of Alba de Banat breed, by gender (g/day)

The average daily gain of the males of Alba de Banat is larger than of females in the same breed, by 6.5% from birth to 28 days, by 3.7% from 28 days to weaning and by 4.6% from weaning to 6 months.

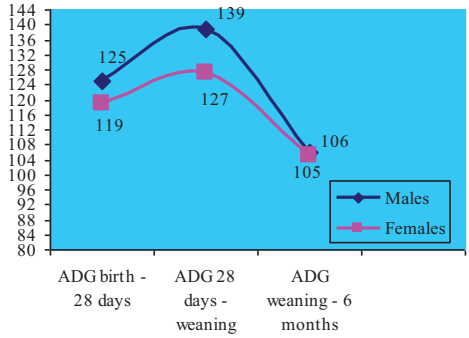

Figure 4. Average daily gain at kids of Carpatina breed, by gender (g/day)

At Carpatina breed, the average daily gain is higher at males than at females by 5% from birth to 28 days, by 9.4% from 28 days to weaning and by 1% from weaning to 6 months. In Figures 5 and 6, are shown the average daily gain curves on simple births and twin births, for the two breeds.

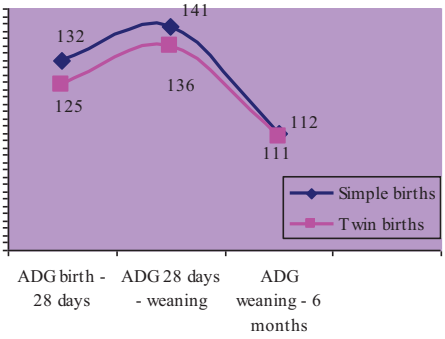

Figure 5. Average daily gain at kids of Alba de Banat breed, on simple births and twin births (g/day)

The data in Figure 5 shows that the kids of Alba de Banat breed from simple births record higher weight gains than those from twin births, as follows: from birth to the age of 28 days with 5.6%, from 28 days to weaning by 3.7% and from weaning to 6 months by 1%.

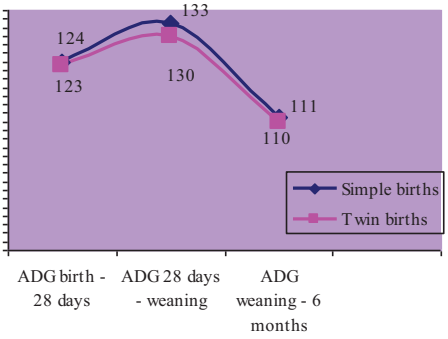

Figure 6. Average daily gain at kids of Carpatina breed, on simple births and twin births (g/day)

Also, at Carpatina breed, there are differences in the weight gain of kids, those from simple births recording increases higher than the twins: from birth to 28 days by 0.8%, from 28 days to weaning by 2.3% and on the entire period from birth until the age of 6 months, by 1%.

CONCLUSIONS

In terms of weight gain of kids, it is superior at Alba de Banat breed, compared to Carpatina; also, the males record an increase in weight larger than the females in both breeds and the kids resulting from simple births have higher weight gains than those from twin births. The weight gain of kids is conditioned by genetic factors, birth weight, gender, individual, type of birth (single or twin) feeding conditions (the mother's milk production and nutrition after weaning) and maintenance etc. It requires the application of amelioration programs in goat breeding in our country, both for milk and meat production, being needed to close the gaps between the results obtained in this field by farmers in Romania and those from European Union countries.

REFERENCES

Călin I., 2004. Technology of rising sheep and goats, Universităţii ”Lucian Blaga” Publishing, Sibiu.

I.C.D.C.O.C. Palas – Constanţa, 2008. Research report, P.S. 417, Stage IV.

Sandu Gh., 1995. Experimental models in livestock breeding, Coral Sanivet Publishing, Bucharest.

Späth H., Thume O., 2008. Rising goats, M.A.S.T. Publishing, Bucharest.

Taftă V., 2002. Production and reproduction of goats, Ceres Publishing, Bucharest.

Taftă V., 2008. Rising sheep and goats, Ceres Publishing, Bucharest.

Zaharia N., 2012. Study on goats populations from North-East of Romania, PhD Thesis, Iaşi.

Zamfirescu Stela, 2009. News in rising goats, Ex Ponto Publishing, Constanţa.

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RESEARCHES ABOUT TOTAL CHICKEN PROTEIN CONTENT

Ioan CUSTURA[1], Minodora TUDORACHE[1], Ilie VAN[1], Maria Antoaneta POPA[2]

[1]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, Email: johncustura2000@yahoo.com
[2]APIA Covasna, Sfântu Gheorghe, Str. Lt. Păiş David, no. 7, cod 520077.
Phone/Fax 0267-311.075 0267-311.148 0267-312.255, E-mail: apia.covasna@apia.org.ro.

Corresponding author email: johncustura2000@yahoo.com

Abstract

Poultry meat earned a very important position on worldwide animal food market due to both its nutritive value low costs compared with other animal protein sources. Poultry meat quality is a particularly complex feature and it is increasingly tackled by taking into consideration consumers' safety which is being now a disqualifying concurrence element on this market.
Considering this situation this study was performed with the aim of finding total chicken meat protein content.
Two chicken hybrids (ROSS 308 and COBB 500) were used during the experiment and influence of hybrid and production season on total chicken meat protein content was observed.
During firs year of experiment total protein content of ROSS 308 hybrids had values between 262.5669 g (season 1) and 265.4952 g (season 2) with difference non-significant statistically. COBB hybrids had a total chicken meat protein content between 312.4330 g and 316.6311 g (NS). Differences between average values of the two tested hybrids were highly significant statistically both in first and second season (respectively $t=12.2578^{***}$ and $t=12.3030^{***}$).
During the second year of the experiment carcass protein content values were between 263.1595 g and 257.9397 g in ROSS 308 hybrid and between 317.4594 g and 320.6270 g in COB 500 hybrid (NS). Differences between the two hybrids for the analyzed parameter were highly significant statistically which allows us to affirm the superiority of COBB 500 hybrid.

Key words: *chicken, protein, broiler.*

INTRODUCTION

Poultry meat quality research concerning poultry meat water content is being a very sensitive issue inside the European Union. What is important is finding not just water content but also protein content and proportion water/protein. Last value is offering information about chicken groups which might be approved or rejected. Methods and requirements have been established by Directive EC no. 543/2008.
Allowed water content of broiler meat inside the European Union is being, depending of chilling method, up to 7% in poultry carcasses and between 2% and 6% in cut parts. Beyond these limits selling of the product is still permitted but under obligation to label it with red capital letters as "water content beyond EC limit".

MATERIALS AND METHODS

A study to find out total broiler protein content using two genetic types of industrial broiler hybrids, ROSS 308 and COBB 500, was perform in poultry production farm Avicola Crevedia.
ROSS 308 is a valuable hybrid with production result up to 2,8 kg average body weight al 47 days of age with sexes housed together and with a conversion index of 1.813 and with a good slaughtering output and also with a better contribution of main cut parts in carcass.
COBB 500 is a hybrid adapted for different climates and production systems. If broilers are raised non sexed body weight might be 2.6 kg at 42 days of age and with a conversion index of 1.76 and with an excellent livability. High quality carcasses are produced following slaughtering, with a high slaughtering output of 78-80%.

The study aimed to find out the influence of genetic type and season on carcass total protein content.

Experiments took place during two years with two sequences in each year (one sequence was raised during the warm season (season 1: April-September) and the second during the cold season (season 2: October-March).

Groups of 100 birds (50-50) were raised in uniform conditions and in extended captivity, in upgraded houses, by sticking to standard technologies of each hybrid; feeding and watering were performed „ad libitum" and slaughtering was performed at 6 weeks of age.

Combined feeds for birds were processed according to nutritional requirements of studied hybrids by three research phases: starter 1-10 days (3055 kcal ME/kg and 24% CP), production 11-25 days (3178 kcal ME/kg and 22% CP) and finishing 26-42 days (3228 kcal ME/kg and 20% CP).

Total carcass protein content was evaluated and detected differences between the two genetic types and also between the two seasons of each year was statistically tested after slaughtering.

RESULTS AND DISCUSSIONS

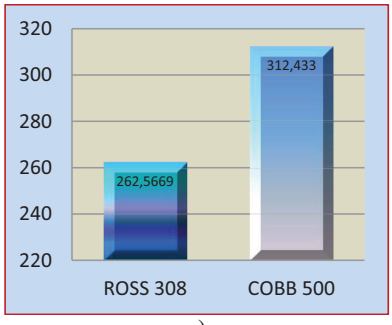
Chicken total protein content was evaluated in this paper as it is used for calculating chicken maximum acceptable water content. We are going to illustrate average values of this feature for the two discussed groups and also the statistical significance of differenced noticed between averages to evaluate quality standards in the house in which study was performed and also to emphasize a likely difference between the analyzed genetic types.

In table 1 we are illustrating value found for "chicken total protein content" in the two groups used in the experiment in year I and season 1.

From analyze of data in table 1 and figure 1a) it is noticed that best average performance was found for carcasses from COBB 500 chickens with 15.96% higher than at ROSS 308 chickens. Significance of differences noticed between averages of this feature was tested using the Student test. So, calculated value of Student test ($t = 12.2578^{***}$) has been showing very significant differences between average performances presented by groups of the two genetic types.

Table 1. Influence of genetic type on total protein content, first year, first and second season

Genetic type	n	$\bar{X} \pm s_{\bar{X}}$	s	c.v.%
First year, first seson				
ROSS 308	25	262.5669 ± 3.6322	18.1612	6.9168
COBB 500	25	312.4330 ± 1.8320	9.1600	2.9318
Differences significance	$t = 12.2578^{***}$ $t_{48;0.05} = 2.01$; $t_{48;0.01} = 2.68$; $t_{48;0.001} = 3.51$			
First year, second seson				
ROSS 308	25	265.4952 ± 3.7698	18.8488	7.0995
COBB 500	25	316.6311 ± 1.7505	8.7525	2.7643
Differences significance	$t = 12.3030^{***}$ $t_{48;0.05} = 2.01$; $t_{48;0.01} = 2.68$; $t_{48;0.001} = 3.51$			



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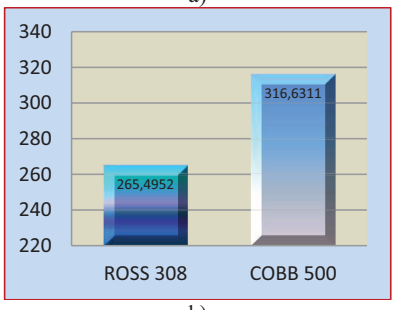

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Figure 1. Total protein content at both hybrids, first year, first season (a) and second season (b)

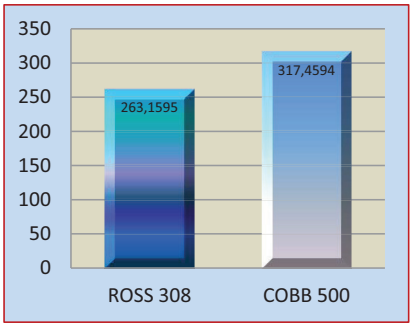
Hierarchy has been the same in year I and season 2 and best average performance has been noticed in chickens of hybrid COBB 500 with 16.16% higher than average performance noticed in chickens ROSS 308 in the environment inside the house in which study was performed. Estimated value of Student test was higher than table value for corresponding

tolerance limits and significance level was 0.001 which is demonstrating that there are very significant differences between analyzed averages ($t = 12.3030^{***}$).
In table 2 and figure 2 we are illustrating value found for "chicken total protein content" in the two groups used in the experiment in year II and seasons 1 and 2.

Table 2. Influence of genetic type on total protein content, second year, first and second season

Genetic type	n	$\bar{X} \pm s_{\bar{X}}$	s	c.v.%
Second year, first seson				
ROSS 308	25	263.1595 ± 2.9769	14.8843	5.6560
COBB 500	25	317.4594 ± 2.2162	11.0811	3.4905
Differences significance	$t = 14.6312^{***}$ $t_{48;0.05} = 2.01$; $t_{48;0.01} = 2.68$; $t_{48;0.001} = 3.51$			
Second year, second seson				
ROSS 308	25	257.9397 ± 2.5732	12.8660	4.9880
COBB 500	25	320.6270 ± 1.7459	8.7294	2.7226
Differences significance	$t = 20.1595^{***}$ $t_{48;0.05} = 2.01$; $t_{48;0.01} = 2.68$; $t_{48;0.001} = 3.51$			

It is noticed that in second year best average performance was also noticed in carcasses from chickens COBB 500 with 17.10% higher than average performance noticed in chickens ROSS 308. Measured value of Student test ($t = 14.6312^{***}$) is suggesting that there are very significant differences between average performances noticed for the groups containing the two genetic types.
Hierarchy has been the same in season 2 and best average performance has been noticed in chickens of hybrid COBB 500 with 19.55% higher than average performance noticed in chickens ROSS 308 in the environment inside the house in which study was performed. Estimated value of Student test ($t = 20.1595^{***}$) was higher than table value which is demonstrating that there are very significant differences between analyzed averages.
The measure in which analyzed quality indexes are the same in year II in production house in which we performed the study is statistically analyzed by testing the significance of noticed differences between feature averages by year and season.



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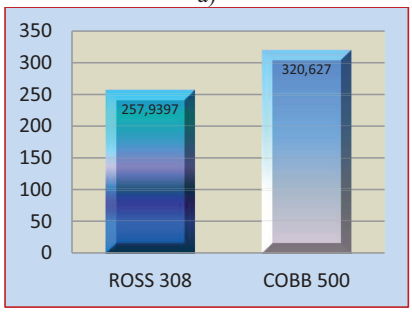

b)

Figure 2. Total protein content at both hybrids, second year, first season (a) and second (b) season

In tables 3 and 4 we are showing values found for Student test and their statistical significance.

Table 3. Testing of differences significance between years, first and second season, ROSS hybrid

Specification	Student value	Student critical value
First season ROSS		
Carcass weight	0.1020^{NS}	$t_{48;0.05} = 2,01$ $t_{48;0.01} = 2,68$ $t_{48;0.001} = 3,51$
Total protein content of chicken (RP)	0.1262^{NS}	
Second season ROSS		
Carcass weight	2.0699^{*}	$t_{48;0.05} = 2,01$ $t_{48;0.01} = 2,68$ $t_{48;0.001} = 3.51$
Total protein content of chicken (RP)	1.6553^{NS}	

Table 4. Testing of differences significance between years, first and second season, COBB hybrid

Specification	Student value	Student critical value
First season COBB		
Carcass weight	0.8660^{NS}	$t_{48;0,05} = 2.01$ $t_{48;0,01} = 2.68$ $t_{48;0,001} = 3.51$
Total protein content of chicken (RP)	$1,7481^{NS}$	
Second season COBB		
Carcass weight	0.0688^{NS}	$t_{48;0,05} = 2.01$ $t_{48;0,01} = 2.68$ $t_{48;0,001} = 3.51$
Total protein content of chicken (RP)	$1,6163^{NS}$	

Analyze of results is revealing that there are no statistical significant differences between averages of chickens of ROSS 308 hybrid between the two analyzed years excepting carcass weight in season 2. So it is recognized that there were no differences about the technologies of production, feeding and assurance of quality standard between previous and next year inside the house in which study was performed. The significant differences about carcass weight in season 2 might attributable to some trial errors.

For COBB 500 hybrid noticed differences between averages between the two analyzed years are not statistically significant.

CONCLUSIONS

Average protein content had different values for the two analyzed hybrids. Value found in season I and year I has been 262.5669 ± 3.6322 grams in ROSS 308 and 312.4330 ± 1.8320 grams in COBB 500 and differences noticed between the two hybrids are also highly significant statistically. Value found in season 2 has been 265.4952 ± 3.7698 grams in ROSS 308 and 316.6311 ± 1.7505 grams in COBB 500, and differences noticed are highly significant statistically between the two hybrids.

In year II and season 1 it was found a value of 263.1595 ± 2.9769 grams in ROSS 308 and 317.4594 ± 2.2162 grams in COBB 500 and differences noticed between the two hybrids are highly significant statistically. In season 2 it was found a value of 257.9397 ± 2.5732 grams in ROSS 308 and 320.6270 ± 1.7459 grams in COBB 500 and differences have been very significant from a statistical point of view.

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REFERENCES TNR

Banu C. et al.., 1997. Procesarea industrială a cărnii. Ed. Tehnică, Bucureşti.

Custură I., I. Van, Minodora Tudorache, Elena Popescu-Micloşanu Antoaneta Maria Popa, 2012. Research on the performances of raising certificate chickens. AgroLife Scientific Journal, Vol. 1, University of Agronomic Sciences and Veterinary Medicine of Bucharest, 147-152, ISSN 2285-5781, ISSN – L 2285-5781.

Sandu Gh., 1995. Modele experimentale in zootehnie. Ed. Coral Sanivet, Bucureşti.

Vacaru-Opriş I. et al., 2000. Tratat de avicultură, vol. I. Ed. Ceres, Bucureşti.

Vacaru-Opriş I. et al., 2004. Tratat de avicultură, vol. III. Ed. Ceres, Bucureşti

Van I. et al., 2010. Creşterea and industrializarea puilor de carne. Ed. Total Publishing, Bucureşti.

***Watt Poultry Statistical Yearbook.

*** Directiva CE 543/2008.

*** www.en.aviagen.com

*** www.cobb-vantress.com.

*** www.thepoultrysh. com, Farmes Weekly Interactive.

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STUDY OF THE INFLUENCE OF STIMULATING FEEDING OF BEES DURING SPRING TIME

Nicolae EREMIA[1], Andrei ZAGAREANU[2], Susana MODVALA[1], Angela CHIRIAC[1]

[1]State Agrarian University of Moldova, 44 Mircesti street, MD 2049 Chisinau, Republic of Moldova; 00 373 22 432380; E-mail: n.eremia@uasm.md
[2]Scientific-Practical Institute of Biotechnology in Zootechny and Veterinary Medicine, MD 6525 Maximovca, Republic of Moldova; 00373 22 359350

Corresponding author email: eremianicolai@rambler.ru

Abstract

In the spring time sometimes Nosema occurs and as a result we have a number of essential losses, such as lots of dead bee families, as well weak families with a small number of bees because of their low resistance. One of the methods by which it is possible to increase growth and productivity of bee families is early stimulating nutrition. The purpose of the investigation is to study the stimulating feeding of bees during spring time with using of probiotic. Using of "Bilaxan" probiotic in bees feeding stimulates increasing of bee families power raised in horizontally hives by 8.29%, queens prolificacy and caped brood with 2.51-31.41% and productivity by 12.24% and respectively in multistage hives with 3.89-5.56%; 8.48-32.45%, and 4.94-12.47%.

Key words: honey bees, probiotics, sugar syrup, bee families.

INTRODUCTION

The main task of apiculture is ensuring food products with superior nutritive and biological values. Mostly, the produced goods depend mainly on the conditions in which the bee families are kept and nurtured and the work organization, selection, and quality of the honey obtained from the apiary specialized queens. The bees collect pollen and nectar from the flowers of plants, which it processes into honey and bee bread. Bees' feed contain all the vital nutritive substances – proteins, lipids, carbohydrates, mineral substances and vitamins (Буренин, et al., 1977). In cases where family food reserve amount is insufficient, bees must be fed. For growth of juvenile sugar syrup is used in a concentration of 50% (1 kg of sugar in 1 L of water) (Кривцов et al., 2000). In the same environmental conditions, with the same growth technology, bee families with equal population achieved different formats in quantitative yields. One of the methods, which can assure the profitability of small and medium hives, is the temporary stimulatory nutrition. By using this nutrition, enhanced egg-laying can be achieved by the laying queen, resulting in increased number of bees and harvested honey from fruit trees and white acacia. For the stimulatory feeding in spring, honey, honey syrup, pollen and sugar syrup can be used (Marghitas, 1997; Marghitas, 2005).

The main function of the bees from the spring generation is focused towards increasing the number of juveniles, in order to assure the maximum number of bees for the main harvesting (Лебедев, 2000). For Carpathian bees it is typical a set of traits for the biological and morphological precious (high prolificacy queens), allowing a short time for the growth of strong and productive families, able to all kinds of harvesting, starting with spring (Малькова et al., 2007). Productivity of bee family is founded in early spring. From this moment the number of eggs laid by the queen in 24 hours must increase every day, due to which the correct amount of food is needed to feed larvae and protein secretion by the royal bee (Маслов, 2007).

For the brood growth stimulation sugar syrup is used, which is usually enriched with vitamins, microelements, floral pollen, bee bread and pine extracts (Ишмуратова et al., 2002).

The utilization of sugar syrup as stimulatory nutrition is fully efficient only when the feed contains protein substances, as the growth of

the juveniles can only happen when there are enough proteins. If there is a lack of proteins in the hive or in nature, then the bees use the protein reserves of their own bodies (Билаш et al., 2002).

It was reported that the prebiotics are significant effects on performance, health, vitality, intestinal ecology and the digestibility observed in many studies, although the mode of action of probiotic is not yet completely explained (Fialho et al., 1998).

Манапов et al. (2009) communicated that lately animal husbandry and veterinary Probiotics are widely used for the prophylaxis of infectious diseases of animals and increase their total resistance. Using probiotics in beekeeping as a feed additive also demonstrated increase in the survival of bees. In the spring, Nosema sometimes occurs and as a result we have a number of essential losses, families of dead bees, weak with few bees due to the low resistance. One of the methods with the help of which it is possible to stop the development and productivity of bee families, is early stimulatory nutrition. The purpose of the investigation is to study the bees feeding stimulants during spring with probiotic use.

MATERIALS AND METHODS

In order to achieve the set objectives, as object for investigations have served hives of Carpathian breed from apiary "Albinărie" maintained horizontally and "Nisporeni" – multi-leveled hives. In order to determine the optimal amount of probiotic per liter of syrup in bee feeding, during spring 5 groups of bee families were formed, including four experimental and one control. Bee families from the experimental group I received 0.5 l of sugar syrup with 50 mg/l "Bilaxan", II - 100 mg/l, III - 150 mg/l , IV - 200 mg/l. Families bee in group V (control I) received 0.5 litres of pure sugar syrup.

The families of bees in the apiary "Albinărie" were fed during spring evenings starting April 19 - 0.5 l sugar syrup to a family every 6 days until the beginning of the main harvest and the apiary "Nisporeni" - one liter of syrup every 12 days. The influence of the "Belaxan" probiotic increases immunity and metabolic normalization process, bees growth, development and productivity of bee families in the spring. During the active season, the checking of bee families was performed every 12 days before the main harvest from the white acacia. From productive characters of bee families were studied: strength, number of capped brood and honey productivity.

The data were processed by means of statistical variations by Меркурьева (1970), Плохинский (1971).

RESULTS AND DISCUSSIONS

The probiotic substances are used for the enhancement of the immune system, recovery normalization of intestinal microflora and metabolic process of the body. As probiotic "Bilaxan" was used - symbiotic feed composed of microorganisms like: strains of *Lactobacillus acidophilus*, *Lactobacillus plantarum*, *Lactobacillus acidophilus bulgaricus* titer of 1x108 CFU/g, *Enterococcus faecium* - 1x107 CFU/g, *Bifidobacterium bifidum* - 1x108 CFU/g - liofilozate cells, antagonistic to pathogenic microflora and pectin, yeast extract, essential phospholipids as natural acidifier.

Research results have shown that during the first spring check (19.04.2013) at the formation of experimental group, families power was 4.7-5.0 spaces between frames populated with bees, capped brood cells were from 64.3 to 80.0 and honey from 4.2 to 8.2 kg (Table 1).

At the control that has been done on 05.01.2013 was found that the strength of families was on average 4.7 to 8.0 spaces between frames populated with bees, capped brood cells was from 96.6 to 136.3 and 4.0-5.3 kg honey. During this period better developed were in group III that was fed with sugar syrup and 150 ml/l "Bilaxan" or with 37.6 hundred cells (td = 2.31) more than in the control group.

It was revealed that prior to the flowering of white acacia (on 13.05.2013) the highest number of capped brood (157.3 hundred cells) was in group III or 31.41% more than in group I (td = 2.66) (Figure. 1). Queens' prolificacy in this group was 1310 eggs in 24 hours and in the experimental group I - 997.5 eggs.

Table 1. Morph-productive indicators of bee families from the LTD „Albinărie" apiary

Groups	Strength, spaces between frames populated with bees	Brood capacity, hundreds cells	Honey, kg
19.04.2013			
I – Sugar Syrup + 50 mg/l „Bilaxan"	5.0±1.00	80.0±18.58	5. 7±1.67
II – Sugar Syrup + 100 mg/l „Bilaxan"	5.0±0.58	67.0±13.43	4.2±0.79
III – Sugar Syrup + 150 mg/l „Bilaxan"	5.0±1.00	65.0±7.94	6.8±2.05
IV – Sugar Syrup + 200 mg/l „Bilaxan"	4.7±0.33	67.0±1.16	8.2±0.81
V – Sugar Syrup pure (witness)	5.0±0.00	64.3±9.20	7.1±0.70
1.05.2013			
I – Sugar Syrup + 50 mg/l „Bilaxan"	6.3±1.33	120.0±23.74	5.0±1.53
II – Sugar Syrup + 100 mg/l „Bilaxan"	8.0±0.58	100.0±18.21	5.3±1.45
III – Sugar Syrup + 150 mg/l „Bilaxan"	7.0±1.16	136.3±13.54	4.33±0.67
IV – Sugar Syrup + 200 mg/l „Bilaxan"	4.7±0.33	109.3±2.333	4.0±1.53
V – Sugar Syrup pure (witness)	7.0±0.00	98.7±9.025	4.0±1.00
13.05.2013 (before the flourish of white acacia)			
I – Sugar Syrup + 50 mg/l „Bilaxan"	7.3±1.33	136.0±31.19	5.3±1.45
II – Sugar Syrup + 100 mg/l „Bilaxan"	9.7±1.20	122.7±22.93	9.3±0.89
III – Sugar Syrup + 150 mg/l „Bilaxan"	9.7±1.20	157.3±11.26	8.3±2.33
IV – Sugar Syrup + 200 mg/l „Bilaxan"	8.3±0.33	141.3±21.80	5.0±0.58
V – Sugar Syrup pure (witness)	9.3±0.33	119. 7±8.51	6.7±0.67
26.05.13 after the harvest from white acacia			
I – Sugar Syrup + 50 mg/l „Bilaxan"	11.67±2.67	132.3±24.85	22.2±6.38
II – Sugar Syrup + 100 mg/l „Bilaxan"	11.33±0.88	97.7±10.20	27.5±1.43
III – Sugar Syrup + 150 mg/l „Bilaxan"	12.67±2.73	131.3±15.25	25.1±4.36
IV – Sugar Syrup + 200 mg/l „Bilaxan"	11.7±1.20	105.0±7.1	22.9±0.35
V – Sugar Syrup pure (witness)	11.7±1.45	127.0±10.9	24.5±0.32

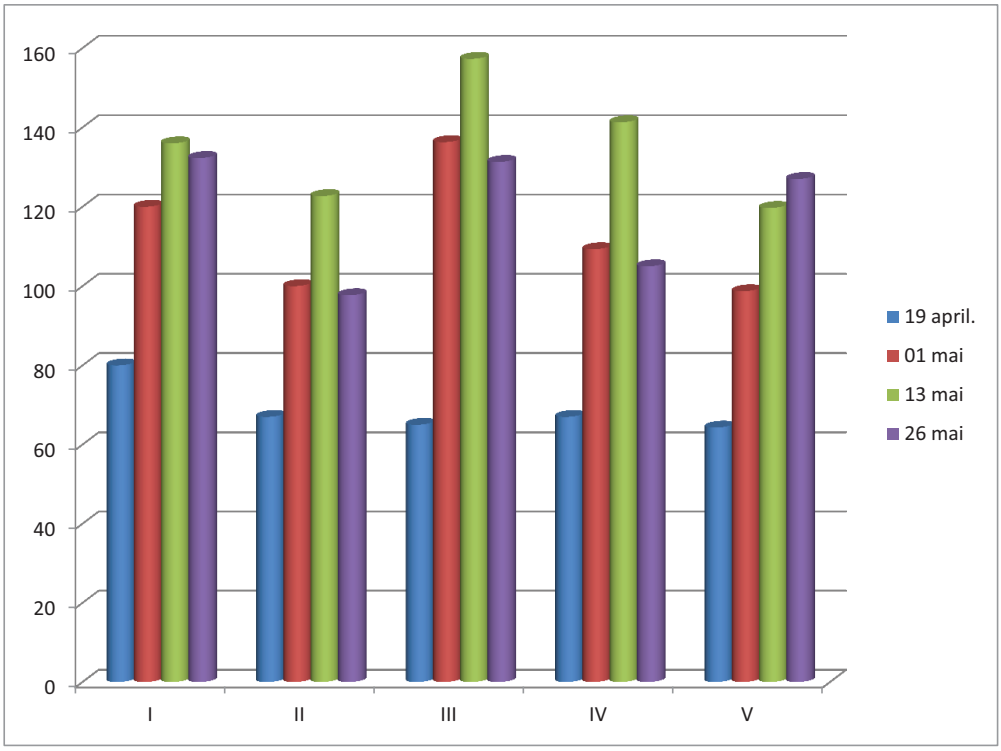

Figure 1. Number of capped brood, hundreds cells

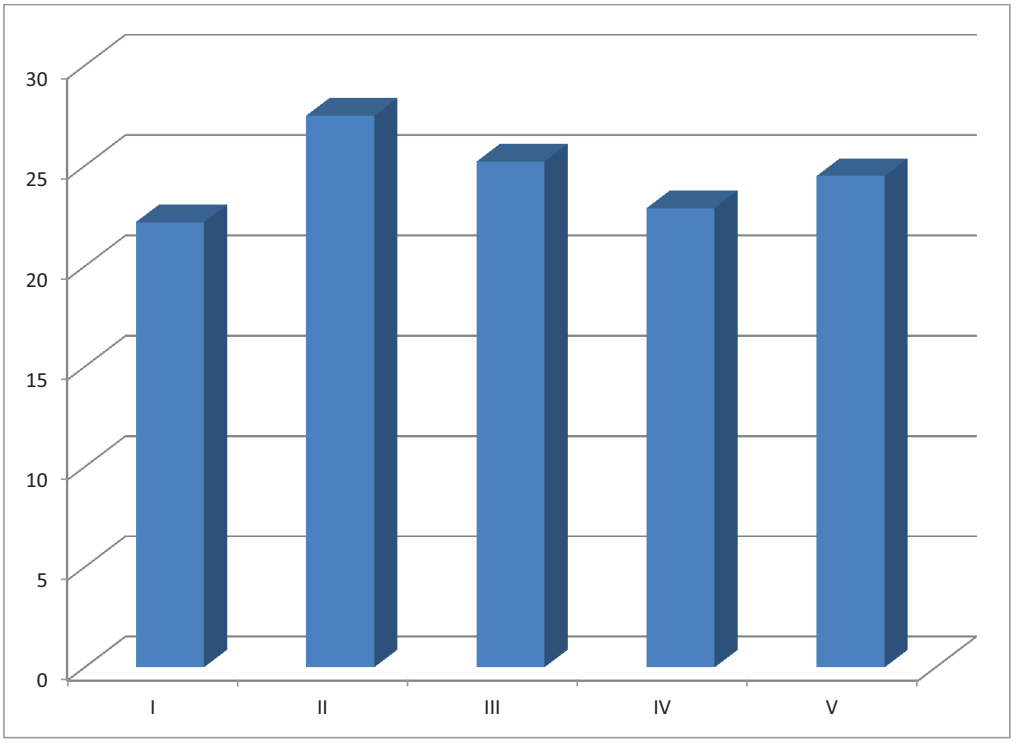

Figure 2. Quantity of collected honey, kg

It was found that after the harvest of white acacia (26/05/2013) the most power had families of group III - 12.67 spaces between frames populated with bees, or 8.29% more than the control group. The maximum amount of honey from the bee families was collected in group II - 27.5 kg or 12.24% more than in the control group (Figure. 2).

In the second experience made with bees families maintained in multistage hives in the apiary "Nisporeni" feeding was done every 12 days using one liter of syrup, starting on 20 of April before the main harvest from the white acacia.

At the moment of groups formation on 20/4/2013, bee families had an average of 7.7-8.0 combs, with power of 6.7 to 7.0 spaces between frames populated with bees, capped brood counted 71.2 -75.0 hundred cells, and the reserve of honey was 2.0-4.0 kg (Table 2).

After 12 days at the next check, there was an increase in the number of capped brood in group II where were administered sugar syrup 100 mg / l "Bilaxan" more with 35.6 hundred cells, or 32.45% compared to the control group (td = 2.16). Also significant increase was noted in group III, which increased 137.7 hundred cells or with 25.52% more than in the control group (td = 2.38).

After harvest of white acacia on 4 of June, 2013 bee families in group II reached spaces between frames populated with bees to 19 or by 5.56% higher than the control group, the family has grown 169.3 hundred cells or by 29.93 % cells more than in the control group (Figure 3). Queens' prolificacy of bee families in group II, in this period was 1 411 eggs in 24 hours, or 326 eggs more than the control group. Bees from the experimental groups stored in an average of 40.4 to 43.3 kg of honey per nest or with 1.9 to 4.8 kg (4.94 to 12.47%) more than in the control group.

Table 2. Morph -productive indicators of bee families from the „Nisporeni" apiary

Groups	Strength, spaces between frames populated with bees	Capped brood, hundreds cell	Honey, kg
20.04.2013			
I – Sugar Syrup + 50 mg/l „Bilaxan"	6.7±0.33	74.0±5.51	4.0±1.15
II – Sugar Syrup + 100 mg/l „Bilaxan"	7.0±0.00	75.0±3.21	2.0±0.58
III – Sugar Syrup + 150 mg/l „Bilaxan"	6.7±0.33	74.0±6.81	2.7±0.67
IV – Sugar Syrup + 200 mg/l „Bilaxan"	7.0±0.00	74.0±6.56	3.3±1.33
V – Sugar Syrup pure (witness)	6.7±0.33	73.67±9.82	3.3±0.88
02.05.2013			
I – Sugar Syrup + 50 mg/l „Bilaxan"	9.0±0.00	134.3±22.24	5.7±0.88
II – Sugar Syrup + 100 mg/l „Bilaxan"	9.3±0.33	145.3±15.94	7.3±0.33
III – Sugar Syrup + 150 mg/l „Bilaxan"	9.0±0.00	137.7±11.05	6.3±1.20
IV – Sugar Syrup + 200 mg/l „Bilaxan"	9.0±0.00	119.0±13.00	6.3±1.20
V – Sugar Syrup pure (witness)	9.0±0.00	109.7±4.05	6.0±1.00
04.06.2013 after the harvest from white acacia			
I – Sugar Syrup + 50 mg/l „Bilaxan"	19.0±0.00	135.0±0.00	40.7±1.12
II – Sugar Syrup + 100 mg/l „Bilaxan"	19.0±0.00	169.3±35.36	41.0±4.84
III – Sugar Syrup + 150 mg/l „Bilaxan"	18.7±0.33	132.0±24.01	43.3±5.34
IV – Sugar Syrup + 200 mg/l „Bilaxan"	18.7±0.33	139.0±26.96	40.4±2.51
V – Sugar Syrup pure (witness)	18.0±1.00	130.3±14.44	38.5±1.21

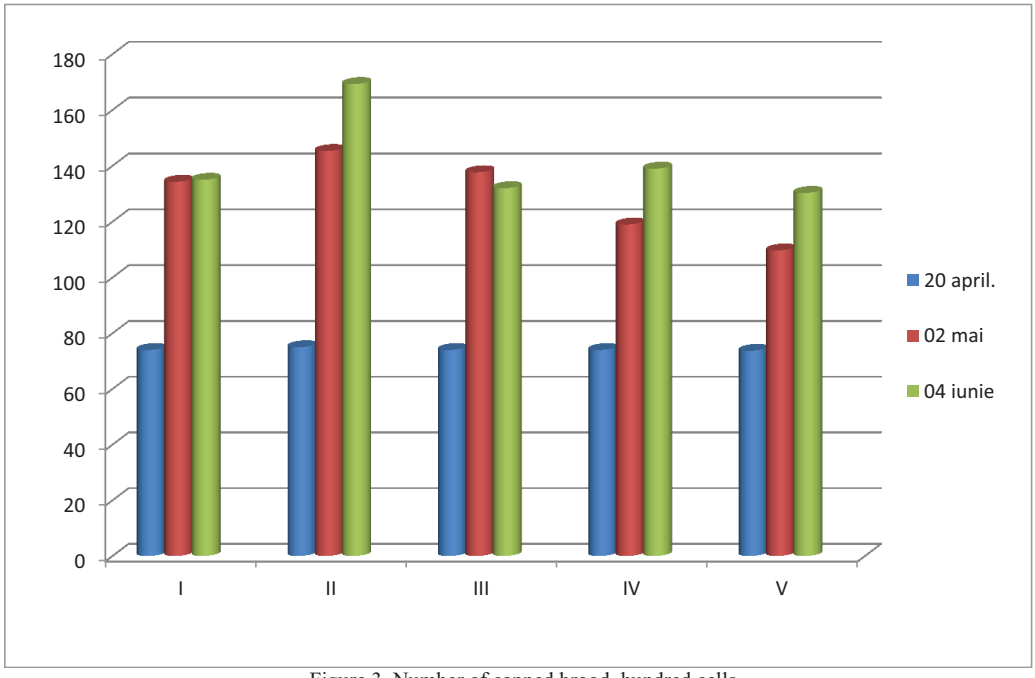

Figure 3. Number of capped brood, hundred cells

The largest quantity of honey from the white acacia has been deposited by families from the 3rd group, averaging 43.3 kg or 12.47% more than the control group (Figure 4).

Therefore the stimulatory feeding of bee families in the spring with the administration of sugar syrup together with the probiotic "Bilaxan" kept in horizontal hives provided a surplus of honey of 12.24% on average and a family in the – in multi-level hives 4.94 to 12.47% more than in the control group.

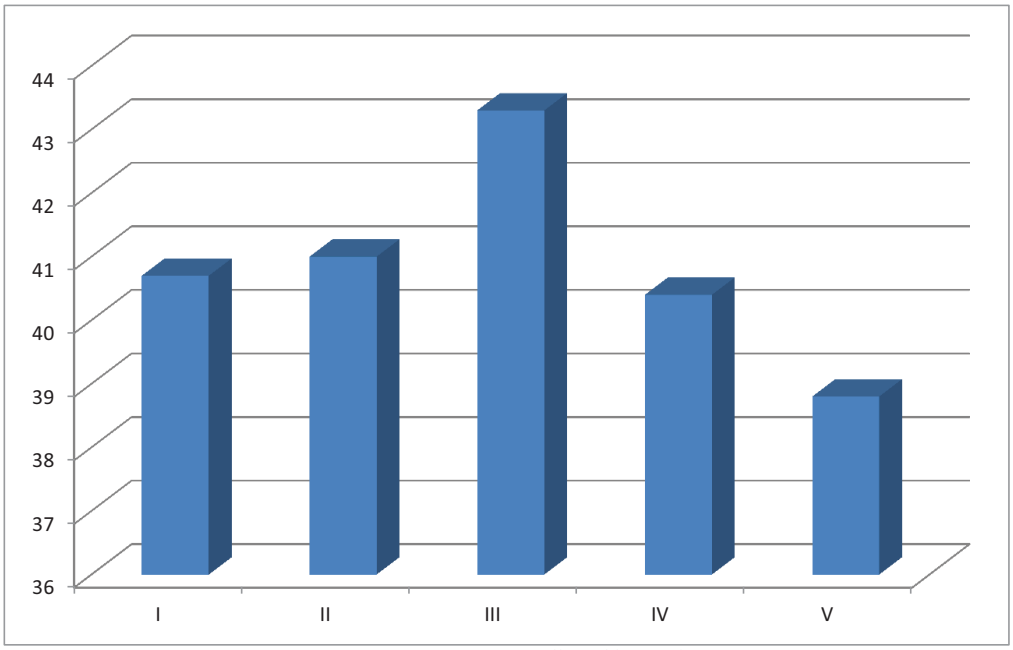

Figure 4. Quantity of collected honey, kg

CONCLUSIONS

1. The optimal dose of probiotic "Bilaxan" for the stimulatory feeding for bees in spring is 100-150 mg/litre of sugar syrup
2. Feeding bee families in spring during the lack of honey harvest without bees flying in the area can be done once, every 6-12 days using 0.5-1.0 l sugar syrup.
3. The use of the "Bilaxan" probiotic in bee nutrition stimulates power growth in families kept in horizontal hives by 8.29%, queens prolificacy and number of capped brood from 2.51 to 31.41% and 12.24% and productivity in multi-levelled hives from 3.89 to 5.56%; 8.48 to 32.45%, and 4.94 to 12.47%.

REFERENCES

Fialho E. et al., 1998. Probiotics utilization for piglets from 10 to 30 kg. The 8th World Conference on animal Production Contributed Papers, 1: 622-633.

Mărghitaş L.A., 1997. Albinele şi produsele lor. Bucureşti, Ceres.

Mărghitaş L., 2005. Albinele şi produsele lor. Bucureşti, Editura Ceres.

Билаш Н., Беневоленская Б., 2002. Заменители корма пчел. Пчеловодство, 2: 24-26.

Буренин Н.Л., Котова Г.Н., 1977. Справочник по пчеловодству. Москва, Колос.

Ишмуратова Н.М., Манапов А.Г., Ишмуратов Г.Ю., Толстиков Г.А., 2002. Препарат Кандисил для стимулирования роста и развития семей в ранневесенний период. Пчеловодство, 2: 20-21.

Кривцов Н.И., Лебедев В.И., Туников Г.М., 2000. Пчеловодство. Москва, Колос.

Лебедев В.И., 2000. Научно-практические аспекты технологии комплексного использования пчелиных семей при производстве продуктов пчеловодства. Материалы международной научной конференции «Пчеловодство- XXI век», Москва.

Малькова С.А., Василенко Н.П., 2007. Чистопородное разведение пчел на юге России. Пчеловодство. 7:12-15.

Манапов А.Г., Губайдуллин Н.М., 2009. Влияние коррекции содержания белка в подкормках и аэроионизация гнезда пчелиных семей на содержание азота в теле пчел и эффективность их работы в теплице. Материалы коорд. Совещ. 9-й науч. - практ. конф. «Интермед». НИИП.

Маслов А.А., Маслова Е.Е., 2007. Подкормка для ранневесеннего развития. Пчеловодство.

Меркурьева Е.К., 1970. Биометрия в селекции и генетике сельскохозяйственных животных. Москва, Колос.

Плохинский Н.А., 1971. Руководство по биометрии для зоотехников. Москва, Колос.

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STUDY ON THE INTERRELATION BETWEEN ANIMAL WELFARE AND PRODUCTION IN DAIRY CATTLE

Mădălin GAVRILĂ, Gheorghe Emil MĂRGINEAN, Livia VIDU

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, Email: gavrilaimadalin@gmail.com

Corresponding author email: gavrilaimadalin@gmail.com

Abstract

The submitted report has not proposed a description of the welfare of cattle, but is mainly aimed at highlighting the fact that the animal is not an "object" that man can exploit at will, without being interested in providing an optimal environment. Animals are living souls who have needs, needs which if understood and if a good relationship between man and animal is kept, one can speak of maximum yields and even record. The first definition of animal welfare was proposed by the Universal Declaration on Animal Welfare and supported by the World Society for the Protection of Animals (based in Boston, USA). This first statement presented, in the first part, animal welfare as "the degree to which the requirements for physical, behavioral and psychological needs of the animal are satisfied." In the second part of the definition,a negative concept is presented, for animals that are dependent on humans, known as the five freedoms simultaneously: 1- ensuring access to fresh water and food; 2-ensure appropriate environment, including watering and comfort; 3- prevention of pain, injury, rapid diagnosis and treatment of wounds; 4- elimination of fear and mental suffering; 5- providing space, facilities and the company of other animals to express normal behavior. The concept of animal welfare must be made common knowledge to all the employees in the farm. The idea that animal welfare depends on their behaviorand their behavior towards animals may induce or prevent fear response and undesirable emotional state should be transmitted and understood.

Key words: *cattle, concept, definition, animal welfare.*

INTRODUCTION

We talk and give attention to the dairy cattles because they are one of the most important farm animele species by being the source that provides a wide range of consumer products like milk and meat mainly, from which derives a lot of preparations, and also a sum of subproducts useful to man, such as the skin and manure. From Figure 1, show that 95% of the world total production of milk, 38% of world production of meat, 75% of the total production of manure and 90% of the raw skins (Velea and Mărginean, 2012).

Milk is the most important aliment obtained from this category of animals, both by its chemical composition and biological value expressed mainly by its the degree of digestibility.

It is well know that the milk contains more than 100 substances that are necessary for human body, including the 10 esential amino acids, 45 minerals, 25 vitamins and last but not least, 10 fatt acids.

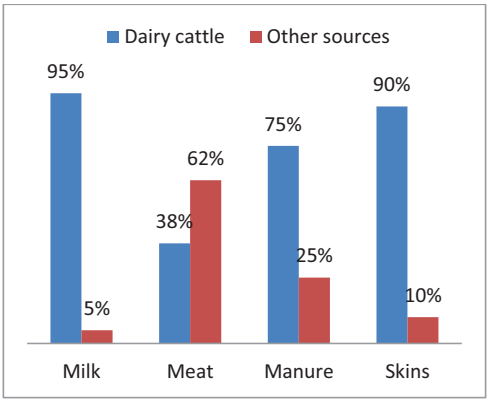

Figure 1. The proportion of products obtained from cattle in world production

Through their physiological functions, dairy cows transform the substances from forages into milk (50%) and meat (20%) with a very high efficiency (Figure 2).

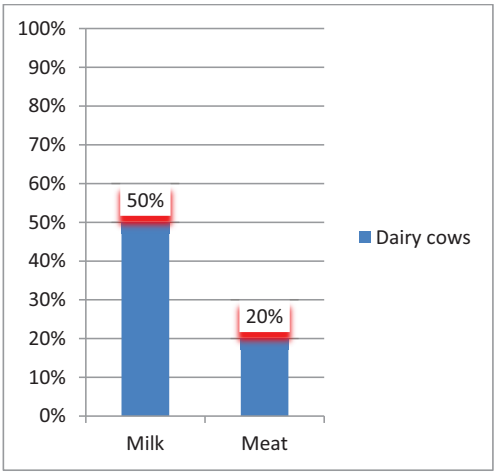

Figure 2. Feed utilisation in dairy cows

Cattle welfare is a very valuable and used concept of the contemporary world.
The importance of this concept was recognized by the United Nations, which already ranks it in the circle of interests of the Eurogroup for Animal Welfare, the Council of Europe, European Union, World Health Organization, and the list goes on.
The importance of the concept of animal welfare was formalized for the first time, by the 33rd protocol of the Treaty of Amsterdam, of the European Union in 1997.
In this treaty was presented the idea based on which, animals are living beings who have senses and intrinsic value.
Besides the zootechnical value of the animals, dairy farmers have to understand that cows need the providing of specific comfort in order to achieve their genetic potential (Battini et al., 2009).
We can not speak of productive performances in dairy cows as long as they received deficient feed rations in terms of energy or other nutrients and were maintained on permanent litter for the last 3 years.
In time, the idea that productive performances are directly related to animal welfare, was better understood, and the interest of more organizations to study, make known and to regulate this concept has grown, so have formed new dairy farming systems, systems that creates a bridge between ethics, engineering and medical sciences.
With our country's integration into the European Union, there were legislative changes of the legal acts of this field, that were emitted by the Council of Europe.These changes are aiming at improving the animal welfare at the dairy farm level, by implementing new assessment methods and a series of specific protocols, largely based on socio-economic reasons.

MATERIALS AND METHODS

The working method consisted in consulting the scientific results obtained at national and international level, until now, in the dairy cows welfare field.

RESULTS AND DISCUSSIONS

We can not have expectations regarding production performances if we do not take animal welfare action on how to feed and maintenance the dairy cows.
Because the animal welfare depends on the housing conditions of the animals and the Managament in the farm, experts of the European Food Safety Authority (EFSA), were involved in supporting the welfare of cattle, by implementing of new legislative basis for systematic risk assessment at the dairy farm level.The European Union was, as well, involved by financing the project titled "The Welfare Quality", within which were established a set of measures to assess animal welfare and at the same time were established strategies to achieve all 12 important criteria, that are part of several areas of animal welfare (Table 1). Afterwards they were systematized in four main criteria, criteria that facilitated communication between consumers, these principles are found in Figure 3. The systematization of the 12 principles in only 4 basic criteria and good information about the impact of animal welfare in farms, in the sense that it is closely related with the quality of products obtained, conclusions that were reached from studies performed before, led to a growing interest in this concept among society, farmers and researchers.

Table 1. Principles and Criteria of animal welfare (Keeling and Veissier, 2005)

The principle		Animal welfare criteria	Examples of potential measurements
Good feeding	1	The absence of prolonged hunger	Body condition assessment
	2	The absence of prolonged thirst	Access to Water
Good housing	3	Rest confort	Frequency of different positions of rest, lift and seating behavior
	4	Thermal comfort	Panting, shaking
	5	The easy movement of the cows	The slipping and falling
Good health	6	The absence of wounds	The clinical state of skin, the carcass quality, pododermatitis
	7	The absence of diseases	Enteric problems, disqualification at slaughter
	8	The absence of induced pain cused by the management methods	The evidence of routine mutilation such as cutting the tail or the horns, efficient stunning at slaughter
Proper behavior	9	The expression of social behavior	Licking, aggression
	10	The expression of other types of behavior	Playing, abnormal behavior
	11	Good human – animal relationship	Nearby and / or avoidance tests
	12	The absence of general fear	Test with new objects

Later, because of the increasing interest among the society, research institutions, educational organizations, enterprises, government agencies and other development institutions were involved in developing and implementing the concept of animal welfare, providing expert support to the dairy farmers.

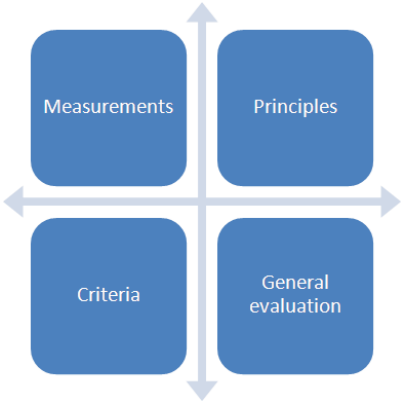

Figure 3. The evaluation is based on estimating the welfare by measurements at the level of the animal that reflect the quality of the housing and the management at which the animal was exposed.
(Blokhuis et al., 2009)

In 2001, Fregonesi and Leaver studied as animal welfare indicators the behavior and health of dairy cows housed on permanent litter compared to the behavior and health of dairy cows that are housed in individual berths.The two researchers watched the comparative indicators of animal welfare within the two systems of housing and concluded that dairy cows that were maintained on permanent litter, have spent a longer period of resting and rumination than cows maintained in individual berths.

Regarding individual berths housing system, the cows were much cleaner, but studies have shown an equality between both systems in terms of milk production, somatic cell number or the motion score that were observed at evaluation.

Productive performances are closely related to the stables where cows are housed.

Regarding the dairy cows, we should be concerned mainly about the udder and the very close relation between the udder health and the next two aspects: condition of the environment in which the animal lives and cow's corporal hygiene. Both aspects are the responsibility of the dairy farmer, in the sense that he must

provide to the animal an optimal environment of housing by providing quality litter, microclimate, the optimal number of heads in the animal house and a suitable system of accommodation.

Natural luminosity in the animal house must be ensured by windows with vertical opening.

Table 2. Minimum values for the natural lighting index (ANSVSA, 2005)

Building density	Lighting index	Lux
Milking cows	1”20	40-60
Maternity	1”15	60-80
Young breeding cattle	1”16	40-60
Young fattening cattle Phase 1	1”10	40-60
Young fattening cattle Phase 2	1”15	40-60
Fattening cattle	1”25	25-30

The microclimate in the animal houses is, also, a very important factor, and the dairy farmer must take into account of it, if he wants to achieve the proposed productive performances.

In Table 3 are presented the standards of temperature, humidity and air currents to ensure an optimum microclimate.

Table 3. Ensuring microclimate in dairy houses (ANSVSA)

Category of the cattle	Temperature (°C)			Humidity (%)		The air currents speed (m/s)	
	Minimum	Maximum	Optimal for calves	Minimum	Maximum	For min. T^o	For max. T^o
Milking cows	6	24	10”14	60	75	0,2”0,3	1
Maternity	12	24	20	55	70	0,1”0,2	0,1”0,2
Young cattle	6	24	8”10	60	75	0,2”0,3	1

Using the data in the table 4, we can accurately calculate the optimal surface which the animal needs to feel comfortable, so as to avoid stress due to overcrowding.

Another factor that is determinant for cows welfare is proper feeding (table 5) and watering. Gjodesen et al. (2010) have stated that the need for water and feed are interdependent.It is true that, when the cows are feeded with forages with a higher water content, the volume of water consumed will be diminished. Feed have a strong impact on health and wellbeing of the cows, and for this reason, the dairy farmers, should give a special attention to it.

Tablew 4. Distribution of the floor space (ANSVSA)

Body weight (kg)	Minimum rest area (m2)	Minimum area without litter (m2)	The total minimum area / animal (m2)
≤100	1.5	1.8	3.3
101 - 199	2.5	2.5	5.0
200 - 299	3.5	2.5	6.0
300 - 399	4.5	2.5	7.0
400 - 499	5.5	2.5	8.0
500 – 599	6.0	2.5	8.5
600 – 699	6.5	2.5	9.0
700 - 799	7.0	3.0	10.0
≥800	8.0	3.0	11.0

Table 5. Nutritional requirements for maintenance of dairy cows (Stoica, 2001)

Body weight (kg)	Dry Matter (maximum kg/zi)	UNL (/zi)	PDI (g/zi)	Ca (g/zi)	P (g/zi)
450	10,36	4,64	225	18	18
500	11,26	5,08	243	20	20
550	12,12	5,46	262	22	22
600	12,96	5,82	279	24	24
650	13,77	6,18	296	26	26

Studies to date have shown that the welfare of dairy cows, is influenced by several factors, among which we mention the proper maintenance of the hooves. If this action is not executed properly, it can cause sub-clinical laminitis, disease that is detectable only when the bleeding is taking place, situation that seriously affects the productiv performances of the dairy cows.

Hard surfaces, also, favors the appearance of limbs disorders, that is manifesting with an incidence of 80% in the hind legs, on the outer surface of the hooves.

DLG Research Institute, showed, in the study on the influence of the surface type of the rest zone, over the limbs disease occurrence, that the surfaces with a softer texture can provide an optimal level of comfort and can significantly reduce the incidence of this kind of affections at the herd level.

CONCLUSIONS

We have to realize that the concept of dairy cows welfare should not remain at a theoretical level, and must be implemented in every farm, through dairy breeders and especially dairy farm workers.

Cattle are animals whose exploatation is profitable if the their well-being is ensured, but, having no reason, they can not choose how to exploit themselves, so, the breeders, must intervene to ensure the optimal living environment and finally a normal relationship with them.

Dairy farmers must understand that once they provide the optimum environment to the cows, they can expect at productive performances, otherwise the results are: deficiency, failure and, finaly, lose of the income.

ACKNOWLEDGEMENTS

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REFERENCES

Battini M., Vieira A.,Barbieri S., Ajuda I.,Stilwell G., Mattiello S., 2009. Invited review: Animal-based indicators for on-farm welfare assessment for dairy goats.

Fregonesi, J. A. and Leaver, J. D. 2001. Behaviour, performance and health indicators of welfare for dairy cows housed in strawyard or cubicle systems. Livestock Production Science, 68. 2-3. 205-216. p.

Gjodesen M.U., Vibeke F.N., Blaga L., Frederiksen H., Masinistru M., Greculescu A., 2010. Sisteme de adapost pentru bovine – volumul 1. Standarde de ferma.

Keeling L., Veissier I., 2005. Developing a monitoring system to assess welfare quality in cattle, pigs and chickens. In: A. Butterworth (Ed.) Science and society improving animal welfare. Welfare Quality® conference proceedings, 17/18 November 2005, Brussels, Belgium, 46-50.

Stoica I., Stoica Liliana, 2001, Bazele nutriției și alimentației animalelor. Editura Coral Sanivet, București.

Velea C., Mărginean Gh., 2012. Tratat de creșterea bovinelor, Volumul 2. Editura Risoprint, Cluj-Napoca.

***ORDIN nr. 72 din 15 august 2005 - privind aprobarea Normei sanitare veterinare ce stabileste standarde minime pentru protectia viteilor ANSVSA.

***http://www.ansvsa.ro

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RESEARCH ON LONGEVITY AND CAUSE OF REDUCTION OF HERD LIFE IN HOLSTEIN COWS

Mădălin GAVRILĂ, Gheorghe Emil MĂRGINEAN, Alexandru KELEMEN

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: gavrilaimadalin@gmail.com

Abstract

Through this research we intend to analyze the Holstein herds in farms of various sizes from Teleorman County to highlight the productive performance of the breed and to emphasize the similarities, respective the differences that were found in the studied farms and furthermore, another goal of the study was to show that the breed is very valuable.The Holstein cows studied for this paper are from various origins (Germain, France, Netherlands). The material studied is represented by the dairy cows that were send to slaughterhouse from 5 farms, representing 1200 heads.The lifetime of the animal is defined by two aspects, namely, the biological longevity and the productive longevity.We can observed, from the data processed and interpreted by us, that the greatest biological longevity was registered in farm 1 (6.27 years), where the productive longevity was 3 years old.The highest frequency of reproductive disturbances was registered in the farm No.4 (58.33%) and farm No. 3 (55%).The data held by us from the 5 farms that were analyzed shows higher rates of reproductive disorders comparative to that established by Curelariu of 43.66% et al (1980) and Vidu of 28,52%(2002).The herds analyzed are characterized by a mean of 5.71 years for the biological longevity with a variability between 5.10 years and 7.24 years, which is consistent with the literature.

Key words: *milk, biological longevity, reproductive longevity, dairy cow.*

INTRODUCTION

A particularly very important indicator for animal welfare is the longevity, as a result of the health welfare. The lifetime of the animal consist of two parts, namely the biological longevity and the productive longevity. Both components of the animal life are influenced by the following factors: the degree of genetic improvement, the direction of exploatation, the applied technology and health.

The biological longevity can be defined as beeing the time elapsed from birth until the death, as a result of natural causes. Normally, lifespan of the cattle is between 15 and 18 years, being influenced by the physiological and environmental factors.

The reproductive longevity or the exploitation period is between the first calving and the final lactation of the cow.It can be expressed by the number of lactations or years of exploitation. High productive longevity is reflected by high yields of milk and by a larger number of calves per cow, which leads to increased economic efficiency (Georgescu, 1990; Vidu, 2002).

From an economic perspective, it is desirable for productive cows to achieve the production peak at an early age, to keep production constant for as long as possible and to obtain at first the lactation a milk production as close to the maximum level.

MATERIALS AND METHODS

The present study aimed to analyze Holstein herds in farms of various sizes in Teleorman County.The material studied is represented by the dairy cows that were send to slaughterhouse from 5 farms, representing 1200 heads.In the analyzed farms, the exploitation technology is playpen, semi-open or closed shelters with cows resting in bunks.The feeding technology is stock feeding and, in some of the studied farms, the distribution of the feed is made with the technological trailer.The milking is done in specially designed for this purpose houses, type tree or Side by side.The working method was based on technical data collection directly from the farms, or from the database of ANARZ.

The data obtained were statistically analyzed, then were compared with the scientific data from the literature and then summarized in this paper.

In order to establish the differences between the studied farms, we applied the Student test.

RESULTS AND DISCUSSIONS

The longevity of dairy cows varies by race.Thus the research conducted over the years have demonstrated a productive longevity between 3,5 years and 4 years in Holstein-Friesian, 4.3 years in Romanian Brown Swis and 4.8 years for Romanian Spotted.

For the breeds reared in Austria, in 2000, the highest productive longevity was recorded for the next breeds: Grauvieh (7.38 years), followed Braunvieh (6.89 years), Pinzgauer (6.74 years), Fleckvieh (6.61 years) and finally Holstein, with the lowest productive period (6.21 years) (Vidu, 2002).

Table 1. Comparative analysis of biological and productive longevity in the studied farms

Farm	Biological longevity				Productive longevity			
	n	$X \pm S_X$	S	V%	n	$X \pm S_X$	S	V%
Farm 1	524	2288±56,01	1350,01	59	524	1066±22,11	550	51,59
Farm2	301	2030±33,11	611	30	301	1590±25,00	460	28,93
Farm3	87	2222±56,15	624,10	28,08	87	1450±42,01	451	31,10
Farm4	71	1816±59,16	544	29,95	71	1248±44,11	390	31,25
Farm5	61	2078±75,6	590	28,39	61	1246±39,11	320	25,68

From the data we statistically processed and interpreted, the greatest biological longevity was observed in farm 1 and was registered in January (6.27 years), where productive longevity was 3 years old. It also can be observed a high variability on the productive and biological longevity (Table 1). Curelariu Niculina et al. (1980), from the analysis of 284 Friesian cows from an elite farm that were send to the slaughter house, founds that biological longevity was 5.6 years and the productive longevity of 2.74 years, which has led to the conclusion that the exploitation technology was ineffective from a zootechnic and economically point of view.

Table 2. Comparison of the biological longevity registered in the analyzed farms

Farm	X	Farm				
		Farm 1	Farm 2	Farm 3	Farm 4	Farm 5
Farm 1	2288	-	3.05 **	1.90 NS	0.53 NS	1.22 NS
Farm 2	2030	3.05 **	-	0.81 NS	2.25 *	0.36 NS
Farm 3	2222	1.90 NS	0.81 NS	-	2.45 *	0.96 NS
Farm 4	1816	0.53 NS	2.25 *	2.45 *	-	1.33 NS
Farm 5	2078	1.22 NS	0.36 NS	0.96 NS	1.33 NS	-

Analyzing the causes of the outputs, it can be grouped into involuntary causes (mortality and slaughter of necessity) and voluntary causes (selective reform).In countries with high achievements in dairy farming, the main cause of exits from the herd is the selective refroma of the cows (Vidu et al. 2005.2014).

Gheorghe Georgescu and co. highlights that, for the Romanian Black Spotted cattle, the reform is made for the next reasons: reproductive disorders (35.6%), circulatory disorders (10.5%), digestive disorders (9.65%) disorders of the rumen (7.45%) mamary gland disorders (5.1%), nutritional and metabolic diseases (4.5%), musculoskeletal disorders (4.3%), leukosis and tuberculosis (1,1%) and other causes (21.9%) (Georgescu et al, 1987).

In Table 2, we have grouped the main causes of reforms in the analyzed farms so that, it can be highlighted the highest procent recorded. From the synthesis in table 2 it can seen that that the most important motif of reform is because of reproductive disorders, followed by limb disorders.The causes of reform are presented in Table 3.

Reproductive disorders. Of all the health disorders that caused the exits of the cows from the herd, for the Holstein cows, we observed the highest frequency for the reproductive disorders (average 43.66%). The highest frequency of reproductive disorders was registered in farms No.4 (58.33%) and No. 3 (55%).In fact, these two farms have livestocks with highest milk production.

Figure 1. Cows from one of the studied farms

Table 3. Causes reform in the analyzed farms

Nr. crt.	The couse	Analized farm									
		Farm 1		Farm 2		Farm 3		Farm 4		Farm 5	
		heads	%	heads	%	heads	%	heads	%	heads	%
1	Agalactia	54	9.37	40	10.81	2	2.5	1	1.58	-	-
2	Pericarditis	131	22.74	15	4.05	10	12.5	2	3.17	-	-
3	Reticulum and foreign bodies	14	2,43	5	1,35	9	11,25	5	7,93	5	12,19
4	Endometritis	3	0,52	2	0,54	2	2,5	7	11,11	2	4,87
5	Womb disorders	20	3,47	1	0,25	7	8,75	8	12,69	7	17,07
6	Ovarian disease	220	38,19	151	40,81	14	17,5	12	19,04	9	21,95
7	Diseases of the udder	15	2,62	42	11,35	19	23,75	14	22,22	7	17,07
8	Limb disorders	48	8,33	56	15,13	10	12.5	6	9.52	9	21.95
9	Repeated abortions. dystocia	1	0.17	9	2.43	2	2.5	2	3.17	1	2.43
10	Nutrition and metabolism diseases	10	1.74	47	12.70	3	3.75	4	6.34	-	-
11	Accidents	60	10.42	2	0.54	2	2.5	2	3.17	1	2.43
	Total	576	100	370	100	80	100	63	100	41	100

Reproductive disorders have origins and different percentages in the farms that were studied, as follows:

- Ovarian disorders have the highest rate of 23.82% of the total reproductive disorders, and, in the analyzed farms, in the farm no. 2 the percentage was 40.91%;
- Udder's disorders ranks second in terms of incidence, with 11.24%; the lowest incidence of Udder's disorders was registered in farm No. 1 (2.62%) and the highest in farm No. 3 (24%) and farm no. 4 (22%);
- Uterine disorders ranks third with a percentage of 7.24%, and the variability of these conditions in relation to the farm are lower (0.25% in farm No. 2 and 12.69% in farm No. 4);
- Endometritis recorded the lowest incidence, averaging 2.52%.

Data held by us regarding the incidence of reproductive disorders in the 5 farms studied shows higher rates comparative to that established by Curelariu et al (1980) and Vidu (2002), respectively 43.66%, against 28.52%.

Pericarditis. This is inflammation of the pericardium and is found in cows mostly in traumatic forme and less as primary pericarditis or in presepticemia form.In the analyzed farms, pericarditis ranks second after the reproduction disorders, in terms of frequency.Thus, this inflammation has an incidence of 11.16%, noting that the farm No. 5 there has been no case, which demonstrates special care in managing the feeding of cows.

Limb disorders. This is manifested most often by inflammation of the hooves.The number of cows examined showed an average incidence of podal disease of 14%.The most intensive care of the hooves was registered farm No. 2 where the frequency was very low.On the contrary, negligence in cleaning and adjustment of the hooves was recorded in the farm No. 5,

were it was recorded a frequency of about 22% for the podal disorders.The data obtained by us are close to those encountered in the literature (14% vs. 13%). (Andronie, 2004).

The accidents. It are random and unexpected events that cause injury, death or reform and are often caused by negligent exploitation in dairy cows.In the analyzed farms, the frequency of accidents was averaged at about 6.13%.Se observă că există o pondere foarte scăzută a accidentelor în ferma nr.2 (0,54%), ca urmare a managementului foarte bun.

Traumatic reticulitis. It is an inflammation of the cow's reticulum caused by trauma of the reticulum wall, produced by a metallic foreign body (nail, wire, etc.) that was swallowed once with the feed.The frequency in the 5 herds studied, had an average of 6%.This value indicates a great deal of carelessness and even negligence in feeding dairy cows, when, for the feeding, bales bound with wire are used.There is also a large frequency for traumatic reticulitis based farm (farm No. 5 12.15%).

Agalactia. The absence of milk in the mammary gland of the Holsetin cows from analyzed farms, was almost 5%.The absence of the secretory disorders is noticeable in some farms analyzed (farm No. 5) and, for the other farms, the frequency is very high (farm No. 2 above 10%, farm No. 19.4%).The nutritional and metabolic diseases. They are the result of a concentrated and unbalanced feedings technologies of the animals.In the analyzed farms, the disease incidence is relatively low (about 3%), due to the fact that, the feeding technology involves the use of the ratios that are optimised relating to the standard requirements of Holstein cattle.However, this kind of disorders are present and the incidence variates from one farm to an other.Thus, in the farm No. 5 does not have been registered such problems, while in the farm No. 2, where milk production performances are the highest, incidence of nutritional and metabolic disorders is very high (12.7%), so, we can conclude that the feeding technology should be assisted by a nutritional sofware.In general, the frequency of this disturbance is four times lower in comparation with the data from literature.Overall, the digestive disorders (pericarditis, traumatic reticulitis, nutritional and metabolic disorders) are ranking second, accounting for 20% of the total disorders, after reproduction disorders, in the analyzed farms.

CONCLUSIONS

The productive longevity and,especialy the reproductive one, have an important significance for both genetic improvement and the economics of dairy farming.

The analyzed herds are characterized by a biological longevity of 5.71 years, with a variability between 5.10 years and 7.24 years,beeing within the literature limits.

The average length of exploitation of Holstein cows is 3.61 years, with variability between 2.73 years and 4.74 years, which proves different housing conditions.

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REFERENCES

Andronie, Cristina, Furnaris F., 2004. Assessment of the protection and the animal welfare and environmental protection, Ed. Tomorrow Foundation Romania, Bucharest.

Curelariu, Niculina et al., 1980. Research on exploitation period of dairy cows in a elite farm. Scientific papers IANB, Series D, XXII, 53.

Georgescu, Gh. et al., 1989. Treaty of diary farming, vol II, Ed. Ceres, Bucharest.

Georgescu, Gh. et al., 1990. Diary farming, Ed. Didactica si Pedagogică, Bucharest.

Vidu Livia, Bacila V., Udroiu, Alina, Popa, R., Popa, Dana, Stanciu Mirela, Tudorache Minodora, Custura I., 2014. Study regarding the

production performance of Montbeliarde dairy cows in the southern area of Romania, Scientific Papers, Seria D, Animal Science, vol. LVII, 2014, ISSN 2285-5750; ISSN CD-ROM 2285-5769; ISSN-L 2285-5750, 216-220.

Vidu Livia, Băcilă V., Udroiu Alina, Vlasceanu Laura, Calin I., 2005. Research on the health of high performance Holstein-Friesian cows, Scientific Papers of annual Sesion - "Achievements and Perspectives European livestock ", Faculty of Zootechnie, Iaşi 22-23 04 2005, vol. 48, ISSN 1454-7368, 1065-1069.

Vidu, Livia, 2002. Research on dairy farming in standard modules farms for the private sector, PhD thesis, USMV Bucharest.

***, 2000. Die OsterreichscheRindzucht Wien.

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MATHEMATICAL MODELLING AND OPTIMISATION TECHNIQUES USED IN DAIRY FARMING

Alexandru KELEMEN, Gheorghe Emil MARGINEAN, Livia VIDU

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, Email: alexandru.kelemen.zoo@gmail.com

Corresponding author email: alexandru.kelemen.zoo@gmail.com

Abstract

This paper aims to present the methods used so far for mathematical modelling and the optimization techniques of the dairy farming sector. Article content is based on the various papers published from 1940, a period which is considered to taken the first steps in mathematical modelling and optimization of technology used in dairy farming. In literature are described three main groups of optimization methods: intuitive methods (empirical, quantitative and qualitative analysis, graphs and charts, etc.), statistical and mathematical (clusters, dynamic series analysis, correlations, etc.) and operational research (linear programming, non-linear programming, square programming, etc.). Currently, both farmers and staff involved in livestock research, have a number of computer softwares, very affordable, allowing quick and efficient analysis of information that build mathematical models developed to optimize dairy farming technology. In addition, these programs allow maximum freedom in terms of how the mathematical models are constructed and their degree of complexity. A good example is the optimization of feed rations and recipes that can be made with specialized softwares or general softwares such as Excel. In both cases, the biological limits imposed restrictions on the model, but the human factor is the one who decides the number of feeds or the number of nutrients that make the object of optimization. Mathematical modelling and optimization activities in this area are of greatest interest because they allow, through a systemic and causal approach of the phenomena that occur in the dairy farm system, achieving economic goals such as minimizing costs, optimizing the use of available resources and maximizing income or profit.

Key words: *dairy, farm system, mathematical modelling, optimisation.*

INTRODUCTION

Dairy farming is an important branch of animal husbandry due to the share on that milk occupy in human nutrition and the nutritional qualities of this type of food. This statement is supported by the following figures: in the world there are about 1.5 billions cattle (for the year 2013, FAOSTAT 2015) and the milk production is about 626 milions tonnes (for the year 2012, FAOSTAT 2015), while in Romania are registered over 2 milions heads of cattle (for the year 2013, FAOSTAT 2015) and the milk production stands around of 4.33 milions tons (for the year 2012, FAOSTAT 2015). In the year of 2012, the cow milk production at global level accounted for 83% of the total milk production, and in Romania 87% (FAOSTAT, 2015).

However, over time, there have been various imbalances, regarding the relationship between supply and demand that have led to the dramatic fall of the prices offered to the dairy farmers. This risk is imminent in the current context that requires the removal of milk quotas starting with April the 1st 2015.

In these circumstances, we consider that one of the ways that the dairy farms can remain profitable, throughout their existence, is to use mathematical modelling to identify the best optimisation techniques for the technologies that are used in dairy farm.

MATERIALS AND METHODS

This paper is a bibliographic study of the results obtained in this area in order to elaborated, in the future, a dynamic macromodel of the dairy farm that allow an integrated optimisation of the used technologies.

For this study, we used the scientific results obtained so far in this field and published in various papers (books, treatises, handbooks, PhD theses and scientific articles published in the scientific symposia dedicated to agriculture

and livestock, available in print in libraries or in digital from the scientific data bases from the internet). After identification of the papers that are of great interest for this article, we selected on those with increased applicability in practice, to be presented here. In addition, our paper contains a short presentation of the main computerizate programs, specialized or not in this area, which can be used in modelling and optimisation of specific technologies for dairy cows.
The period of time studied in this work is that contained between the years 1940 and 2015.

RESULTS AND DISCUSSIONS

In the case of dairy farms, optimization is the action which seeks, by using various methods available, the most effective way of combining production factors, so that the end result is the best possible outcome in the given context. Considering these aspects, the dairy farm must be regarded as a system with the particularity that has to be seen both from an ecological perspective, but also an economical one.
Regarding the ecological side of this type of system, the dairy farm is a productive zoosystem which differs from a natural ecosystem because it assumes a much higher energy consumption, uses several sources of energy and does not account for biogeochemical natural cycles (Gruia and Păstîrnac, 1991). In addition, there are structural and functional differences in the sense that the control subsystem of the dairy farm, as a zooproductiv system, is being done by human intervention (Gruia and Păstîrnac, 1991). The bio-ecological perspective of the farm system, plays an important role in mathematical modelling performed for the system optimization, because it imposes a number of restrictions to the model. Dairy farm system is an integrated economical system because of the "natural" evolution in the market economy context and also it has feedback capacity in the sense that it respects the demand-offer principle. This means that the dairy farm economic system is in competition with other economic systems, more or less similar, so there is a need of a continue optimisation of the production processes to support the development capacity of the dairy farm in time. Under these circumstances, the dairy farm has to be seen as an economic system that has the particularity that the optimization process is strongly influenced by the biological restrictions.
Initially, the optimisation process was very subjective, relying heavily on the experience of the dairy farm manager, but since 1940s, the mathematical modelling of the bio-productive processes and the farm's systemic approach, allowed development of the production functions which have highlighted the relation of dependence between the production factors, represented by the resources involved in the production process, and the result in the form of the finished product. Such a production function can be represented by the following mathematical expression: $Y = f(x_1, x_2, \dots, x_n)$, where the dependent variable "Y" represents the production obtained in dairy farm and the independent variables " $x_1, x_2, \dots, x_n$ " signify the resources used.
The advantage of using production functions to optimize the production process of the dairy farms is the possibility of recognizing the best variant for combining the production factors in terms of quality and quantity.
In the field of optimizing the production, pioneers are considered Eilhart and Mitscherlich which, together with the mathematician Baule, in 1909, have defined the relationship "production factor - production" as being a nonlinear logarithmic function. (Drăgănescu, 1984).
The first specialized work which connects the exploitation of dairy cows and production functions has belonged to Jensen and was published in 1940 (Heady and Dillon, 1966).
In this paper, Jensen points out that, besides the necessity of knowing the nutritional requirements of dairy cows according to their productive potential, it is also required for us to know how the production is affected when the nutrients intake is increased or decreased (Jensen, 1940). Another problem at which the author tries to find an answer is regarding the way that the milk yield varies depending on the amount of concentrates administrated to the cows. Thus, the question is whether between milk production and level of concentrates administrated by ration is a constant relationship or a gradual one (Jensen, 1940).

Considering these issues addressed by Jensen, one can claim that this paper is the first one which introduces the concept of optimization, in its true sense, in the field of dairy cattle.

In the period after publication of the paper of Jensen, there have appeared a number of scientific works on finding an optimum (represented by the change in milk production) regarding the ratio between the quantity of grain and the quantity of forage in the ration. In this context, Heady and Dillon quotes in their book "Agricultural production functions", that was published in 1966, the experiment conducted by Huffman and Duncan in 1949. The two authors have studied the impact of replacing a quantity of TDN from hay, with an equal amount of TDN from concentrates. The result was an increase in milk production, which concluded that milk production function could be rather non-linear.

The next paper on this subject belongs to Ashe and was published in 1950. The results showed that the relationship between the input and output (regarding the milk yield) has a linear character for cows fed with a maximum of 1800 kg of grains / lactation, in the case of cows fed with a surplus of 1800 to 2700 kg of concentrate the increase in production is much smaller, while a grain intake over 2700 kg per cow has no positive effect on milk production (Heady and Dillon, 1966).

Since the 1970s, the attention of researchers began to move increasingly towards the development of the concept of optimizing the technologies for dairy farms by using production functions (Diaconescu, 1995), so, the number of papers published for the evolution of this area began to grow. In 1981, Balaine, Person and Miller published the paper entitled "Profit in Dairy Cattle functions and effect of measures of efficiency and prices" in order to define the profit function based on performances of cows, to establish the relationship between profit functions used and determine the effects that prices may have on characteristics of the functions and classification of cows in the context of the trend of using the economic efficiency achieved in farm as a selection criteria for the genetic improvement of cows. The conclusions reached by the authors are: 1) is advisable to define the economic efficiency, in the case of dairy cows, as a linear function of income minus expenses per herd life time, because a linear function has a more closed correlation with other functions and is easier to understand, 2) the changes in relative prices have little effect on the classification of cows that are candidate for selection, 3) income variables (milk, fat and protein yield) have the highest correlation (> 0.44) with the daily profit, and expenses, defined as mastitis treatments (-0.21), herd life (0.19) and feed consumption (0.27) had the highest correlation with the profit function.

The trend in the '70s continued in the next decade, and researches on production functions have been enhanced by several researchers.

In this regard, we recall the article published by E. Groeneveld and M. Kovac in 1990, showing a calculation procedure based on several methods such as the method of least squares and systems of mixed equations, with applications in the animal genetic improvement.

A dominant feature of the use of production functions to optimize the dairy farm technologies is the fact that it focuses on achieving economic efficiency at the expense of technological aspects, so the production factors are dosed to achieve profit maximization (Diaconescu, 1995). This is eloquently supported by the work entitled "Profitability of dairy cow herd life". The authors, Congleton and King, have used a dynamic model to estimate the impact of increasing herd life on economic efficiency of the farm. So, the model estimates the economic efficiency variation on the base of the interaction between several factors, like the age of the cows, milk production, demand for labor, cost of treatment, etc.

We consider that targeting the efforts to optimize the dairy farm technologies to maximize the farm's economic efficiency, is appropriate given the argumentation made earlier in this paper, which has shown that dairy farm is primarily an economic system, being integrated in the market economy through the self-regulation mechanism which it has developed. In this context, we want to remember that the perspective which defines the dairy farm as a productive zoosystem should not be ignored, because it is the main

peculiarity of livestock and agricultural economic systems. This approach of the dairy farms can be easily understood and accepted, by both theorists and practitioners in the field of dairy farming, by showing how linear programming can be applied in the optimisation of feeding technologies for cows.

Mathematical programming is a very effective method for solving economic problems by its ability to identify the optimal solution from a set of possible solutions, so the production can achieve maximum growth under conditions of rational use of resources involved in the process (Drăgănescu and Drăgănescu, 1966). A practical problem solved by linear programming involves the following steps: 1) the identification of variables, 2) determination of the objective function, 3) the writing of objective function and 4) the highlighting of the non-negativity condition. The objective function is a linear function in the sense that the equations of the mathematical model are equations of first degree, and the goal is to maximize or minimize a process of economic, technical or biological nature.

Dairy production optimization by using the linear programming, can be done with the Simplex method that was developed by Dantzig in 1947.

In the case of dairy farms, the linear programming is typically used to optimize feed rations for cows.

In practice, regardless of the method used for finding the solution, solving the linear programming calculations is difficult and time consuming (increases the likelihood of errors). For this reason, the use of a computer program, that allow finding the optimal solution in a very short time, is highly recommended. The most accessible program of this type is the MS Excel software. Next, we present a practical example of feed ration for cows that was optimized by using the Solver, an Add-in from Excel that calculates the solutions for linear programming problems by using Simplex method.

In the Excel worksheet, the data is systematized as in the Table 1, and then the formulas are inserted as in the cell range from G4 to J7 plus the formula from the F7 cell. The next step is to select from the "Data" menu, the icon "Solver", action after which a window will opened, requesting the next information: 1) "Target cell" or objective function (the J7 cell); 2) "Equal to" or the objective function type. For this case, "Min" will be selected; 3) in the "By Changing Cell" section, the cells that correspond to the variables (F4:F6) will be selected; 4) and in the "Subject to the Constraints", the restrictions of mathematical model will be noted, which, in this case, are:
G7:I7 >= G1:I1; G7:I7 <= G2:I2; F5 <= 6;
F4 >= 0; F5 >= 0; F6 >= 0.

Table 1. The systematization of data in the worksheet and formulas used

	A	B	C	D	E	F	G	H	I	J
1					The nutritional requirements of a 650 kg cow with 20 kg of milk/day	Min.	16.3	15.3	1420	
2						Max.	19.5	16.3	1560	
3	DM (kg)	MNU	IDP (g)	Cost/kg	TYPE OF FEED	Quantity (kg)	DM (kg)	MNU	IDP (g)	COST
4	0.26	0.21	15	0,003 €	Corn silage		A4*F4	B4*F4	C4*F1	D4*F4
5	0.89	0.57	70	0,110 €	Alfalfa hay		A5*F5	B5*F5	C5*F2	D5*F5
6	0.88	1.00	120	0,170 €	Mixture of concentrated feed		A6*F6	B6*F6	C6*F3	D6*F6
7					TOTAL	F4+F5+F6	G4+G5+G6	H4+H5+H6	I4+I5+I6	J4+J5+J6

The next step consists in selecting the "Solve" button and the display of the solution, if there is one. In the case shown here, the solution is the one from Table 2. To illustrate better the importance of using such a method in dairy farming, we can compare the cost obtained by using linear programming to optimize the feed ration with the cost of a feed ration that was optimized by the classical method of "attempt".

Table 2. The optimum solution for the feed ratio

	A	B	C	D	E	F	G	H	I	J
1					The nutritional requirements of a 650 kg cow with 20 kg of milk/day	Min.	16,3	15,3	1420	
2						Max.	19,5	16,3	1560	
3	DM (kg)	MNU	IDP (g)	Cost/kg	TYPE OF FEED	Quantity (kg)	DM (kg)	MNU	IDP (g)	COST
4	0.26	0.21	15	0.003 €	Corn silage	30,0	7,80	6,30	450	0,090 €
5	0.89	0.57	70	0.110 €	Alfalfa hay	5.3	4,68	3,00	368	0,579 €
6	0.88	1.00	120	0.170 €	Mixture of concentrated feed	6,3	5,28	6,00	720	1,020 €
7					TOTAL	41,3	17,76	15,3	1538	1,689 €

To this end, keeping the same nutritional requirements, we optimized purely technical the same feed ration (Table 3). Optimizing by "attempt" involves combining the available forages in various proportions based on the experience and the intuition of the person that optimizes the feed ratio until the nutritional requirements of the cow are satisfied.

One can easily see that there is a significant cost difference between the two feed ratios (0.152 euro per cow per day), although nutritional requirements are satisfied, both for maintaining the vital functions as well for production.

Analyzing this situation, we can conclude that the economy obtained, thanks to applying the linear programming in zootechnical practice (optimizing the feed ratios), for the case discussed here, is of approximately 47 euros per lactation (305 days) for each cow in the farm. Based on these results, it can be stated that along with the increasing of the dairy farm size, the economy achieved increases, if these optimization techniques are used.

Table 3. Feed ratio optimized through the "attempting" method

	A	B	C	D	E	F	G	H	I	J
1					The nutritional requirements of a 650 kg cow with 20 kg of milk/day	Min.	16,3	15,3	1420	
2						Max.	19,5	16,3	1560	
3	DM (kg)	MNU	IDP (g)	Cost/kg	TYPE OF FEED	Quantity (kg)	DM (kg)	MNU	IDP (g)	COST
4	0,26	0,21	15	0,003 €	Corn silage	27.0	7.02	5.67	405	0.081 €
5	0,89	0,57	70	0,110 €	Alfalfa hay	7.5	6.68	4.28	525	0.825 €
6	0,88	1,00	120	0,170 €	Mixture of concentrated feed	5.5	4.84	5.50	660	0.935 €
7					TOTAL	37.5	18.54	15.45	1590	1.841 €

In dairy farms, linear programming can be applied to other activities, such as crop structure optimization, herd structure optimisation or to the genetic improvement technology. In genetic improvement, linear programming is a method for selection of animals that will be used for breeding, taking into account a number of restrictions on the availability of resources, marketing strategies of the farm or the market trends (Jansen, 1984). Thus, Jansen argues in his article, "Linear Programming in Selection of Livestock", published in 1984, that the estimated performances of the animals can be integrated into objective functions (e.g. maximization of the dairy farm's profit), procedure which brings a higher benefit for the decision making process, compared to the results that may be

obtained by using the simple equations method that disregard the model's restrictions or production alternatives.

Another method for optimizing the activities in the dairy farm is by applying the graphs theory in the process of organization and planning the work of employees. This method allows the rationalization of work time through a sequential and global approach of the work process in order to optimize the total labor consumption (Iosif et al., 1984). As in the case of linear programming, the optimization calculations required for this segment, are facilitated by using computer programs. As a result of the application of this method in practice, the benefits obtained are the maximum possible reduction of the duration of the working process, the increased productivity of labor and reducing costs.

Since the 1990s, appear specialized computer programs for agriculture and livestock. The role of these programs was initially storing various information collected in the form of data banks, but the emergence of new programs, especially those for data processing has enabled a better use of them. Thus, implementation of the "Expert Systems" computer programmes allowed more efficient use of the computing technique and also the use of very effective electronic sensors in animal husbandry (Diaconescu, 1995).

The expert systems, also known as "information-based systems" were designed to solve complex problems and allow drawing conclusions and optimal management decisions based on computer processing (analysis, synthesis) of the raw data available. E. Teigenbaum from Stanford University, quoted by Șt. Diaconescu in 1995, gives the following definition of the expert systems "... a "smart" computer program, which, by using information and interface procedures, solve difficult problems that require a significant human experience for them to be resolved. The information required to solve such levels, plus the interface procedures used, are the concern, for such a model, of the best practitioners in the field."

In general, such a system is made up of several sub-systems (Figure 1).

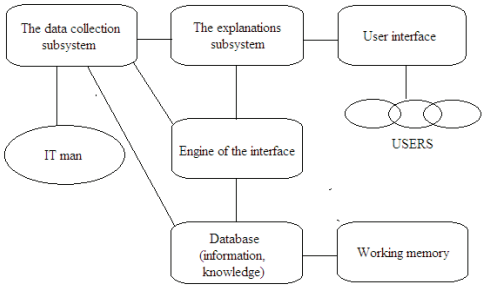

Figure 1. General scheme of an expert system (after Farnir, 1992, cited by Diaconescu, 1995)

Using expert systems, designed to optimize the exploitation of dairy cows, bring some advantages:

- reduce the cost of dairy farming by reducing the need of experts in this field;
- the expertise of an expert system is available at any time;
- can combine the expertise of several specialists in dairy farming;
- developing solutions is timely;
- the solutions are not influenced by human subjectivity;
- is a perfect source of knowledge for the farm manager and not only;
- uses complete and in an intelligent manner the available databases in the dairy farm.

The first steps in the use of expert systems in dairy farms were done by using systems of a lesser extent, in the sense that they were designed to solve specific issues or specific technological segments of dairy farming as: reproduction (Domecq et al., 1991), milking system (Hogeveen et al., 1995), forages (Patacq, 1987), animal health (Benas, 1986), the herd management (Pellerin, 1994), designing and development of dairy farms (Samer et al., 2012), veterinary medicine (Pastell and Kujala, 2007), etc.

Along with the trend of making expert systems that are specialized in the various technologies, there is the tendency of developing complex expert systems that integrate all dairy farm technologies, so that, the solutions developed by the program, to be truly integrated by taking account of the interrelations that exist between the phases of production in the dairy farms. The first steps in this direction are made by Hogeveen et al. in 1991 (Diaconescu, 1995), which creates a general scheme of the modules

and paths of a complet expert system for dairy farms (Figure 2). The functioning of these complete expert systems requires that each module elaborates specific solutions in the field that was designed for (specific algorithms for: breeding, animal health, milking technology, feeding technology, etc.) and then, by unifying these solutions, to issue findings and recommendations in the form of decisions.

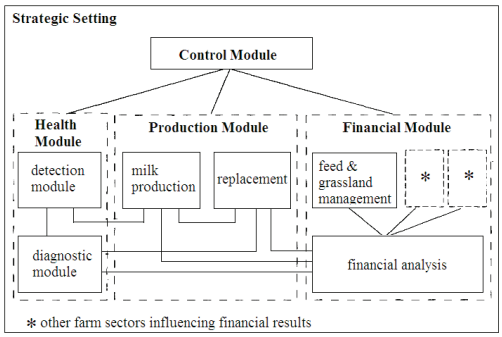

Figure 2. Search paths in a rule-based and a model-based systems (Hogeveen and all, 1991)

One of the first programs of this type is presented in the paper "DXMAS: An expert system software providing advice to dairy operators" that was published in 1993 by Schmisseur and Gamroth. This program was created to identify management problems in dairy farms. As a result, it has been shown that it is able to imitate the decisions and conclusions of dairy experts in 95 management issues. It has been estimated that, by correcting these deficiencies, the dairy farmer can get an income growth ranging, at that time, between $ 25 and $ 450/cow/lactation (Schmisseur and Gamroth, 1993).

A year later, is presented a evaluation of the LAIT-XPERT VACHES program, whose expertise is based on experience of three experts in nutrition and dairy farm management. The program can calculate targets for production, costs of feeding, reproduction, etc; identify problems in feeding technology, genetic improvement, herd management, etc., based on the 950 rules and to issue findings from analysis carried out with an accuracy of 92.3% (Pellerin et al., 1994).

Starting with the year 2000, in dairy farming field occurs the concept of "Precision Feeding", which can be defined as being the optimization of the feeding technology, so that, nutrient losses at dairy farm level to be minimized, and the pollution and costs reduced. This can be achieved by administrating rations with the real nutritional value (what the cow really consume) equal to the theoretical nutritional value, estimated by formulation (Sova et al., 2014). Thus, in the last 15 years, a series of studies have been carried out for the implementation and development of this concept (Bateman et al., 2001; Cerosaletti et al. 2004; Trenel et al., 2009; Spanghero et al., 2010; Lascono et al., 2011).

From 2010 a new trend appeared in dairy farming, that optimize the production processes by treating the cows in the herd at an individual level. This is done automatically by sensors which take and transmit in real time the information collected from the cow on: body temperature, cow's position, daily distance made by the cow, etc. The data collected is processed by computer, and the results are used to develop management strategies in the short, medium and long term (Rutten et al., 2013; Liang et al., 2013). This concept is called "Precision Livestock Farming" (André et al., 2011) and, currently, is the last optimisation technique used in dairy farming.

CONCLUSIONS

Dairy farm is primarily an economic system which has as the main feature, the fact that model's restrictions are of biological nature.

The concept of optimization of dairy farms technologies appeared in the '40s and since, it has gone through several stages: production optimization by using production functions, use of the linear programming, applying the theory of graphs, development of the expert systems and, currently, the tendency to treat dairy cows individually (Precision Dairy Farming Concept).

The objective of optimization of production in dairy farm is mainly of economic nature and secondary of a technological nature.

New studies are needed on the development and implementation of the "Precision Dairy Farming" concept in Romania, in the near future.

REFERENCES

André G., Engel B., Berentsen P.B.M., Vellinga Th.V., Oude Lausink A.G.J.M., 2011. Quantifying the effect of heat stress on daily milk yield and monitoring dynamic changes using an adaptative dynamic model. J. Dairy Sci. 94:4502-4513.

Ashe A.J., 1950. Response of milk production to increased grain feeding. Farm. Econ. 174:4474-4476.

Balaine D.S., Pearson R.E., Miller R.H., 1981. Profit functions in dairy cattle and effect of measures of efficiency and prices. J. Dairy Sci. 64:87-95.

Bateman H.G., Clark J.H., Patton R.A., Peel C.J., Schwab C.G., 2001. Accurancy and precision of computer models to predict passage of crude protein and amino acids to the duodenum of lactating cows. J. Dairy Sci. 84:649-664.

Benas B.F., 1986. The expert systems: an application to the diagnosis of mastitis in dairy cattle. PhD Thesis. Vet. Ecole. Nat. Vet. Toulouse, France.

Cerasaletti P.E., Fox D.G., Chase L.E., 2004. Phosphorus reduction throught precision feeding of dairy cattle. J. Dairy Sci. 87:2314-2323.

Congleton Jr. W.R., King L.W., 1984. Profitability of dairy cow herd life.J. Dairy Sci. 67:661-674.

Diaconescu Șt., 1995. Research on optimization of the technologies of dairy farming in various exploatation systems. PhD Thesis, U.S.A.M.V. Bucharest.

Domecq J.J., Nobel R.L., McGilliard M.L., Pasquino A.T., 1991. Expert system for evaluation of reproductive performance and management. J. Dairy Sci. 74:3446-3453.

Drăgănescu C., 1984. Animals Exploitation - Applied Ecology. Publishing House Ceres, Bucharest.

Drăgănescu C., Dăgănescu C., 1966. Calculation of feed rations. Publishing House Agro-Silvică, Bucharest.

Groeneveld E., Kovac M., 1990. A generalized computing procedure for setting up and solving mixed linear models. J. Dairy Sci. 73:513-531.

Gruia R., Păstîrnac N., Livestock farm treated as zooproductiv ecosystem. Publishing House Ceres, Bucharest.

Heady E. O., Dillon J. L., 1966. Agricultural production functions. Iowa State University Press. Cushing-Mallay, Inc., Ann Arbor, Michigan.

Hogeveen H., Noordhuizen-Stassen E.N., Tepp D.M., Kremer W.D.J., Van Vliet J.H., 1995. A knowledge-based system for diagnostic of mastitis problems at the herd level. 1. Concepts. J. Dairy Sci. 78:1430-1440.

Hogeveen H., Noordhuizen-Stassen E.N., Schreinemakers J.F., Brand A., 1991. Developement of an integrated knowledge-based system for management support of dairy farms. J. Dairy Sci. 74:4377-4384.

Huffman C.F., Duncan C.W., 1949. The nutritive value of alfalfa hay. III. Corn as a supplement tu an all-alfalfa hay ration for milk production. J. Dairy Sci. 32(5):465-474.

Iosif Gh., Zahiu Letiția Frățila Gh., 1984. The economics and organization of production of milk. Publishing House Ceres, Bucharest.

Jansen G.B., Wilton J.W., 1984. Linear programming in selection of livestock. J. Dairy Sci. 67:897-901.

Jensen E., 1940. Determining input-output relationship in milk production. Farm Management Reports – No.5, Washington D.C.

Lascano G.J., Heinichs A.J., Tricarico J.M., 2011. Substitution of starch by soluble fiber and Saccharomyces cerevisiae dose response on nutrient digestion and blood metabolites for precision-feed dairy heifers. J. Dairy Sci. 95:3298-3309.

Liang D., Wood C.L., McQuerry K.J., Ray D.L., Clark J.D., Bewley J.M., 2013. Influence of breed, milk production, season and ambient temperature on dairy cow reticulorumen temperature. J. Dairy Sci. 96:5077-5081.

Patacq J.P., 1987. Systems expert "help with the decision for the establishment of a feeding schedule" Bull. Tech. Inform. – Intelligence artificielle et. Systems experts en agriculture 515:424-425

Pastell M.E., Kujala M., 2007. A probabilistic neural network model for lameness detection. J. Dairy Sci. 90:2283-2292.

Pellerin D., Levallais R., St-Laurent G., Perrier J.P., 1994. LAIT-XPERT VACHES: An expert system for dairy herd management. J. Dairy Sci. 77:2308-2317.

Rutten C.J., Valthuis A.G.J., Steeneveld W., Hogeveen H., 2013. Invited review: Sensors to support health management on dairy farmers. J. Dairy Sci. 96:1928-1952.

Trénel P., Jensen M.B., Decker E.L., Skjoth F., 2009. Technical note: Quatifying and characterizing behavior in dairy calves using the IceTag automaic recordering device. J. Dairy Sci. 92:3397-3401.

Samer M., Hatem M., Grimm H., Daluschitz R., Jungbluth T., 2012. An expert system for planning and designing dairy farms in hot climates. CIGR Journal. Vol. 16, No. 4.

Schmisseur E., Gamroth M.J., 1993. DXMAS: An expert system program providing management advice to dairy operators. J. Dairy Sci. 76:2039-2049.

Sova A.D., LeBlanc S.J., McBride B.W., DeVries T.J., 2014. Accuracy aiion of total mixed rations fed on commercial dairy farms. J. Dairy Sci. 97:562-571.

Spanghero M., Berzaghi P., Fortino R., Masoero F., Papetti L., Zanfi C., Tassone S., Gallo A., Colombini S., Ferlito J.C., 2010. Technical note: Precision and accurancy of in vitro digestion of neutral detergent fiber and predicted net energy of lactation content of fibrous feeds. J. Dairy Sci. 93:4855-4859.

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EVOLUTION OF MORPHOPRODUCTIVE PERFORMANCE OF LAYING HENS EXPLOITED IN AUTHORIZED BREEDING SYSTEMS

Lucia Iuliana NISTOR (COTFAS), Andreea Cătălina NISTOR, Marius Giorgi USTUROI

University of Agricultural Sciences and Veterinary Medicine "Ion Ionescu de la Brad" Iasi

Corresponding author email: iulia_cotfas@yahoo.com

Abstract

Public perception about the nutritional qualities of table eggs obtaining from alternative systems compared to conventional one is based on the idea that eggs produced in alternative systems are superior in quality to those obtained in growth batteries. In the foreground but falls to assure the welfare of laying hens in battery cages adoption 'improved' , which provides ethological needs of laying hens during production . The purpose of this study is to analyze the impact of the welfare on the performance as body weight, egg production and laying intensity ,at Lohmann Brown laying hens during 50 weeks of operation. The determination of performance indicators was performed using specific methods weighing scales Weighmate regular Junior , records and intensity of egg production of laying computerized system Touch Viper Climate and Production. The research was conducted on two groups of hens exploited in classic system as improved battery and alternative system free range. Performance indicators were recorded and the data were statistically processed , establishing systems impact on body weight , egg production , and the intensity of laying. Compared to conventional systems where body weight of birds at the age of 20 weeks was 1545.571 ± 15 369 g, in alternative one the body weight was 1652.429 ± 29.663 g ;in terms of egg production was 1.17 % lower than the production standard for free range system and 0.03 % in group battery operated, about laying intensity was 97.14 % at week 34,in the free range group and 97.43 % at week 29 in the group operated batteries. Alernative systems has multiple benefit on the health of laying hens but not on their productivity , morphological and productive performance of the hybrid , both classical system and free range one is due to genetic stability and environmental factors .

Key words:*eggs, laying hen, performance, free range, improved battery.*

INTRODUCTION

Alternative systems of growth, such as free range have increased in recent years not only to meet the latest consumer food changes, and respond to their concerns about the of well-being condition of laying hens during the productive life (Anderson K.E., 2009).

Growing system is a very important external factor influencing both morphological and productive performance of laying hens and quality characteristics eggs obtained (Englmaierová et al., 2014) .

Productions and their quality are related to physiological status of laying hens, which is the best indicator that expresses the condition of welfare (Travel et al., 2011).

In this context with the ban in the European Union in 2012, was allowed classical battery operation only caged hens improved or alternative systems, such as systems with loft and outdoor access, improve quality of productive life of laying hens (Tauson et al., 1999; Leyendecker et al., 2001a).

In conventional systems is achieved notable performances, including higher eggs production, index improved of feed conversion and lower mortality (Voslarova et al., 2006; Valkonen et al., 2010), but high production of eggs occurred when small groups of chickens were housed in cages improved, but feed intake was higher (Appleby et al., 2002).

The results of Tanaka and Hurnik (1992) indicate that egg production of hens is similar, and relatively high in both systems, conventional and free range, but alternative systems provides a more comfortable environment for birds than batteries .

Therefore poultry specialists are forced to focus on growing alternative systems that replicate the natural environment of life of the hens, but must ensure conditions for a while externalizing the productive potential they possess (Usturoi, 2004).

In this context, the aim of this study is to analyze the impact of the welfare provided in the 2 systems increase the performances as:

body weight, egg production and laying intensity, of Lohmann Brown laying hens during 50 weeks of operation.

MATERIALS AND METHODS

The biological material studied was the Lohmann Brown laying hens, in the period April 2012 - March 2013 distributed as follows: free range system operated (group FR) and improved battery (group B) (Table 1).

Table.1 Experimental research scheme

Specification	Experience groups	
	Group FR	Group B
Hybrid used	Lohmann Brown	
Growth system	Free – range 7000 hens	Improved battery 32000 hens
Insured surface	in hall = 7 hen/ m² in paddock = 4 m²/ hen	750 cm²/hen
Followed indicators	- body weight	
	- egg production	
	- laying intensity	

The determination of performance indicators was made by using specific methods weighing scales Weighmate regular Junior, records and intensity of egg production of laying computerized system Viper Touch Climate and Production .

The investigations were carried out over a 50 weeks of the production period of laying hens.

On the 2 groups of hens exploited - improved batteries - conventional system and free-range-alternative system weighings were performed every 10 weeks, aiming their body weight in the two systems. The other two parameters that were recorded was egg production number and intensity of laying.

The recorded data were statistically processed (arithmetic mean, standard deviation and coefficient of variation average V%).

RESULTS AND DISCUSSIONS

Data analysis performed on the 3 parameters investigated in the 50 weeks, there was a higher weight to free range laying hens compared with the improved battery system where body weight of birds at the age of 20 weeks was 1545.571±15.369 g, the weight of the hybrid Lohmann Brown was 1652.429±29.663 g in alternative system, with an ascent that weight at 70 weeks was 2051.286±27 970 in alternative system compare to 2032.000±25 430 g in the conventional one.

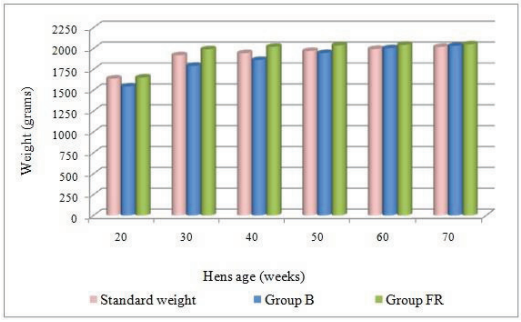

Figure 1. Evolution of body weight of the Lohman Brown hybrid in operated agreed systems

Regarding the evolution of this parameter, regular individual weighings were performed on individual samples from each group, body weight scales measuring with the Weighmate Junior .

Rise in body weight in laying hens is due to access to the paddock outside the hall for the free range, and recipe management.

Production parameters were determined was eggs production and laying intensity exploited in the 2 growthing systems .

Cumulative egg production was 1.17% lower than the production standard for free range system in week 70 of productive life of birds, and 0.03% in group of improved battery operated, as confirmed by the literature .

The Golden J.B. in 2012 states that egg production registered a productive cycle is 357 eggs/hen in conventional system and 304 eggs/hen in free range system, but environmental factors and genetic stability of the hybrid, contribute to achieving these productions .

The peak of laying was reached in week 34, the group free range, laying intensity was 97.14% compared to 97.43% at week 29 in the group operated batteries. In 2009, Arbona, said that in the 65 weeks of the hybrid operation Lohmann Brown in the two systems it was 81.9% battery, and 77.7% for the free range system.

Table 2. Egg production in the two systems agreed (eggs/hen)

Groups of experience	Hens age	Eggs/hen		Standard (eggs/hen)
		weekly	cumulative	
B	20	3.1	3.10	3.9
	30	6.78	66.16	65.8
	40	6.60	132.70	130.7
	50	6.17	197.10	193.1
	60	5.45	252.60	252.1
	70	5.50	306.80	306.9
FR	20	2.26	2.26	1.4
	30	6.7	61.6	58.3
	40	6.6	128.7	122.3
	50	5.7	190.2	183.7
	60	5	242.9	241.1
	70	4.4	289.7	293.1

Table 3 . Intensity of laying in the two systems agreed (%)

Groups of experience	Hen age	Total production (no.)	Laying intensity (%)	
			Realized	Standard
B	20	99200	44.29	45.0
	30	216210	96.81	94.8
	40	209850	94.23	93.3
	50	195694	88.14	89.8
	60	172460	77.86	85.1
	70	173547	78.57	79.4
FR	20	15820	32.29	20.0
	30	46599	95.71	93.7
	40	45791	94.29	91.8
	50	39438	81.43	88.1
	60	34505	71.43	82.2
	70	30188	62.86	73.8

CONCLUSIONS

Alternative systems provide multiple benefits on the health of laying hens but not on their productivity, morphological and productive performance of the hybrid, the improved battery and free range is due both welfare conditions, environmental factors and genetic stability of the hybrid .

The body weight of hens exploited this dynamics for during the 50 weeks, due the good bioconversion of food but also additional sources provided by outside paddock with grass from free range system.

Egg production in the two systems has increased from one stage to another, to guide, but both free range system and the improved batteries in week 70 it was lower than that of technological guide with 1.17% in free range system and 0.3% in the improved battery.

Regarding the intensity of laying, at the age of 40 weeks was 0.9% higher in the improved battery, than 2.7% in free range system. Generaly , the morpho-productive performance of laying hens, in the two systems approved, may not be assigned to a particular operating system .

Battery system brings an improvement in production due to the well-being improved batteries, balanced consumption, and better feed conversion.

The alternative system as free range offers the possibility of manifesting all the instincts (pecking, scratching) at the expense of higher food consumption to ensure both productivity and ethological specific activities, but not least products with superior nutritional qualities.

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REFERENCES

1. Anderson, K. E. 2009. Overview of natural and organic egg production: Looking back to the future. J. Appl. Poult. Res. 18:348–354.
2. Appleby M.C., Walker A.W., Nicol C.J., Lindberg A.C.,Freire R., Hughes B.O., Elson H.A. 2002. Development of furnished cages for laying hens. British Poultry Science, 43, 489–500.
3. Arbona, D.V., J.B. Hoffman, and K.E. Anderson, 2009. A comparison of production performance between caged and free-range Hy-Line Brown Layers. Poultry Sci. Suppl. 88: Abstract # 255P.
4. Englmaierová M., Tůmová E., Charvátová V., Skřivan M., 2014. Effects of laying hens housing system on laying performance, egg quality characteristics, and egg microbial contamination; Czech J. Anim. Sci., 59, (8): 345–352.
5. Golden J.B., D.V. Arbona, and K.E. Anderson, 2012. A comparative examination of rearing parameters and layer production performance for brown egg-type pullets grown for either free-range or cage production japr.oxfordjournals.org/content/21/1/95.full.pdf
6. Leyendecker M., Hamann H., Hartung J., Kamphues J., Ring C., Glunder G., Ahlers C., Sander I., Neumann U., Distl O. 2001b. Analysis of genotype-environment interactions between layer lines and housing systems for performance trails, egg quality and bone strength. 2nd Communication: Egg quality traits. Zuchtungskunde, 73, 308–323.
7. Tanaka T., Hurnik J.F. 1992. Comparison of behavior and performance of laying hens housed in battery cages and an aviary. Poultry Science,71, 235–243.

8. Tauson R., Wahlstrom A., Abrahamsson P. 1999. Effect of two floor housing systems and cages on health, production, and fear response in layers. Journal of Applied Poultry Research, 8, 152–159.
9. Travel A., Nys Y., Bain, M. 2011. Effect of hen age, moult, laying environment and egg storage on egg quality. In: Yves Nys (Editeur), M. Bain (Editeur), F. Van Immerseel (Editeur), Improving the safety and quality of eggs and egg products. Vol.1 Egg chemistry, production and consumption (p. 300-329). Woodhead Publishing in Food Science, Technology and Nutrition (213).Cambridge, GBR: Woodhead Publishing.
10. Usturoi M.G., 2004. Production of eggs for consumption. Publisher"Ion Ionescu de la Brad " Iasi .
11. Valkonen E., Venalainen E., Rossow L., Valaja J. 2010. Effects of calcium diet supplements on egg strength in conventional and furnished cages, and effects of 2 differentnest floor materials. Poultry Science, 89, 2307–2316.
12. Voslarova E., Hanzalek Z., Vecerek V., Strakova E., Suchy P., 2006. Comparison between laying hen performance in the cage system and the deep litter system on a diet free from animal protein. Acta Veterinaria Brno, 75, 219–225.

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REDUCTION OF AMMONIA LEVELS WITH ZEOLITE APPLICATION IN BROILER PRODUCTION

Hasan OZ[1], Atılgan ATILGAN[1], Ali COSKAN[2]

[1]SuleymanDemirel University, Agriculture Faculty, Agricultural Structure and Irrigation Department, 32260 Isparta, Turkey, Phone: +90 246.211.39.22, Fax: + 90 246 211 8696, Email: hasanoz@sdu.edu.tr
[2]SuleymanDemirel University, Agriculture Faculty, Soil Science and Plant Nutrition Department, 32260 Isparta, Turkey, Phone: +90 246.211.85.92, Fax: + 90 246 211 8696, Email: alicoskan@sdu.edu.tr

Corresponding author email: hasanoz@sdu.edu.tr

Abstract

The content of the air surrounding animals is important in terms of poultry production. Particularly increasing concentration of some harmful gases such as ammonia, carbon dioxide and methane cause unfavorable conditions for animals. In the insufficient ventilation conditions these gases should be taken into consideration.
In this research, the ammonia reduction effect of zeolite incorporation to litter was investigated at the poultry house which 22 m long, 10 m wide, capable to produce 3000 chicken at once. Twenty-five percent of zeolite (w/w) was applied to litter as 3 m wide band throughout the short edge of the poultry house to prevent rise of ammonia concentration. EFM/C coded electrochemical ammonia sensors were used to determine ammonia level whereas 16 bit AD/DA converter was used to log data on PC using self-developed software. The first sensor located in the middle of zeolite applied zone, second sensor located just conjunction between zeolite applied and non- applied zones and the others located on the centerline that parallel to long edge, 1.5 m far from each other.
While planning the experiment, reduction on ammonia level was predicted by means of zeolite application. Results obtained showed that the zeolite has a potential to be used in poultry house to prevent rise of ammonia concentration. However, due to the rapid diffusion capabilities of ammonia gas, the differences between measurement sites determined rather low. Therefore, measurement should repeat in tight-separated measurement sites.

Key words*: ammonia, harmful gases, poultry, zeolite.*

INTRODUCTION

Improving ambient conditions is one of the major parameters to improve productivity of broiler production. Therefore a number of researches focused on new approaches that allow reducing ammonia level of the surrounding atmosphere of poultries. Broiler production commonly performed on the litter in Turkey (Sarıca et al., 1996; Sarıca and Çam, 1998). Although many different litter materials are available, the cheapest and easily accessible regional products are preferred (Moore et al., 1996; Sarıca and Çam, 1998; Eleroğlu and Yalçın, 2004). To obtain the expected performance of broilers is closely connected to the appropriate environmental factors as well as types and management of the litter. Moreover type of the litter also effective on the performance, welfare, health and behavior of animals along with product quality (Benabdeljelil and Ayachi, 1996; Ritz et al., 2005; Torok et al., 2009; Garcia et al., 2012; Şekeroğlu et al., 2013). Sawdust accepted as the most suitable litter material for poultry; however, the price of sawdust is raised in recent years due to it has been using for different purposes, consequently production cost of enterprises is raised (Sarıca et al., 1996). Thus, researchers are encouraged to devise new materials that will not negatively affect broiler production performance with reasonable cost issues (Poyraz et al., 1991; Eleroğlu and Yalçın, 2005). A number of studies carried out to determine influence of different litter materials that may substitute sawdust litter on broiler production performance and carcass properties of poultries (Kaygisiz and Corekci, 2003). Some researchers (Eleroglu and Yalcin, 2005) are focusing divers chemical additives to

reduce microorganism abundance and ammonia level. The litter material where the production activities carried out should be clean and odorless to prevent bothering the animals. To this end, researchers are trying to optimize litter quality by a number of different approaches to improve animal welfare. Zeolite that used as a binding agent for animal feed; also be used as an additive to litter due to it has high water-holding capacity, a positive effect on litter aeration, an effect of reducing gas and odor formation (Eleroğlu and Yalçın, 2004). Ammonia formation in litter is closely related by urease activity that synthesized by particular microorganism that act to convert urea to ammonia and carbon dioxide in the presence of adequate moisture. Produced ammonia is volatile and commonly located just 3-8 cm above the litter whereas ammonium ions are water soluble and may stay in the litter (Şekeroğlu et al., 2013). Rising litter humidity and ammonia concentration in the atmosphere surrounding animals are negatively affecting growth rate of animals (Akşit et al., 2000). Optimum litter moisture content reported by Sainsbury (1992) as 24-25%; while, other researchers (Reece et al., 1980; Caveny et al., 1981) reported reduction of body weight, air bladder inflammation and some viral diseases in case of rising ammonia level above 25 ppm. Moreover, in case of ammonia level rise up to 50-100 ppm, eyes of the employees are also effecting from ammonia, with tears appearance and irritation.

In this research effect of 25% (w/w) zeolite incorporation to the litter was investigated to reduce ammonia formation under limited or non-aerated conditions.

MATERIALS AND METHODS

The research carried out at deep litter poultry house that 22 m long, 10 m wide and 3.3 m high with the capacity of 3000. Long axis of the poultry barn positioned East-West direction. The base was covered with lean concrete. Course sawdust was used as a litter throughout the experiment. Barn walls built from lean concrete blocks 20x20x40 cm in size and, therefore, heat insulation was quite poor. Between the supporting columns of southern and northern walls, series windows are located on both sides for ventilation. Two types of feeders are used during each production cycle as bottom placed or hanging tube feeders considering the age of animals. To provide water requirement of the animals a nipple type drinker was used. Barn heated by radiant type heaters, additionally greenhouse heaters (Bouderus) was used as auxiliary heat source in the coldest days.

Ross hybrid chicks which widely used in poultry production were used as a test organism and chicks transferred to barn when they were 1 day old. Thomason et al., (1987) reported that due to the chicks are not able to manage body temperature in beginning stage, ambient temperature should be carefully adjusted. Thus within first two weeks ambient temperature should be higher than 27-30 °C whereas should not rise over 32-35 °C. Considering that information ambient temperature of barn in the beginning stage adjusted between 30-32 C and gradually decreased at the rate of 2-3 C in week until reaching recommended temperature for adult chickens.

Twenty-five percent of zeolite (w/w) was applied to litter as 3 m wide band throughout the short edge of the poultry house to prevent rise of ammonia concentration. EFM/C coded electrochemical ammonia sensors (Electronic Devices Limited, UK) were used to determine ammonia level whereas 16 bit AD/DA converter was used to log data on PC using self-developed software. The first sensor located in the middle of zeolite applied zone, second sensor located just conjunction between zeolite applied and non- applied zones and the others located on the centerline that parallel to long edge, 1.5 m far from each other (Figure 1). Sensors were located to determine differences between zeolite applied and non-applied sites as well determine the ammonia gradient by means of distance from zeolite applied sites. Two more sensors were placed on 1.7 m high from bottom to determine ammonia concentration in the air where the employee was breathing.

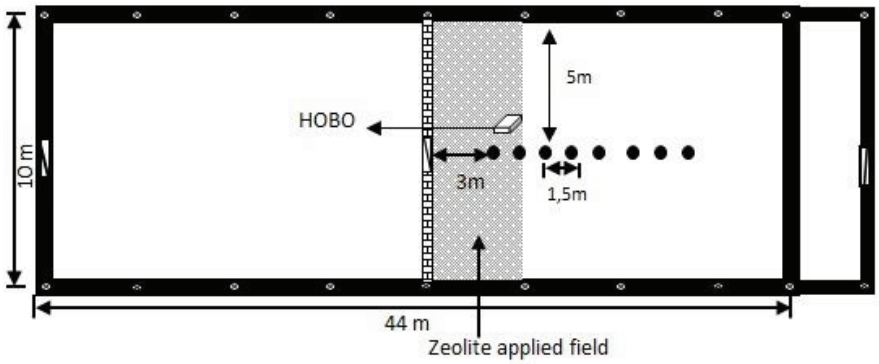

Figure 1. Placement plan of the sensors in barn (Figure is not scaled)

RESULTS AND DISCUSSIONS

In the experiment, cultivation in poultry house was continued for 45 days; however, measurement was not cover all cultivation period. Because, in the earlier stage of cultivation, litter was not enough dirtied by poultry dung; thus ammonia emission in the barn atmosphere was quite low, even lower than the detectable threshold value of the sensors. In the later stage of cultivation, ventilation was necessary due to higher outside temperature; therefore, ammonia concentration was rarely exceeded 60 ppm. It was not possible to suspend ventilation on the day time and thus, at the late afternoon ventilation windows are closed and then opened at the morning. Data from ammonia sensors started to log 2-3 hours before closing ventilation windows, whereas log closed 2-3 hour later then windows opened. Figure 2 presents the ammonia measurement of three days average at 1/2 hour interval that representing overall situation about ammonia level in the barn.

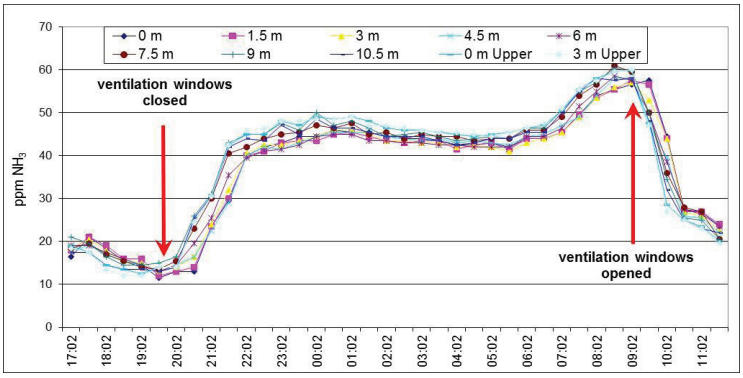

Figure 2. Ammonia values of representative measurement day (please note 0 m upper and 3 m upper are stand for employee nose level ammonia concentration)

Ammonia concentration of barn atmosphere along with ambient temperature was starting to rise dramatically just after closing the ventilation windows. It was expected situation but ammonia increment settled up couple hours later and ammonia level became constant between 40 to 50 ppm nearly all night long. Early in the morning ammonia level started to increase once more and reached slightly higher than 60 ppm. Results obtained were not clearly revealed the emission-reducer effect of zeolite application. Between the measurement sites

there was minor differences by means of ammonia values; however these differences was not significant to conclude precise suggestions. On the other hand, reasons for not determining the difference between the measurement sites seems to be closely related to diffusion of ammonia gas from one measurement sites to other. Thus based on this experimental design it is hard to make inferences revealing that zeolite incorporation is beneficial or useless.

Eleroğlu and Yalçin (2004) reported that intensive ventilation condition prevents reliable measurements on ammonia level in the barns. Supporting that evaluation Sarıca and Demir (1998) did not reported positive effect of zeolite application on ammonia level controlling in well ventilated poultry houses. Bintaş et al., (2014) moved subject to different point and revealed that zeolite incorporation is not effective on controlling ammonia emission from litter and improving animal welfare.

Three days average values for each measurement sites are presented in Figure 3. Ammonia concentration was increased with distance from zeolite applied zone. Considering trends on ammonia level by means of distance from zeolite zone (Polinom. 30 cm; Figure 3), it can easily said zeolite application is effective on ammonia concentration suppressing. Nose-level ammonia concentration (170 cm) was higher than all measurement points except 9th. The 9th point gives the highest ammonia level among the sites which is one of the farthest sites. The last point which 10.5 m far from zeolite applied zone shows lower value than 7.5 and 9 m points. This because that point just close to entrance of barn and while employees are entering the barn fresh air comes in. Lower values in nose-level point would be another evidence for beneficial effect of zeolite.

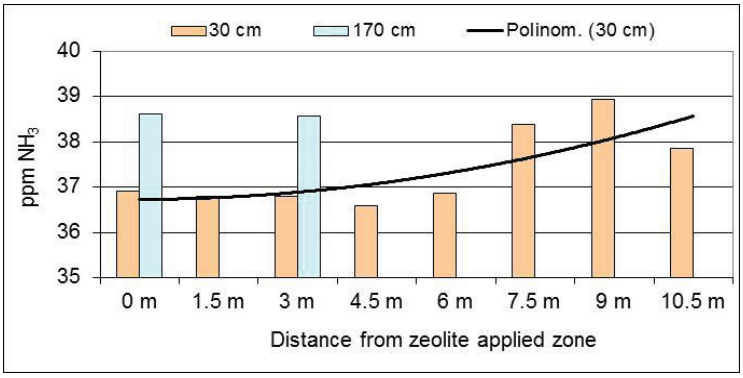

Figure 3. Mean ammonia values by means of distance from zeolite applied zone

The determined temperatures in the barn presented in Figure 4. Results revealed that the air temperature in barn were higher than recommended value of 21-22 °C. The experiment carried out in spring time and outside temperature increased rapidly when cultivation near to end. Last week of cultivation, all windows tightly closed at the night time to be able to evaluate effect of zeolite application, this would be another factor elevating air temperature in the barn. There was no difference in temperature between the measuring points as expected.

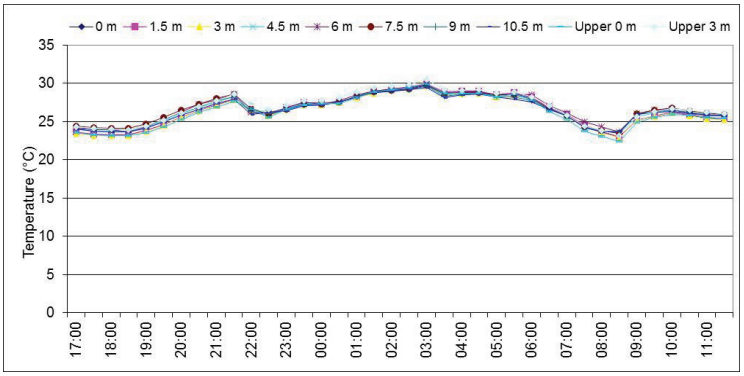

Figure 4. Air temperatures of poultry house

CONCLUSIONS

Overall results obtained from the experiments revealed that zeolite may use as an ammonia suppressing agent. However, due to the mean ammonia concentration fluctuating within only 3 ppm band, based on present data given here it is hard to conclude any reliable decision. Thus, further experiment should carry out using air tight compartments to prevent ammonia diffusion from one site to other. Although ventilation seems to be the most effective method for reducing ammonia concentration, yet it should be noted the negative effects of air quality.

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REFERENCES

Akşit M., Bozkurt M., Alçiçek A., 2000. Farklıformdayemlerlebeslenenetlikpiliçlerdealtlığade ğişikdüzeylerdezeolitilavesininperformansvealtlıköze lliklerüzerineetkileri.HayvansalÜretim, 41: 84-90. (Turkish press)

Benabdeljelil K., Ayachi A., 1996. Evaluation of alternative litter materials for poultry. J. Appl. Poultry Res., 5 (3): 203-209.

Bintaş E., Küçükyilmaz K., Bozkurt M., Çatli A.U., Çinar M., Topbaş S., Koçer B., Ege G., 2014. Altlığa ilave edilen doğal zeolitin etlik piliçlerin performansı ve refahına etkileri. Tavukçuluk Araştırma Dergisi, 11(1): 10-15. (Turkish press)

Caveny D.D., Quarles C.L., Greathouse G.A., 1981. Atmospheric ammonia and broiler cockesel performance. Poultry Sci., 60: 513-516.

Eleroğlu H., Yalçın H., 2004. Zeolitle karıştırılan altlığın etlik piliçlerde besi performansı ile bazı altlık parametreleri üzerine etkileri, Tavukçuluk Araştırma Dergisi, 5 (1): 31-40. (Turkish press)

Eleroğlu H., Yalçın H., 2005. Use of natural zeolite-supplemented litter increased broiler production. South African Journal of Animal Science, 35 (2):90-97.

Garcia R.G., Almeida I.C.L., Caldara F.R., Nääs I.A., Bueno L.G.F., Freitas L.W., Graciano J.D., Sim S., 2012. Litter materials and the incidence of carcasslesions in broilers chickens. Brazilian Journal of PoultryScience, 14 (1):27-32.

Kaygısız F.H., Çörekçi Ş., 2003. Broiler Üretiminde zeolitli altliğin tekrar kullanilabilirliğinin fayda-maliyet analizi. İstanbul Üniv. Vet. Fak. Derg., 29(1): 43-50. (Turkish press)

Moore P.A., Daniel T.C., Edwards D.R., Miller D.M., 1996.Evaluation of chemical amendments to reduce ammonia volatilization from poultry litter.Poult. Sci., 75:315-320.

Poyraz Ö., Özçelik M., Çep S., Bahadıroğlu M.E. 1991. The use proportions of diatomite as litter on broiler production. J. Vet. Med. Assoc., 45-47.

Reece F.N., Lott B.D., Deaton W.J. 1980. Ammonia in the atmosphere during brooding effects performance of broiler chickens. Poultry Sci., 59: 486-488.

Ritz C.W., Fairchild B.N., Lacy M.P., 2005. Litter quality and broiler performance. cooperative extension service. The University of Georgia College of Agricultural and Environmental Sciences, Bulletin 1267, 2005.

Sainsbury D.. 1992. Poulty health and menagement. Blackwell Science Ltd. Osney Mead, Oxford. Third Edition.

Sarıca M., Saylam S.K., Öner F., Karçay N., 1996. Altlığazeolitilavesininetlikpiliçlerdebüyümevealtlıköz elliklerineetkileri.Hayvancılık Kongresi'96, İzmir, (1):346-352. (Turkish press)

Sarıca M., Çam M.A., 1998. Broiler üretimindealtlığıntekrarkullanımınınverimvealtlıközel

liklerineetkileri.DoğaTürkVeterinerlikveHayvancılık Dergisi, 22(3):213-219. (Turkish press)
Sarıca M., Demir Y., 1998. Etlikpiliçyetiştiriciliğindealtlığazeolitilavesininkümes içiçevrekoşullarıveverimözelliklerineetkileri.Ondoku zMayısÜniversitesi, Ziraat Fakültesi Dergisi, 13: 67-78. (Turkish press)
Şekeroğlu A., Eleroğlu H., Sarıca M., Camcı Ö., 2013. Litter materials and litter material management used in production on the ground. Tavukçuluk Araştırma Dergisi, 10: 25-34.
Thomason D.D., Lepley M.K.C., Dendy M., 1987. American Soybean Association Poultry Brooding, USA, 65p.
Torok V.A., Hughes R.J., Ophel-Keller K., Ali M., MacAlpine R., 2009. Influence of different litter materials on cecal microbiota colonization in broiler chickens. Poult Sci., 88: 2474–2481.

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THE INFLUENCE OF LACTATION ON THE MILK YIELD OF ESTONIAN RED AND MOLDAVIAN BLACK SPOTTED CATTLE BREEDS

PARASCA ALEXANDRU[1], PARASCA ANNA[2], SERGHEI CHILIMAR[3]

[1] SPIBAHVM, Maximovca
[2] SPIBAHVM, Maximovca
[3] State Agrarian University of Moldova, Chisinau

E-mail: parasca86@mail.ru

Abstract

The research was focused on the influence of lactation on the milk yield in terms of quantity and quality, as well as on the speed of milking and total content of fat and protein in the milk of Estonian Red and Moldavian Black Spotted cattle breeds in the first and third lactation. The researches were conducted in the cattle breeding farm of the Experimental Technological Station „Maximovca" and in the laboratory of Cattle Breeding and Exploitation of the Scientific and Practical Institute of Biotechnology in Animal Husbandry and Veterinary Medicine. We studied the following indices: milk yield by performing control milking, assessment of fat, protein and lactose content and milk density by laboratory analysis of the milk, milking speed, the amount of total fat and total protein.

***Key words**: Analysis, Cattle, Milking speed, Quality, Total fat, Total protein.*

INTRODUCTION

Cattle breeding is an ancient occupation, which today has become a representative one, as it allows to obtain a variety of products - milk, meat, leather, biomass, etc. At present, high yields of milk obtained from cattle, with a high content of fat, protein and lactose represent the result of human labour. The specialists in selective breeding have worked for centuries with local, moderately productive, cattle creating specialized dairy breeds as Holstein breeds. Taking into account current market conditions, when it is necessary to obtain high and qualitative yields in short terms, it is crucial to know the productive and qualitative characteristics of cattle breeds at different ages in current breeding conditions. As for the milk production and its quality [9], it can be mentioned that milk production, fat, protein and sugar content change as the animals age. If one intends to buy dairy cows and to raise them in small and medium-sized farms, it is necessary to know the productive and qualitative characteristics of the cow breeds raised in the country in order to achieve an essential economic efficiency.

MATERIALS AND METHODS

The researches were performed in the cattle breeding farm of Experimental Technological Station „Maximovca" of the Scientific and Practical Institute of Biotechnology in Animal Husbandry and Veterinary Medicine. As research material, we took the cows raised in the farm and divided them into groups. The first group included Estonian Red breed, the second group – Moldavian Black Spotted breed and also the cows were distributed depending on the lactation number: the first and third lactation. The cows of Estonian Red breed were grouped as follows: 12 cows in the first lactation and 17 cows in the third lactation. As for Moldavian Black Spotted breed, the situation was as follows: 20 cows in the first lactation and 14 cows in the third lactation. All animals were kept in identical breeding conditions according to the loose housing system. We studied the following indices: milk yield by performing control milking, assessment of the fat, protein, lactose content and milk density doing the analysis of milk in the Laboratory of Cattle Breeding and Exploitation of the SPIBAHVM using the apparatus „Ecomilc Total". Milking speed was determined in the milking parlor, using a pine tree milker and a timer to control the length of time, and then comparing the obtained amount of milk and the time. The amount of total fat and total protein was determined by multiplying the milk yield by the percentage of fat and protein, then dividing by 100.
The obtained data were processed biometrically using the Excel program. Milk density was converted from degrees of the aerometer (1.02677 ˚A – for Estonian Red breed) = % - in percentage 26.77%.

RESULTS AND DISCUSSIONS

According to the analysis of data on the milk yield of the breeds used in research, we obtained the results shown in Figure 1.

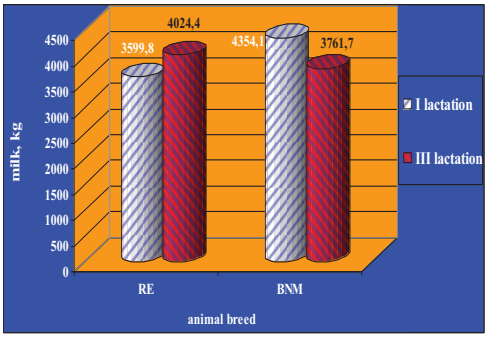

Figure 1. Milk yield characteristics

The data in Figure 1 show that the highest amount of milk was obtained from cows of Estonian Red breed in the third lactation - 424.58 kg of milk and from cows of Moldavian Black Spotted breed – in the first lactation - 592.4 kg of milk, but many researchers consider that the maximum milk yield can be obtained in the IIIrd and IVth lactation from cows of Black Spotted breeds [8].

High nutritional value of milk fats as well as the ease of their assessment served as reasons to consider the fat content as basic criterion to weigh the breeding and productive value of the dairy animals [10].

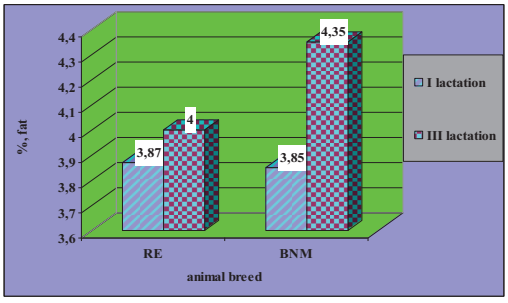

Figure 2. Characteristics of the fat content of milk

According to data presented in Figure 2, we can mention that the fat content of milk of Estonian Red breed is by 0.13% higher at cows in the third lactation compared to the first lactation as for the Moldavian Black Spotted breed it is also higher in the third lactation by 0.05%.

As reported by the authors [9], there is a direct connection between milk fat content and milk density, and if milk is degreased, its density increases, and vice versa when increasing the fat content - milk density increases too. Figure 3 indicated milk density of the studied dairy cattle depending on the breed and lactation number of these cows.

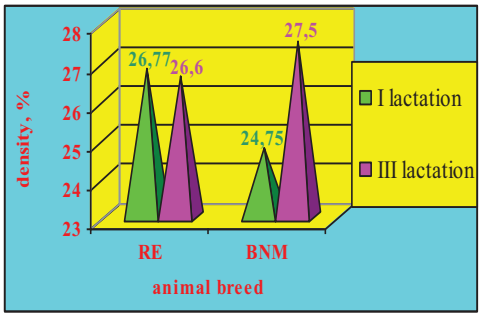

Figure 3. The density of milk in percentage

The data in Figure 3 show the density of milk, which is by 0.11% higher in the milk obtained from cows of Estonian Red breed in the first lactation, while for the cows of Moldavian Black Spotted breed, milk density is by 2.75% higher in the third lactation.

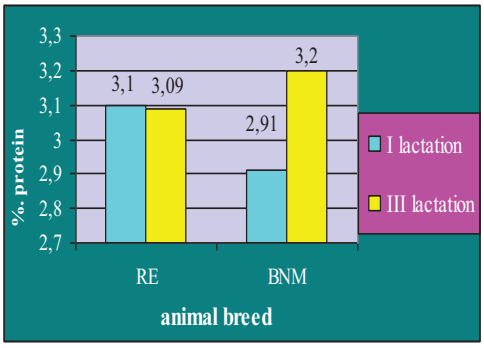

Figure 4. Protein content of milk

The cows of Estonian Red breed have higher protein content in the first lactation - by 0.1%, while the cows of Moldavian Black Spotted recorded a greater percentage of protein in the third lactation - by 0.29%.

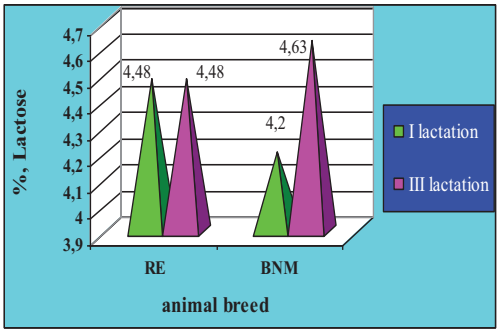

Figure 5. Lactose content of milk

According to the figure above, the lactose content of milk of Estonian Red cows is 4.48, regardless of lactation number, while the highest

lactose content of milk - 4.63 was obtained from cows of Moldavian Black Spotted breed in the third lactation, which is by 0.43 % higher than in the first lactation. In conditions of intensive technologies, the cows are milked using a milking equipment and special attention is given to the selection of aptitude for mechanical milking. Increased speed of cow milking reduces labour costs and time to obtain 1 q of milk, fact which influences the economic efficiency of breeding cows for milk yield.

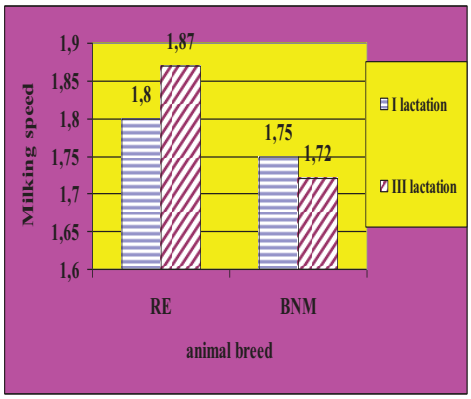

Figure 6. Milking speed

The results presented in Figure 6, show that the milking speed of cows of Estonian Red breed in the first lactation is lower than the milking speed of cows in the third lactation by 0.062 l/min, while in the case of Moldavian Black Spotted breed the milking speed of cows in the third lactation is by 0.023 l/min lower than the milking speed of cows in the first lactation.

The authors [9] consider that when increasing the speed of milking, the obtained milk has a higher fat content. Therefore, we calculated the amount of total fat in kg as it is necessary in the production of butter and other dairy products, which require an increased amount of fat content in milk.

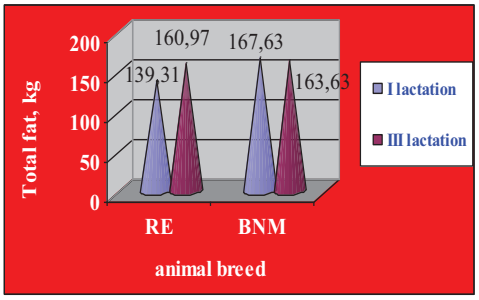

Figure 7. Total fat amount in the milk obtained from the entire lactation

According to Figure 7, the greatest amount of fat in the milk from the entire lactation of Estonian Red breed was obtained from the cows in the third lactation, i.e. by 21.66 kg more than from the cows in the first lactation. As for the Moldavian Black Spotted breed, the largest amount of fat in the milk from the entire lactation yield was obtained from the cows in the first lactation, approximately by 4.00 kg more than the total amount of fat obtained from cows in the third lactation.

In the European Union, according to the traditions of cheese-producing countries, the milk that is used to produce cheese contains a greater amount of protein compared to drinking milk, therefore they choose to breed cows producing milk with high percentage of protein. Taking into consideration all the facts mentioned above we calculated the total amount of protein in milk obtained from the studied cows.

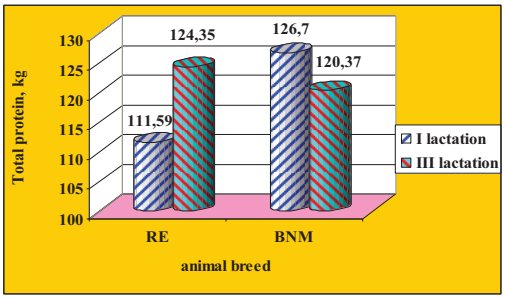

Figure 8. Total amount of protein in the milk obtained from the entire lactation

Figure 8 shows that the largest amount of total protein in the milk of Estonian Red breed was obtained from cows in the third lactation, which is by 12.76 kg of protein more than from the cows in the first lactation. The cows of Moldavian Black Spotted breed recorded the largest amount of total protein in the first lactation, which is by 6.33 kg of protein more than from the cows in the third lactation.

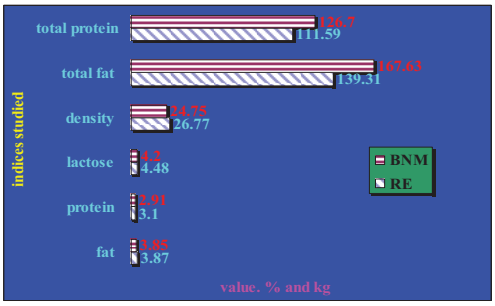

Figure 9. Indices studied for the mentioned cow breeds in the first lactation

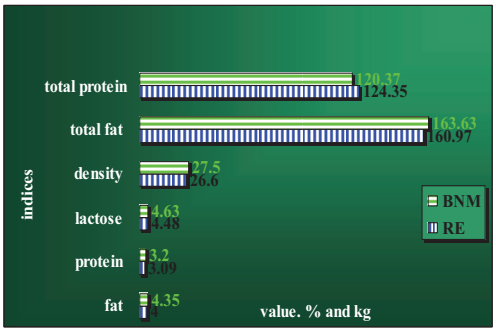

Figure 10. Indices studied for the mentioned cow breeds in the third lactation

CONCLUSIONS

According to the research results we can draw the following basic conclusions:

1. It was found that depending on the lactation number and animal breed, milk yield indices, its quality and content of total fat and total protein varies from one lactation to another, and namely:

2. Regarding the milk yield per lactation, the highest milk yield was obtained from cows of Moldavian Black Spotted breed - 4354.15 kg of milk in the first lactation and the lowest milk yield was obtained from cows of Estonian Red breed - 3599.83 kg of milk in the first lactation.

3. Fat percentage, milk density, percentage of protein, and lactose percentage are superior in the milk of cows of Moldavian Black Spotted breed.

4. As for the milking speed, the highest speed was recorded by the cows of Estonian Red breed in the third lactation.

5. With reference to the fat content of milk, the cows of Moldavian Black Spotted breed are superior in both lactations.

6. The amount of total protein in the milk from the entire lactation shows that the largest amount of total protein was obtained from cow's milk of Moldovan Black Spotted breed in the first lactation.

REFERENCES

Bucătaru N, Radionov V., 2001. Increasing cattle for milk. Inform AGRO

Chilimar S. et al., 2001. Type intrarasial Black Spotted cattle in Moldova International Symposium "50 years of higher education in Iasi livestock"

Dranca D., 2008. How should show a good cow milk. - Farm, Nr. 1

Drummers N.V., 1983. Dairy business. - Ear Publishing, Moscow

Georgescu Gh et al., 1982. Cattle breeding technology. - Publisher didactic and pedagogical Bucharest

Georgescu Gh., 1988. Treaty of cattle. - Publishing Cherry, Bucharest

Lupan B., Sergiu Chilimar, Vasile Ujică, 1997. Technology cattle. - Central Printing House, Chisinau

Markov K., Altman A., 1963. What factors influence the composition of milk. - Publishing USDA RSFSR, Moscow

Novikov E., 1981. Molocinoe productivnosti carov v cnighe scotovodstvo. - M: tom. I

Radis D., 2004. Dairy farm in the Netherlands. Rev. "Farm"

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THE CURRENT STATUS AND THE PERSPECTIVES OF DUCK HUSBANDRY AT GLOBAL LEVEL AND IN OUR COUNTRY

Elena POPESCU MICLOȘANU, Gabriel Cătălin GÎLEA, Alexandru KELEMEN

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, Email: catalin_gabriel1989@yahoo.com

Corresponding author email: catalin_gabriel1989@yahoo.com

Abstract

In the context of the global demographic boom, we notice a more and more obvious interest of livestock farmers' both in streamlining animal farming and in the biodiversity of domestic species from which we may obtain a series of food products with high nutritive and organoleptic value. This phenomenon is supported, as far as duck husbandry is concerned, by the numerical evolution of the livestock for these species at global level in the interval 2009-2013. Thus, the latest data available at present (for the year 2013, FAOSTAT 2015) indicate that the livestock being reared in the world exceed 1,335 million ducks. They gain a more and more significant ratio among the domestic poultry livestock reared on a large scale, filling the third position in Europe after chicken and turkey and the second position in Asia. On the Asian continent, which has the longest tradition of consuming food products obtained from ducks, we notice a 13% increase, in Europe the increase is of 4%, in Africa of 5%, in North and South America of 7%, while Oceania is the only region where the livestock decreased by 20%, which is not insignificant at all due to the small livestock that are reared there. In this context, an international level, there is an average increase of 9%. Romania, too, follows this upward trend, the duck livestock increasing by 5% in the above-mentioned interval.

An important aspect of duck husbandry is represented by the diversity, quantity and quality of the productions it provides: meat, foie gras (considered a delicacy), eggs, feathers and down of very high quality. The global production of duck meat is about 4.341 million tons, 11.5% of which is produced in European countries. The trade in this poultry totals imports of over 11.3 million ducks, 11.7% of which is performed in Europe. The main problem encountered by livestock farmers in farming certain species of duck is represented by the relatively low performances recorded in the reproduction activity, which decreases the farm's profitability and makes it difficult to improve the poultry populations.

Key words*: ducks, foie gras, livestock, meat productions, trade.*

INTRODUCTION

Ducks are poultry that love water, they are reared in large flocks in people's households, as well as in farms with various levels of intensification. They are highly variable in terms of breeds, which is why farming them may have several purposes such as: meat, foie gras, feathers, down or eggs. These fowls' consumption of concentrate feed is low, as they make very good use of green mass. Thus, in the first part of their life, their feed includes a mixture of concentrated fodder, then juicy fodder is gradually introduced in feeding. Legumes such as clover and lucerne, as well as poaceae can be consumed by ducks directly through grazing. In order to obtain larger carcass, or to obtain foie gras, the ducks are force-fed. This may be done using corn grains boiled with 2% extra fat, or corn mash which includes 1.5-2% fat, 0.8% salt and 1% premix. The force-feeding interval ranges between 20-25 days. In order to obtain a foie gras weighing 350-400 g approximately 15-17 kg of corn are consumed (Vacaru Opriș, 2002). Rearing this species contributes to diversifying poultry production, in order to increase the assortments that the consumers can choose from. This is very important in the context in which classic poultry species such as chicken or broiler turkey have already achieved outstanding performances, which renders necessary the development of research in this field.

MATERIALS AND METHODS

The paper was elaborated upon a thorough bibliographic study, based on the scientific

results obtained to date in the field of duck husbandry. Various bibliographic sources were referred to, such as: books, textbooks, scientific articles, other internet data about the numeric evolution of the duck livestock, the quantitative aspect of yields and the economic balance. In the statistical processing of the data, we used the Excel MS software, which decreased the time required for this activity and allowed us to avoid calculation errors.

RESULTS AND DISCUSSIONS

At global level, a wide variety of breeds is reared, even different species of ducks, as well as hybrids, which may be ranked according to the specialised direction of their production into: meat breeds, egg-producing breeds and decorative breeds. Another classification criterion is body weight, according to it there are heavy and light breeds. Hybrids are the products obtained by interspecific or intraspecific crossbreeding, breeds or lines, in order to obtain an added yield due to the heterosis phenomenon.

The Pekin breed. It originated in China, wherefrom, in the 16th century it was imported to the UK, and then it spread throughout Europe. The body weight is 3.5 kg in males and 3.0 kg in females, and the egg production varies between 160-220 eggs. In extensive and semi-intensive farming, the egg-laying season opens in February and lasts until August, extending even until October. The plumage is white, and incubation takes 28-30 days. In our country, the breed was imported several times, the latest import being made in the 1980s, when the pure lines 001 and 005 were brought from Cherry Valley in the UK, which were used in producing intraspecific and interspecific hybrids with the Muscovy breed. (Popescu-Micloşanu 1990)

The Muscovy breed. It originated in South America, it appeared naturally by taming the Cairinia moscata breed. It was brought to Europe by Christopher Columbus in the 16th century. The breeds exhibits a significant gender dysmorphia, mainly the male weighs between 4.5-6 kg and the female between 2.5-3.5 kg. The egg production ranges between 70-80 eggs, and in controlled microclimate 120-150 eggs may be obtained. In terms of colour varieties, this breed displays the following colours: black, white and pied. Incubation takes 34-36 days, at the age of 11-12 weeks the males weigh 3-3.5 kg, and the females 2-2.15 kg.

The Rouen breed originates from North-West France, the Rouen town region, it is a heavy duck breed, with a slightly elongated body shape, the chest is wide, it has good features for meat production, the males achieve weights ranging between 3.5-4 kg, and the females 3-3.5 kg. The female lays 80-90 eggs of cream of greenish colour. The plumage is wild. (Bunaciu, 2009)

The Aylesbury breed was obtained in Aylesbury county town in the UK. In this region, the breed was obtained through the selection of individuals with high body weight, whose offspring grow fast, the breed fattens easily and produces high quality meat. It is highly appreciated in Germany, where it has been reared for 150 years. The body weight is 3.5 kg for ducks, and 4 kg for drakes; the fattened fowl may reach 5 kg. Ducklings are fattened easily and yield fine meat. Ducklings also have a fast growth rate, so that at the age of 8-11 weeks they weigh 2-2.5 kg. The egg production is of 80-180 eggs with an average weight of 90-115 g/egg, and a white-yellowish or greenish colour. Egg-laying begins in December – January and continues until July. The egg fecundity ratio is satisfactory. The plumage is white. (Vacaru-Opriş, 2000)

The Campbell breed. It was created at the end of the 19th century. There are many colour varieties, such as: khaki, white and pied. At the age of 8-10 weeks, ducklings weigh between 1.5-1.9 kg, and at adult age, males weigh 2.2-2.5 kg, and females 1.8-2 kg. The breed is very good egg-layer, with a high yield ranging between 250-300 eggs, in 1929 a record number of 357 eggs was recorded. The breed is used in obtaining egg-laying or meat producing hybrids.(Popescu-Micloşanu, 2009)

The Indian Runner breed. It originated in India, wherefrom it was brought to Europe in mid-20th century, its body weight is of 2 kg in males and 1.8 kg in females. It is a good egg-layer, yields range between 180-200 eggs, weighing 65 g and having a white or cream shell colour. In terms of colour varieties, the breed displays: the wild variety, pied, white, brown and trout colour. (Usturoi, 2008)

Ducklings at the age of 8-10 weeks weigh between 1.5-1.8 kg.

Ornamental breeds. Recently, both at global level and in our country, these breeds have expanded rapidly and they include: the Carolina, Mandarin, Dwarf, Crested, Labrador or Cayuga breeds. They are appreciated for their plumage beauty, structure and colour as such, but also for embellishing parks and yards.

To obtain foie gras, as well as meat, the Pekin and Muscovy breeds are reared globally and their crossbreeding yields interspecific hybrids. The intraspecific hybrids based on the Pekin breed are those that achieve the highest performances in terms of broiler production, and they are the most widely spread at global level. Thus, the hybrids produced by Cherry Valley Farms in the UK may reach a delivery weight of over 3 kg, on a low specific consumption, over an interval 10-15 days shorter than the local duck breeds in various countries, thus they are in demand due to these performances.

The Star 53 hybrid of a French company, has as father the GL 50 strain, with sexual maturity at the age of 25 weeks, and as mother the GL 30 strain, with maturity at the age of 24 weeks and a production of 230 eggs per egg-laying season of 44 weeks, ducklings reaching a weight of 3 kg at the age of 42 days, with a specific consumption of 2.4 kg. 49 days from hatching, ducklings weigh 3.4 kg, with a specific consumption of 2.6, which is why they are also in high demand due to the increased performances they achieve.

Among the Muscovy duck hybrids reared for meat ducklings with very high yield characteristics, we may quote those produced by the company Grimaud Frères in France. When Muscovy ducks and common ducks mate naturally, the fertility ratio is usually very low. At present, the artificial insemination method is used to increase fertility. (Usturoi, 2005)

The R.32 black hybrid. It is a Muscovy duck hybrid obtained by crossbreeding males from the "Dominant" line with females from the "Typical" line, it has black plumage, dark grey skin colour, male slaughter age of 80 days, and female 70 days, the slaughter weight of 3.8 kg in males and 2.1 kg in females, the specific consumption is of 2.85 kg.

The R.51 hybrid. Muscovy duck hybrid created by crossbreeding males from the "Cabreur" line with females from the "Casablanca" line, it has white plumage and yellow skin. The slaughter age is the same as in the case of R.32, the male eviscerated carcass weighs 3 kg, and the female one 1.7 kg. The specific consumption is of 2.8kg. (Popescu-Micloşanu 1990)

The 31 barred hybrid. Muscovy duck hybrid obtained by crossbreeding males from the "Dominant" line with females from the "Dynamic" line, plumage upon hatching is barred, and at slaughter age it is grey with black spots. The male eviscerated carcass weighs 3.7-4.0kg and the female one 1.8-2.0kg. the slaughter age and specific consumption are the same as in the case of the other hybrids. (Popescu-Micloşanu 1990)

The Romanian meat and foie gras hybrid, Mulard. It was obtained by crossbreeding Muscovy males with Pekin females, the hybrids have a fast growth rate, with good force-feeding features, they may reach 3.5-4kg, after force-feeding, foie gras weighing 350-400g may be obtained, with a consumption of 16-18 kg corn. (Usturoi, 2005)

Ducks are valued for their diverse productions, as they yield: meat, eggs, foie gras, feathers and down. The meat production is influenced by breed, age, gender, hybrid, feed maintenance or administration, using force-feeding or not. Duck meat is consumed due to its organoleptic features, as well as to its low intramuscular fat content, being leaner than chicken meat, having 2% lipids content in the muscles and being rich in polyunsaturated fatty acids. The colour is redder due to the high red muscle fibre ratio ranging between 70-90%.

A 100g serving of skinned duck breast meat contains 140 calories and 11.2 g of fat.

A great advantage of moderate consumption of duck meat is given by duck fat. The melting point for duck fat is only 14 degrees Celsius, much lower than the human body temperature, and for this reason it is easy to eliminate, as compared to the high melting points of beef, pork and chicken fat which are 45, 38 and 37 degrees Celsius. The low melting point allows the meat to be very delicious even when it is served cold.

The egg production is also important. According to M. Bălăşescu (1980) the chemical

structure of a duck egg is the following: 70.1% water, 13% protein, 14.5% lipids, 1.4% glucose and 1% mineral salts. The caloric value of a duck egg is of 131 calories/egg or of 190-230/100g egg.

The duck egg contains iron, zinc, potassium, retinol, vitamin K and can help people suffering from insomnia, digestive problems or hypertension. The disadvantage is that the duck egg contains a high level of cholesterol, so it will be consumed more rarely and only very well cooked. The eggs laid by these species must be consumed after proper thermal treatment was applied (boiling/frying), or they are consumed as liquid eggs (pasteurised), thus lowering the possibility of becoming infested with certain diseases such as Salmonella. (Popescu-Micloşanu, 2007)

Eggs for human consumption are obtained from light breeds, considered good egg-layers (Campbell, Indian Runner).

Feathers and down are used in the animal flour industry, as well as in manufacturing quilted clothing, pillows or duvets, for which white down is preferred.

The foie gras production is generally obtained from hybrids after force-feeding, a procedure which leads to liver becoming even 10 times heavier than normal. The phenomenon is due to the accumulation of lipids in the hepatic tissue. The global production of foie gras in 1998 was of 16,800 tons and has increased rapidly and constantly in the past 20 years. Almost 80% of the global foie gras production is obtained by France and Hungary. Bulgaria and Spain are also large producers.

Romania, as well as its neighbours, can also produce and market foie gras efficiently, both for domestic consumption and for export, where the demand is continually increasing.

A statistical situation related to the global foie gras production in 2005 shows that the overall production was of 23,500 tons, the main producer being France, with 18,450 tons, followed by Hungary with 1,920 tons and Bulgaria with a production of 1,500 tons.

Table 1. The global foie gras production in 2005

Country	Foie gras production (tons)	Ratio to the total
France	18,450	78.5%
Hungary	1,920	8.2%
Bulgaria	1,500	6.4%
USA	340	1.4%
Canada	200	0.9%
China	150	0.6%
Other countries	940	4%
Total	23,500	100%

Force-feeding may be done manually and industrially. Manual force-feeding is a method that takes more time, between 5-10 minutes, the advantage being that the behaviour and state of each individual can be observed, obtaining satisfactory average yields. Among the disadvantages there are the high corn consumption and the workforce. (Usturoi, 2005)

Industrial force-feeding is of three types: force-feeding on corn grains, on corn mash and the auto-force-feeding method, the amount of labour being smaller and yields being higher.

The current status of duck husbandry, both at global level and in our country, is good, the data from the past 13 years and the analyses performed indicate that livestock is constantly growing, reaching 22.27% at global level.

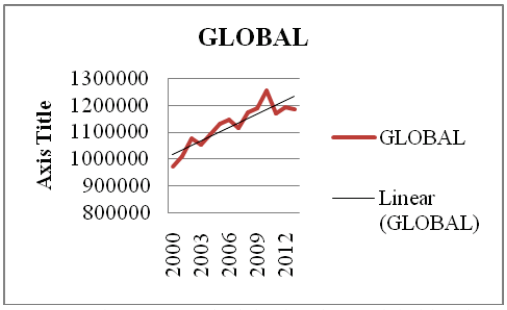

Fig.1. The status of duck husbandry at global level (thou heads)

In Romania, the livestock is small due to the fact that duck consumption in our country is quite low.

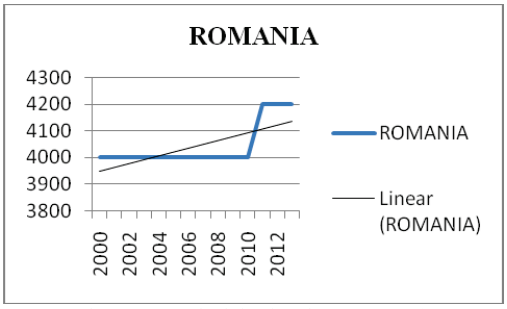

Fig. 2. The status of duck husbandry in Romania (UM)

At continent level, Asia yields the best results, its livestock increasing from 860,857,000 heads in 2000, to 1,045,055,000 in 2013, the increase being of 21.40%.

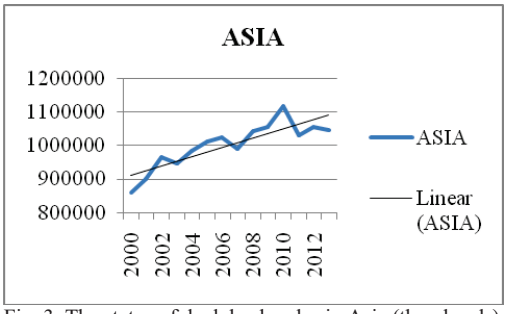

Fig. 3. The status of duck husbandry in Asia (thou heads)

The Asian continent is followed by the European one, in 2000 in Europe there were approximately 67,728,000, increasing by 28.43% and reaching 86,983,300 heads in 2013.

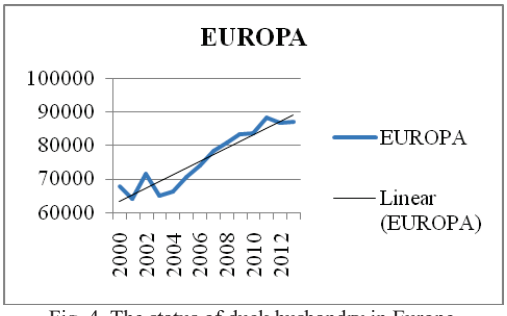

Fig. 4. The status of duck husbandry in Europe (thou heads)

Africa is the third continent globally with a livestock of 25,514,000 in 2013, the increase being quite high, of almost 50% since 2000, when 17,095,000 heads were reared in Africa.

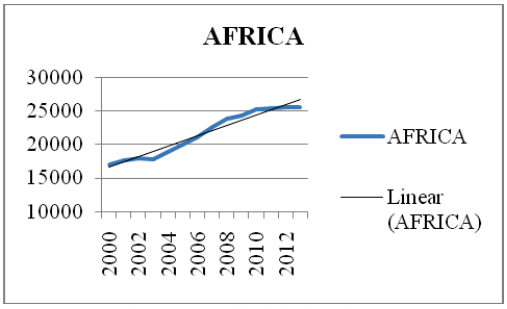

Fig. 5. The status of duck husbandry in Africa (thou heads)

In South America the status is also good, Fig.6 showing that there is a 31.21% increase since 2000, when the livestock amounted to 7,118,000 heads, until 2013, when it reached almost 9,400,000 heads.

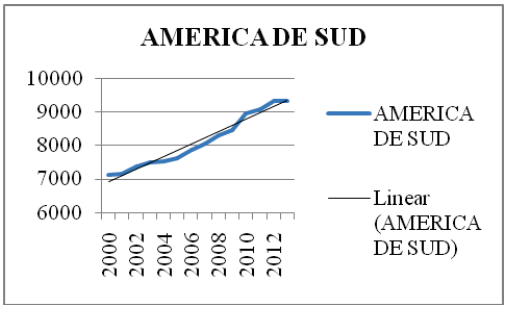

Fig. 6. The status in South America (thou heads)

In North America, in 2013, there were approximately 8,751,000 ducks, the livestock increasing by 13% since 2000, when 7,751,000 heads were reared.

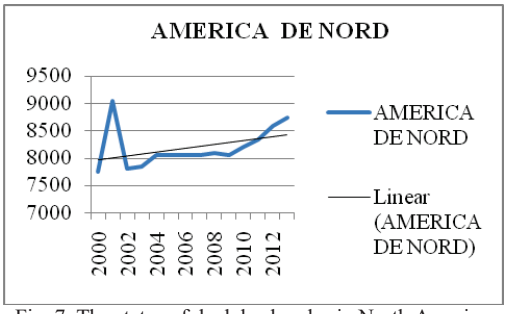

Fig. 7. The status of duck husbandry in North America (thou heads)

In Oceania, we encounter the highest increase of approximately 75%, but the livestock reared here is much smaller that it is on the other continents, thus the increase is insignificant at global level.

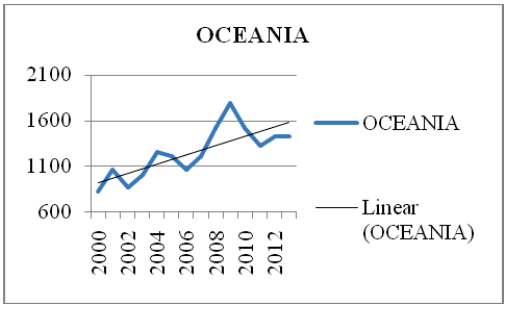

Fig. 8. The status of duck husbandry in Oceania

The livestock recorded in Oceania have evolved from 818,000 heads in 2000, to 1,428,000 in 2013.

CONCLUSIONS

From the analysis we performed, we notice a global increase in duck livestock in the past 13 years. Among the continents, the largest livestock are found in Asia, the Asian peoples having a long tradition of rearing and consuming duck meat and eggs. Asia is also the largest duck exporter in the world, and there are countries that process the duck eggs incubation on board ships, reaching the destination during the ducklings hatching.

Europe also has an important duck livestock, which has increased more than the global one, by over 28 % in the interval 2000-2012.

We notice that the first two foie gras exporters are France and Hungary.

In Romania, the increase in livestock amounts to 5%, from 4,000,000 to 4,200,000. In time, the livestock tends to develop, which is also due to the ever higher demand for duck meat, as well as to the outstanding characteristics of duck meat.

Decorative breeds will also develop due to the fact that they are liked more and more globally, and the demand is higher and higher. The livestock tend to keep increasing due to the fact that ducks meet the requirements of poultry products diversification and have a low feed consumption, they can be reared very well in extensive system, on lakes and pastures, thus making very good use of feed sources that are not used by other animal species and do not compete with human food.

REFERENCES

Bunaciu, Petru, 2009. Poultry reproduction, Printech Publishing House, Bucharest.

Popescu-Miclosanu, Elena, 1990 Research on the interaction of nutrition and microclimate factors on productive parameters in dack broiler

Popescu-Miclosanu, Elena, 2009. Duck and goose husbandry. Rentrop & Straton Publishing House, Bucharest

Popescu-Miclosanu,Elena, 2007. Poultry husbandry for egg production, Printech Publishing House, Bucharest.

Usturoi, Marius, Giorgi, 2005. Poultry breeding technologies, Alfa, Iasi.

Usturoi, Marius, Giorgi, 2008. Poultry husbandry, Ion Ionescu de la Brad Publishing House, Iasi.

Văcaru-Opriş, Ioan, 2000. Treaty on poultry farming, vol I, Ceres Publishing House, Bucharest.

Văcaru-Opriş, Ioan, 2002. Treaty on poultry farming, vol II, Ceres Publishing House, Bucharest.

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ROMANIAN SPORT HORSES: EFFECTS OF COMPETITION LEVEL, SEX AND BREEDER ON THE NATIONAL DRESSAGE RANKING

Anca ROMAN-POPOVICI, Dan SUMOVSCHI, Ioan GÎLCĂ

Faculty of Animal Science, University Of Agricultural Science And Veterinary Medicine
„Ion Ionescu de la Brad" No. 3, Street Aleea Mihail Sadoveanu, Zip code 700490, Iași, Romania,
Phone: +40232.407.407, Fax: +40232.260.650, Email: roman_anca2004@yahoo.com,
sumovschi.dan@gmail.com, igilca@uaiasi.ro

Corresponding author e-mail: roman-anca2004@yahoo.com

Abstract

The study of the average performance regarding the results in national competitions for the horses from the Romanian Sport Breed (RSB) is particularly important. Thus, by studying the averages nationally competitive results we can form an idea about the performance level in dressage for this population.
The objective of this study was to analyze the differences between the horses from RSB regarding competition level, sex and origin, and to investigate the impact of these factors on the average competition results for the last 3 years.
For this research we examine all the horses legitimated FER from RSB participants in national dressage tests, divided in 2 groups for origin variable and 3 groups for competition level and sex variables.
Data were manipulated and analyzed using SPSS Version 21 for Windows (IBM, USA).
The results of the statistical analyze show that for the sex variable in case of the RSB legitimated FER from national state studs evaluated as being significantly different ($p<0.001$) for variable competition results at FE level in 2012, ($p<0.01$) for variables competition results at BA level in 2012 and competition results in 2012, ($p<0.05$) for variable competition results.
Also, the results show that from the distribution of RSB legitimated FER participants in national dressage tests, 62.5% participate at FE level (the lower difficulty level) and only 12.5% at BA level (the highest difficulty level). Must be noted that the last year in which the RSB horses participated at BA level was 2012, and from 2012 to 2014 the number of RSB horses participants in national dressage tests been halved.
In conclusion we can say that the present research work demonstrate that the role of RSB horses in dressage is abruptly decreasing and the only levels in which this horses are still present are the lower difficulty levels.

Key words: *competition, level, dressage, competition results, Romanian sport horses.*

INTRODUCTION

When we talk about breeding sport horses for performance in dressage or show jumping, the performance level obtain by these horses in competitions is particularly important. This is the basic criterion in terms of profit and profitability achieved from the economic point of view of this branch of farming.
The recent studies show that young horse performance tests have high genetic correlations with later competition results, but competition data are more accurate predictors when selecting for only one discipline (Hellsten et. al., 2006; Ducro et. al., 2007).
Good competition results in dressage and/or show jumping are the main objectives for breeding sport horses all over the world (Olsson et. al., 2008). The study of the average performance regarding the results in national dressage competitions for the horses from the Romanian Sport Breed (RSB) is particularly important because in this way we can form an idea about the performance level in dressage for this population.

MATERIALS AND METHODS

For this research we examine the entire population of horses from the Romanian Sport Horse (RSB) breed that are legitimated to Romanian Equestrian Federation (FER) and participants in national dressage competitions from 2012 to 2014.
This RSB horses come from both state studs and private studs and farms, consisting of 16 individuals, of whom 13 individuals represent RSB horses from state studs and 3 individuals representing RSB horses coming from private

studs and farms, with both parents from Romanian breeds.

In this research we had 4 independent variables divided in two groups for breeder variable (state studs and private studs and farms), three groups for sex variable (geldings, stallions and mares), four groups for competition level variable (EF levels, DC levels, BA levels and EF+DC levels) and six groups for competition period variable (2012, 2013, 2012-2013, 2014, 2013-2014 and 2012-2014).

The horse distribution for competition level and competition period variables taken is shown in Figure 1.

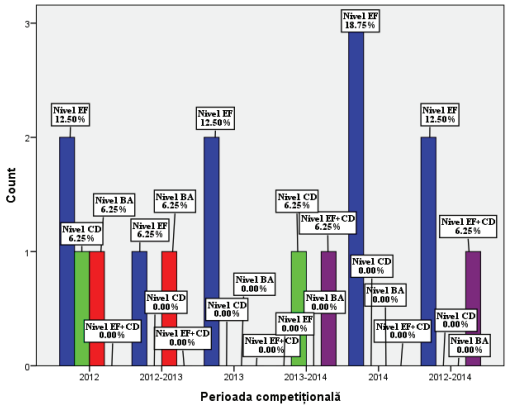

Figure 1. Horse distribution according to competition level and competition period

All the data were assessed based on FER reports, also serving in the calculation and assessment of results of competitions each year separately, for each and every horse in part.

The competition results of each horse were judged in part as follows (Figure 2.):

- for the first 5 places were offered points for the obtained place (5 point for first place, 4 points for second place, 3 points for third place, 2 points for fourth place and 1 point for fifth place);
- number of points obtained was multiplied by 1, 1.5 or 2 along with the raising of tests difficulty;
- the scores were calculated for each horse regardless of the rider because we want to realize an analysis of the horse sports performance and not those of the rider;
- we considered and awarded points only for the first 5 places in each test because under FER Regulation prizes are accorded to 25% of the number of starts, but at least to the first 5 finishers (http://www.fer.org.ro/pdf/regulament-competitional-2013.pdf).

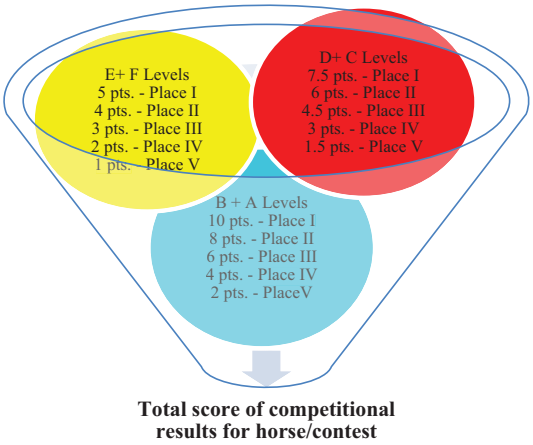

Figure 2. Assessment of competition results

Data was manipulated and analyzed using SPSS Version 21 for Windows (IBM, USA) the following statistical analyzes being performed:

- Independent Sample T-Test;
- One-Way ANOVA;
- Descriptive statistics on the distribution of variables, mean, median, graphs etc.

The value of alpha was set at 0.05 for all statistical tests.

The objective of this study was to analyze the differences between the horses from RSB regarding competition level, sex and origin, and to investigate the impact of these factors on the average competition results for the last 3 years.

RESULTS AND DISCUSSIONS

The results show that for:

- sex variable: - 43.75% of the horses are mares, 31.25% are stallions and 25% are geldings;
- breeder variable: - 81.25% of the horses come from state studs and 18.75% from private studs and farms;
- competition level: - 62.5% of the horses compete on EF levels, 12.5% on DC levels, 12.5% on BA levels and 12.5% on EF+DC levels;

- competition period: - 25% of the horses competed only in 2012, 12.5% only in 2013, 18.75% only in 2014, 12.5% from 2012 to 2013, 12.5% from 2013 to 2014 and 18.75% from 2012 to 2014.

For the RSB horses from state studs that are legitimated FER and competing in dressage tests we observed that the highest value of the factor score for competition results was recorded for the mare Magnolia with an overall score of 59.5 points, while the lowest value was recorded for the gelding Taifun with a total score equal to 0 (Table 1). The average performance for this factor is 23.62 points. (Table 3)

Table 1. The values of the competition result scores of RSB horses from state studs that are legitimated to FER and competing in dressage

Name	Sex	Competition period	Score for competition results
APOLLO	s	2014	2
DESTOINIC	s	2012-2013	38
DOMINO	s	2012	46
ELMO	s	2013-2014	33,5
GRETA	m	2013-2014	37.5
LEONA	m	2012-2014	46
LUP	g	2012-2014	6
MAGNOLIA	m	2012-2014	59.5
NUFĂR	g	2012	4.5
OLIMPIA	m	2014	6
PANTERA	m	2012	27
REFLEX	g	2012	1
TAIFUN	g	2013	0

For the RSB horses from private studs and farms that are legitimated FER and competing in dressage tests we observed that the highest value of the factor score for competition results was recorded for the mare Zaina with an overall score of 19 points, while the lowest value was recorded for the mare Afrodita with a total score of 8 points (Table 2).
The average performance for this factor is 12 points (Table 3).

Table 2. The values of the competition result scores of RSB horses from private studs and farms that are legitimated to FER and competing in dressage

Name	Sex	Competition period	Score for competition results
AFRODITA	m	2013	8
ORLIC	s	2012-2013	9
ZAINA	m	2014	19

The results shows that the total score for competition results for all 16 horses taken into study vary between 0 and 59.5 points, with an average of 21.44 points (Table 3 and Figure 3).

Table 3. Descriptive statistics for the competition results variable

Competition results	N	$\bar{X} \pm S_{\bar{X}}$	s	V%	*Min.*	*Max.*
RSB State Studs	13	23.62±5.84	21.06	89.17	0	59.5
RSB Private Studs and Farms	3	12±3.51	6.08	50.69	8	19
Total Effective	16	21.44±4.88	19.53	91.12	0	59.5

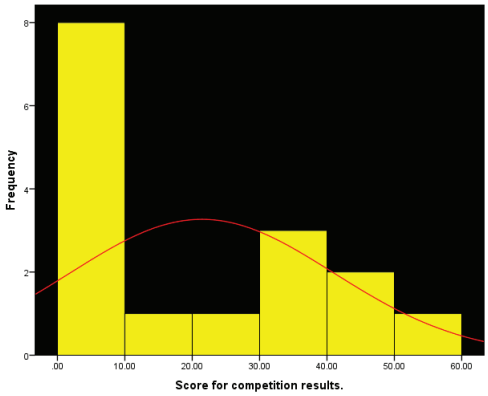

Figure 3. Normal distribution of the score for competition results in the studied population

For the sex variable the results from the statistical analysis shows that the score for competition results doesn't present statistically significant differences for the 0.05 significance level ($F = 3.153$, $p = 0.077$).
Therefore, we can say that, regarding the score for competition results there are no statistically

significant differences between mares, geldings and stallions.

Table 4. Descriptive statistics for the sex variable

	N	$\bar{X}$	s	$\mp S_{\bar{X}}$	Min.	Max.
mare	7	29.00	7.50	19.85	6	59.5
gelding	4	2.87	1.41	2.83	.00	6
stallion	5	25.70	8.55	19.13	2	46
Total	16	21.43	4.88	19.53	.00	59.5

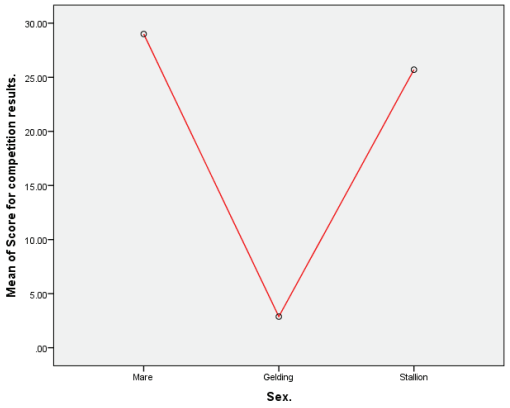

Figure 4. Means Plots of the score for competition results for sex variable

Nevertheless the results from the statistical analysis shows that the score for competition results for the RSB horses from state studs presents a statistically significant differences for the 0.05 significance level (F = 4.596, p = 0.038).

Therefore, we can say that the score for competition results for the RSB horses from state studs is significantly higher (p = 0.048) for mares ($\bar{X}_1$=35.2) compared to geldings ($\bar{X}_3$=2.87), but no statistically significant differences exist between stallions and gelding (p=0.134), nor between mares and stallions (p=1.000).

Table 5. Descriptive statistics of the RSB horses from state studs for the sex variable

	N	$\bar{X}$	s	$\mp S_{\bar{X}}$	Min.	Max.
mare	5	35.20	9.03	20.20	6	59.5
stallion	4	2.87	1.41	2.83	.00	6
gelding	4	29.87	9.64	19.28	2	46
Total	13	23.61	5.84	21.05	.00	59.5

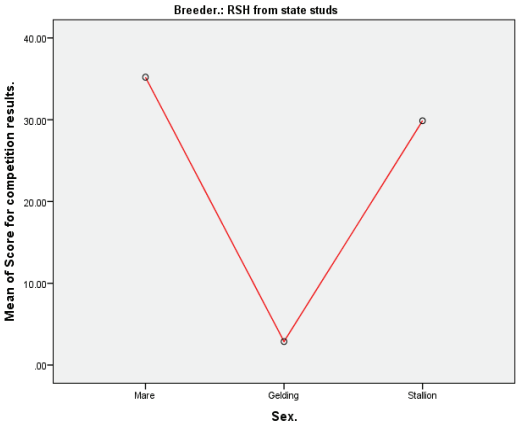

Figure 5. Means Plots of the score for competition results of RSB horses from state studs for sex variable

For the breeder variable the results from the statistical analysis shows that the score for competition results doesn't present statistically significant differences for the 0.05 significance level (t = 1.704, p = 0.113).

Therefore, we can say that, regarding the score for competition results there are no statistically significant differences between horses from state studs and horses from private studs and farms.

Table 6. Descriptive statistics for the breeder variable

	N	$\bar{X}$	s	$\mp S_{\bar{X}}$	Min.	Max.
State studs	13	23.61	5.84	21.05	.00	59.5
Private studs and farms	3	12.00	3.51	6.08	8	19
Total	16	21.43	4.88	19.53	.00	59.5

For the competition level the results from the statistical analysis shows that the score for competition results presents a statistically significant differences for the 0.05 significance level (F = 4.163, p = 0.031).

Therefore, we can say that the score for competition results is significantly lower (p = 0.028) for horses that compete in EF levels ($\bar{X}_1$=12.4) compared to horses that compete in BA levels ($\bar{X}_3$=42) and also comparing to

horses that compete in EF+DC levels (p=0.014, $\bar{X}_4$=46.5), but no statistically significant differences exist between the horses that compete in EF levels and the horses that compete in DC levels (p=0.482).

Table 7. Descriptive statistics
for the competition level variable

	N	$\bar{X}$	s	$\mp S_{\bar{X}}$	Min.	Max.
EF levels	10	12.40	4.58	14.49	.00	46
DC levels	2	21.00	16.50	23.33	4.5	37.5
BA levels	2	42.00	4.00	5.65	38	46
EF+DC levels	2	46.50	13.00	18.38	33.5	59.5
Total	16	21.43	4.88	19.53	.00	59.5

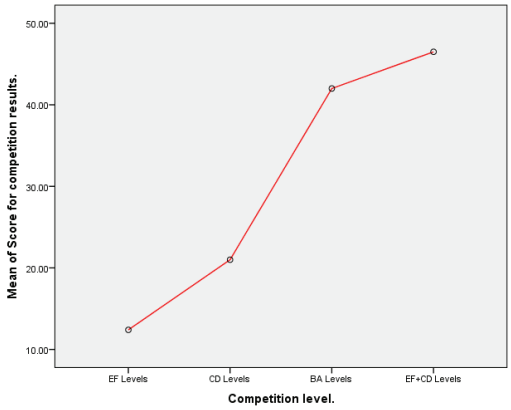

Figure 6. Means Plots of the score for competition results for competition level variable

For the competition period variable the results from the statistical analysis shows that the score for competition results doesn't present statistically significant differences for the 0.05 significance level (F = 1.278, p = 0.346).
Therefore, we can say that, regarding the score for competition results there are no statistically significant differences between horses that competed only in 2012, the ones that competed only in 2013, only in 2014, the horses that competed from 2012 to 2013, the ones that competed from 2013 to 2014 and the ones that competed from 2012 to 2014.

Table 8. Descriptive statistics
for the competition period variable

	N	$\bar{X}$	s	$\mp S_{\bar{X}}$	Min.	Max.
2012	4	19.62	10.51	21.02	1.00	46
2012-2013	2	23.50	14.50	20.50	9.00	38
2013	2	4.00	4.00	5.65	.00	8
2013-2014	2	35.50	2.00	2.82	33.50	37.5
2014	3	9.00	5.13	8.88	2.00	19
2012-2014	3	37.16	16.06	27.82	6.00	59.5
Total	16	21.43	4.88	19.53	.00	59.5

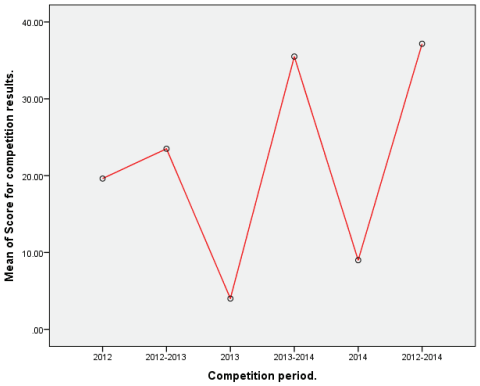

Figure 7. Means Plots of the score for competition results for competition period variable

CONCLUSIONS

From those shown so far it can be seen that the majority of RSB horse participates in the lowest difficulty tests (62.5% in FE levels tests) and only 12.5% in the test with the highest level of difficulty (BA levels tests). It should be observed that the last year in with an RSB horse participated in BA levels tests was 2012, and in the period between 2012 and 2014 the number of participants from this breed halved.
In conclusion we can say that regarding the average score for competition results for RSB horses participants in national dressage competitions from 2012 to 2014 is not influenced by the type of breeder (private or state), but we have to specify that for the private farmers we included in the study only

horses with both parents from Romanian breeds.

For the future is impetuously necessary to research how the level of performance achieved in dressage tests is influenced by the RSB horses from different type of breeder (private or state), the parents breed and also a comparison of their results to other breeds that take part in Romanian national dressage championship.

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REFERENCES

Ducro B.J., Koenen E.P.C., van Tartwijk J.M.F.M., van Arendonk J.A.M., 2007. Genetic relations of First Stallion Inspection traits with dressage and show-jumping performance in competition of Dutch Warmblood horses, Livestock Science, 107:81-85.

Hellsten E.T., Viklund A., Koenen E.P.C., Ricard A., Bruns E., Philipsson J., 2006. Review of genetic parameters estimated at stallion and young horse performance tests and their correlations with later results in dressage and show-jumping competition, Livestock Science, 103:1-12.

Olsson Elisabeth, Nasholm Anna, Strandberg Erling, Philipsson J., 2008. Use of field records and competition results in genetic evaluation of station performance tested Swedish Warmblood stallions, Livestock Science, 117:287-297.

http://www.fer.org.ro/pdf/regulament-competitional-2013.pdf

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THE SWINE PRODUCTIVE CAPACITY DEPENDING ON USED BREEDS FOR THE HYBRID PRODUCTION

Ilie ROTARU

State Agrarian University of Moldova, 42 Mircesti street, Chisinau, Republic of Moldova

Corresponding author email: i.rotaru@uasm.md

Abstract

This paper explains the swine productive capacity study and the swine growth during the lactation period and the breeding under the animal genetic type influence. It was concluded that by crossing the Yorkshire x Pietrain breeds, a young swine breed has been fetched, defined by a more advanced intensive growth; made by boars and sows similar to Landrace breed resulting in a bigger body mass of the studied swine family at the age of two months, explained by the manifestation of the heterosis phenomenon.

Key words: *boar, breed, growth, heterosis, swine.*

INTRODUCTION

Research carried out worldwide proves that nowadays the main technique of increasing productivity is hybridization. The hybrids obtained from crossing breeds, types and specialized lines have a higher productivity, with 8-10% than pure breed animals and with 5-6% towards half-breeds from crossing breeds (Rotaru, 2013; Rotaru et al., 2015).

In order to obtain higher and more efficient productivity quantitatively and qualitatively there are certain methods for proficient exploitation of heterozis phenomenon.

Swine populations formed and improved in our country, as well as import genotypes could be very useful in this process, being provided efficiently, this is why, genetic resources must be rationally used in swine growth and hybridization system (Rotaru, 2009).

For improving genetic swine background, a selection of individuals used on reproduction is made, in order to create parental forms capable of producing descendents with a valuable productive potential, among products resulted, there are chosen those who submit special characters.

Based on reported facts, the aim of the research was to study the reproductive capacity of swine according to breeds used for producing hybrids (Ladosi, 2010; Polen, 2007).

Scientific postulates submitted were focused on testing productive qualities of swine by using boars on Danish selection Duroc and Pietrain and determining growth and development performances on young biracial swine during lactation period until the age of 2 months.

MATERIALS AND METHODS

The research was made at P.E "Moldsuinhibrid". The subject of investigations was the swine for breeding Yorkshire, Landrace- maternal form and Duroc, Pietrain-paternal form.

Table 1. The scheme of obtaining young biracial swine of reproduction

Lot	**Parental forms**				**Young selected swine**	
	Maternal	Sow no.	Paternal	Boar no.	Young boars	Sows
I- witness	Landrace	14	Landrace	5	6	11
II-experim.	Yorskhire	11	Duroc	4	6	16
III-experim.	Landrace	14	Pietrain	4	5	23

In order to obtain young Yorkshire x Duroc x Pietrain biracial swine there were selected 11 Yorskhire sows and 14 Landrace sows, which were tested according to their body mass and length at 2, 4, 6 months. The young swine was set to analogical conditions of sustenance and nutrition during the testing period. Before artificial seeding sows were assessed according to their own performances to determine the body mass, body length and fat depth using electronic scales, ribbon and ultrasonic equipment. For the insemination there were used 4 Duroc boars and 4 Pietrain boars. Experimental swine was appointed pursuant to evaluation marks in elite and I categories.

The productivity of swine was appreciated on calving according to prolificacy (number of living piglets), the weigh of a piglet and piglet lot at birth. At piglet weaning there could be determined the fertility of swine, based on the number of weaned piglets.

The average weigh of a piglet at birth was calculated by weighing individually each boar and sow using electronic scale.

The development of piglets in lactation and growth period was appreciated according to body mass and average daily gain, indexes being determined individually on each animal.

The young swine was selected for reproduction after 2 months (6 boars and 16 Yorskhire x Duroc sows and 5 boars and 23 Landrace x Pietrain boars) appreciated according to their own performances. The control lot (Landrace x Landrace) was represented by 6 boars and 11 sows.

The results obtained following the investigations were processed statistically by calculating parameters of variation series, arithmetic average (M), average error (m) and variation coefficient (Cv).

RESULTS AND DISCUSSIONS

Growth and fattening units of swine could perform and be competitive, only by practicing intensive system based on selection and hybridization technologies, feeding and modern exploitation. Using these technologies can assure continuous growth of carcass and meat quality by reducing feed consumption per kg and work force.

Table 2. Testing swine on body mass, kg

Age	Breed					
	Yorkshire			Landrace		
	$\overline{X} \pm S\overline{x}$	Limits	C_v	$\overline{X} \pm S\overline{x}$	Limits	C_v
2 months	18.55±0.21	17-19	3,71	18.00±0.30	15-20	6.16
4 months	47.09±0.83	42-50	5,81	44.86±1.44	29-52	12.05
6 months	81.36±2.09	71-95	8,52	79.50±2.78	51-85	13.07

The results of determining body mass presented in this table, show that together with increasing the age of sows, in different periods, the intensity of growth differs. During the growth period between 2 and 4 months, absolute gain was of 28.54 kg, while between 4 and 6 months, the body weigh on Yorkshire breed increased with 34.27 kg.

Such results were obtained also on Landrace breed.

The selection effect, but also hybridization depends on the quality of biologic material used for this aim, which must be valued according to their own performances until seeding. The results of these papers are presented in table 3.

Table 3. The appreciation of sows own performances on seeding

Breed						
Yorkshire				**Landrace**		
	$\bar{X} \pm S\bar{x}$	**Limits**	**C_v**	$\bar{X} \pm S\bar{x}$	**Limits**	**C_v**
Body mass, kg	119.46±3.18	104-132	8.82	104.93±3.21	80-133	11.44
Breed						
Yorkshire				**Landrace**		
Body length, cm	134.55±1.07	128-142	2.65	135.0±0.55	131-138	11.44
Fat depth, mm	14.18±0.35	13-16	8.23	13.64±0.33	12-16	8.91

The sows were appreciated according to their own results before seeding, determining their body mass, stem length and fat depth. Following the research, there was proved that between breeds, no significant differences based on body length and fat depth were signaled, but in what concerns body mass the differences were of 14.53 kg, which is considered significant. (B>0.95).

Sows proved a characteristic development for breeding animals, and fat depth complied within the limits of 12-16 mm, the average varying between 13-14 mm, which corresponds to the requirements of biologic material used for this experiment.

Table 4. Sows productivity

Lot	**Parental combinations**	**No.**	**Prolificacy, head**	**1 Weight piglet at birth, kg**	**Piglet weigh lot at birth, kg**
I – witness	L x L	10	11.50±0.54	1.37±0.04	15.24±0.57
II – witness	Y x D	4	11.25±1.37	1.32±0.08	14.27±0.99
III- witness	L x P	12	10.58±0.78	1.37±0.05	14.18±0.90

The data from this table prove that sows prolificacy on experimental lots vary within the limits from 10.58 to 11.50 on piglets, the difference between Landrace the combination of L x P was equal with 0.92 piglets, being insignificant. The results concerning the weight of a piglet at birth, prove that between lots there were no significant differences, varying between 1.32-1.37 kg. The lot weigh of piglets at birth is higher with 1.06 kg based on a prolificacy higher that 11 piglets.

The results analyzed lead to the conclusion that indexes used for the appreciation of swine productivity are more optimal so that young swine could be selected for reproduction from them.

Table 5. Sows fertility

Lot	**Parental combinations**	**Weaning**		**Piglet lot weight, kg**
		No. of piglets, head	**1 weight at birth, kg**	
I – witness	L x L	10.90±0.46	8.57±0.17	93.00±3.62
II – experim.	Y x D	10.50±0.96	8.02±0.40	84.25±8.80
III – experim.	L x P	10.00±0.67	8.37±0.60	81.67±6.84

The data form the table confirm the fact that the number of piglets at seeding is decreasing from 0.60 in control lot, until 0.75 in experimental lot II, but there could be mentioned that the fertility of sows is good enough in every lot.
The average weight of a piglet varies from 8.02 kg in lot II of piglets obtained by crossing Yorskhire and Duroc breeds, and 8.57 kg in control lot of piglets, the differences varying within the limits of 0.35-0.55 kg. Such differences were noticed on lot weigh of piglets at seeding.

Table 6. Piglets development during embryonic period

Lot	Parental combinations	Average weight of piglets at birth, kg					
		Boars			Sows		
		No	Mass	Limits	No	Mass	Limits
I – martor	L x L	6	1.42±0.02	1.30-1.50	18	1.39±0.02	1.3-1.5
II –experim.	Y x D	6	1.41±0.03	1.30-1.50	16	1.37±0.02	1.2-1.5
III–experim.	L x P	5	1.50±0.03	1.40-1.60	23	1.37±0.02	1.2-1.5

The embryonic development of piglets appreciated by the weigh of a piglet at birth, confirm that it varies from 1.3 until 1.5 kg on boars and sows, the average being higher in experimental lot III. Important differences between lots were not noticed, but there could be mentioned the fact that at birth piglets had a weigh that could be appreciated as being in accordance with optimal indexes of development.
During lactation period, the piglets must develop intensively, but such factors as swine genotype could influence this process.

Table 7. Development of piglets during lactation period

Lot	Parental combinations	Piglet weigh on weaning, kg					
		Boars			sows		
		No	Mass	Limits	No	Mass	Limits
I – witness	L x L	6	10.0±0.00	10.0-10.0	18	9.03±0.24	7.8-10.0
II – experim.	Y x D	6	8.30±0.33	7.10-8.90	16	7.32±0.85	7.10-8.9
III –experim.	L x P	5	8.66±0.21	8.30-9.00	23	8.38±0.50	2.3-10.8

The results presented in this table prove that on boars, important differences between lot were not noticed, these being insignificant and equal with 0.36 kg.
The best results on sows were obtained in control lot and experimental lot III. Differences between I and II lot were equal with 1.71 kg, III and II-1.06,I and III -0.65 kg. The limits in every lot were 2.2-2.5 kg.
In selection units, for the appreciation of development level of young swine, there is analyzed the average weigh of a piglet at 2 months, period which includes the duration of lactation and piglets growth. The results obtained in this period are presented in table 8

Table 8. Results of piglets growth until 2 months

Lot	Parental forms	Average weigh of a piglet at 2 months, kg					
		Boars			sows		
		No	Mass	Limits	No	Mass	Limits
I – witness	L x L	6	18.23±0.85	17-21	11	16.46±0.41	14-19
II –experim.	Y x D	6	17.83±1.14	16-21	16	17.31±0.85	14-26
III-experim.	L x P	5	19.80±0.97	17-23	23	18.39±0.59	15-25

In the growth period from birth until 2 months, the development of piglets was within optimal limits, but a smaller average weigh of a sow, was registered in control lot, equal with 16.46 kg, and one bigger in experimental lot III -18.39 kg, formed from biologic material obtained by crossing Landrace and Pietrain breeds (B>0.95), the results being insignificant. The limits were between 16 and 23 kg on boars and 25 kg on sows.

The results of growth and development of young swine during lactation and growth period until 2 months, prove that the number obtained could be selected from the necessary number of boars and sows for reproduction, because it correspond with the requirements submitted by the instruction of evaluation marks of swine, especially the aim presented during the research.

The ratio of meat in carcass at hybrids is influenced by the growth intensity of young swine during seeding, because the formation of muscular tissue happens more intensive during post embryonic period.

It must be mentioned that beginning with 2 months the weigh of sow fits within the normal limits.

The results of growth speed appreciation on young swine are presented in table 9.

Table 9. Growth speed on young swine

Lot	Parental combinations	Average daily gain,g							
		boars				sows			
		No	$\bar{X} \pm S\bar{x}$	Limits	C_v	No	$\bar{X} \pm S\bar{x}$	Limits	C_v
I – witness	L x L	6	282.0± 13.9	388-458	8.79	11	191.6± 7.79	148-221	13.48
II-experim.	Y x D	6	190.0± 11.7	153-220	15.08	16	186.1± 8.84	140-275	18.99
III-experim.	L x P	5	214.2± 11.8	180-253	12.4	23	204± 70	160-266	11.61

The results presented in this table prove that the average daily gain on boats in experimental lots varied from 190 g in lot II until 214 g in lot III and 186 at 204 g corresponding to differential swine being insignificant. This confirms the fact that the development standard of young swine from experimental lots is almost at the same level, but we can observe a tendency of increase in experimental lot III.

CONCLUSIONS

1. Yorkshire and Landrace sows, according to body weigh and length during the growth period from 2 months until seeding fits within the standard of elite class and I, and according to fat depth in elite class.
2. Their own performances and reproductive qualities of Pietrain and Duroc boars fit within the standard of this breeds according to the age of each animal.

3. The development of boars and sows during lactation period was more intensive in experimental lot III and the body weigh at seeding was 8.38 kg on swine and 8.66 kg on boars, while the body mass of young swine from experimental lot II was of 7.32 kg on swine and 8.30 kg on boars, the difference being significant and equal with 1.06 kg on boars and 0.36 on sows.
4. During the growth period from birth and until 2 months young swine developed within normal limits, but the body weight of swine was smaller than control lot equal with 16.46 kg, and swine from experimental lot III had a weigh of 18.39 kg, the difference being insignificant and equal with 1.93 kg (B>0.95). Such differences were noticed on boars between experimental lots.

REFERENCES

Rotaru I., 2013. Swine meat growth and production" Chisinau: Print-Caro, 243

Rotaru I., 2009. Problems and perspectives in development of swine growth in the Republic of Moldova, Proceedings IV Balcan Conference of animal Science, Trakia University, Stara Zagora, Bulgaria, 73-77.

Rotaru I. Ceban A., Eremia M., 2015. Swine growth system and hybridization". Chisinau, Print-Caro, 36

Ladosi I., 2010. Reproduction problems on young swine, Farm Journal no 2

Polen T., 2007. Successful hybrid swine characteristics, Farm Journal, no 4.

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PROPOLIS EXTRACT USE DIN INCUBATION TECHNOLOGY FOR HENS' EGGS TREATMENT

Elena SCRIPNIC, Nicolae EREMIA

State Agrarian University of Moldova, 44 Mircesti street, MD 2049 Chisinau, Republic of Moldova; 00 373 22 432380, email: e.scripnic@uasm.md, eremianicolai@rambler.ru

Corresponding author email: e.scripnic@uasm.md

Abstract

The eggs treatment process is one from which the result of incubation largely depends. In incubation for eggs disinfection are used different methods that affect the bacterial load. Choosing the most effective methods of eggs disinfection will depend on how used substances will influence on embryonic and postembryonic development of poultry. Currently the use of substances having disinfectant effect which would not have negative influence during embryonic and postembryonic development is one of the latest trends. As an alternative to chemical treatment methods it was proposed the use of propolis extract in hens' egg incubation. The aim of research was to determine the influence of propolis extract on hatching indices used in incubation of hens' eggs. At the end of the experience and data recording there was established that the maximum index of eggs hatchability was received as a result of using as a disinfectant the propolis extract in an amount of 2 ml/70 ml of water and 4 ml/70 ml water daily during the whole period of incubation. Hatchability was higher in first experimental group compared to the control group and second experimental group.

Key words: eggs, hens, incubation process, treatment.

INTRODUCTION

Achievements of science and best practices prove conclusively that one of the reserves of increase in hatchability, improving the quality of day-old chicks and their future viability and productivity is not only the continuous improvement of the conditions of eggs incubation, but the search for methods of stimulating the embryonic development.

It is found that a single treatment in critical periods of development in the embryonic or early postnatal ontogeny period influences the entire subsequent development program for the animal organism and, consequently, on their productivity (Бессарабов, 1983; Шакирева, 1997).

Several methods and disinfectants are available for hatching eggs disinfection in poultry. There are produced different antibacterial disinfectants that have an important role in poultry eggs hatching practice. There are used different methods of eggs treatment as: treatment by using formaldehyde; UV treatment; ozone therapy; nebulization with hydrogen peroxide and other. Substances for disinfection of hatching eggs are divided into: chemical; physical and biological. All the methods are more or less used because of their influence on egg shell bacteria. The most spread method of eggs disinfection is chemical, and the most popular disinfectant is formaldehyde. There is an actual tendency for avoiding formaldehyde because of its negative action on living beings as it is carcinogen.

Studies have shown that using biological methods in poultry eggs disinfection had a positive effect by increasing hatching indices. The disinfectant used, in itself, must fulfill different requirements: to have a broad spectrum (to be able to destroy a wide range of micro-organisms), to be active at low concentration, safe for human users as for eggs, without any corrosive action on metals, etc. As a natural disinfectant may be used propolis, as it has antibacterial properties. This product was used in different trails in eggs hutching. There was shown that propolis extract used in eggs hutching presented antibacterial and antifungical effect besides not being harmful to the embryo development

allowing high hatchability rates (Vilela et al., 2012; Shahein et al., 2014).

Propolis is a resinous mixture that honey bees collect from tree buds, sap flows, or other botanical sources. It is used as a sealant for unwanted open spaces in the hive. Propolis is used for small gaps. It is dark brown in color, but it can be found in green, red, black, and white hues, depending on the sources of resin found in the particular hive area.

Preliminary scientific studies show some types of propolis have in vitro antibacterial and antifungal activity with active constituent including flavonoids like galangin and hydroxycinnamic acids like caffeic acid.

Propolis and its ethanolic extract are usually used for treatment and prevention of different diseases. Propolis has antibacterial, antiviral, antifungal, anti-inflammatory, anesthetic and immunomodulating properties (Eremia, 2014; Majieneet al., 2004).

The main aim of this study was to determine the influence of propolis extract produced in our country in poultry eggs hatching on hutching indices.

MATERIALS AND METHODS

The present experiment was carried out in the Laboratory of Poultry and Eggs hutching, State Agrarian University of Moldova.

The propolis was collected from the central zone of Republic of Moldova. Brown propolis extract was produced beforehand and stored in a dark place at $+4^0$C.

To determine the influence of propolis extract eggs were collected from the parental flock of broiler chickens and were placed in incubation.

In the experiences were formed three groups: one control group and two experimental groups. Each group of eggs was placed in separated incubator. In each batch were placed in incubation of 120 eggs, before appreciating eggs quality parameters as: egg weight; index of eggs format; diameter of air chamber. To assess the quality indices 30 eggs were collected from each batch. Incubation was performed using identical regimens for chicken eggs hatching.

Because of propolis volatile properties it was placed in a container directly in the incubator. The propolis extract was added daily using 2 ml of extract diluted in 70 ml of distilled water for experimental group I and an amount of 4 ml diluted in 70 ml of distilled water in the second experimental group.

At the end of the incubation the hatching indices and the quality of the chickens were determined. Eggs with dead embryos were broken and age of embryonic death was determined.

All the results were processed and analyzed using Microsoft Excel program.

RESULTS AND DISCUSSIONS

For determination of the hatching performances the eggs quality indices were analyzed (Table 1).

Table 1. Hatching eggs quality indices

Group	Eggs format index (%)	Diameter of air chamber (mm)	Egg weight (g)		
			Nr. of weightings (days)		
	$\dot{X} \pm S\dot{x}$	$\dot{X} \pm S\dot{x}$	Before hatching	6	14
			$\dot{X} \pm S\dot{x}$		
Control	72.5 ± 0.5	17.7 ± 0.3	55.4 ± 0.6	53.5 ± 0.6	51.2 ± 0.6
Experimental I	72.5 ± 0.5	18.4 ± 0.4	54.9 ± 0.5	53.4 ± 0.6	51.2 ± 0.6
Experimental II	73.2 ± 0.3	18.1 ± 0.3	55.3 ± 0.6	53.5 ± 0.6	51.4 ± 0.5

Index format in groups ranged from 72.5% to 73.2%, the data showed that the index of format of hatching eggs of hens meet the requirements. Analyzing the diameter of the air chamber it can be concluded that it ranged from 17.7 to 18.4 cm, which proved that the eggs used in the experiment had shelf life no more than six days which meets the technology requirements. Another index is the egg weight that characterizes embryonic development and its evolution during the incubation period, knowing that significant changes of weight characterize the failure of incubation regime. In the experience hatching eggs weight had values that were within 54.9-55.3 g (Figure 1).

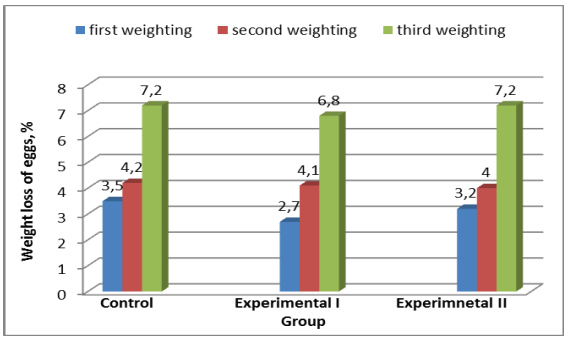

Figure 1. Weight loss of eggs during incubation, %

Analyzing weight loss shown in the diagram is noted that the total weight loss in different groups were different, registering 6.7% -7.7% values throughout the incubation period, maximum weight lost had the eggs in the control group but it should be mentioned that the total loss of weight in all groups were within the rules for this index (Table 2).

Table 2. Results of eggs hutching

Group	Total hatched eggs	Eggs fertility (%)	Eggs with dead embryos (%)		Dead chicks (%)	Hatchability of fertile eggs (%)	Hatchability of total eggs (%)
			6-14 days	15-20 days			
Control	120	95.8	6.0	3.5	3.5	86.9	83.3
Experimental I	120	92.5	3.6	-	2.7	93.7	86.7
Experimental II	120	96.7	5.1	1.7	4.3	88.8	85.8

The fertility of hatching eggs placed in the incubator ranged from 92.5% to 96.7%. Maxim eggs with dead embryos were observed from 6 to 14 days in the control group and accounted 6.0%, in other groups this index was low ranging from 3.6%-5.1%. It should be noted that the lowest mortality rate was 3.6% in first experimental group or 2.4% lower than in the control group. In the second experimental group, however this figure is lower compared to the control group, but higher compared with first experimental group.

Chicks hatching results showed that the maximum number of hatched chicks was obtained in first experimental group - 93.7% higher to the control group by 6.8% and 4.9% to the second experimental group (Figure 2).

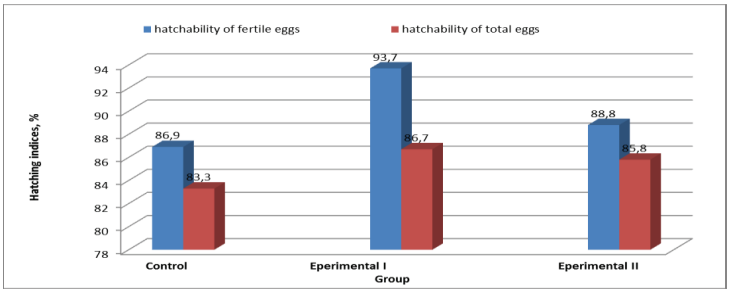

Figure 2. Hatching indices

At the days 15 to 20 of hatching, maximum percentage of eggs with dead embryos were observed in the control group (3.5%), while in experimental group II the index was lower (1.7%), in the experimental group I mortality was not observed. At hatching in all groups was recorded mortality rate that ranged from 2.7% to 4.3%,the lowest was 2.7% in the first experimental group (Figure 3).

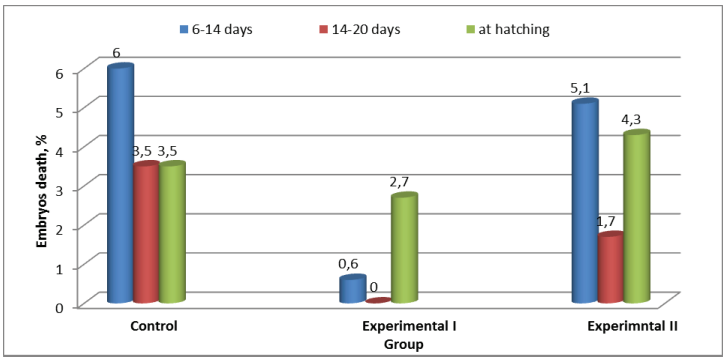

Figure 3. Embryos death during the hatching period

Another important indicator is the chicks' quality. Maximum number of checks of first quality was obtained in second experimental group -77.7% by 9.7% and 9.4% higher than in the other groups (Table 3).

Table 3.Chicks quality

Group	Total number of chickens	Quality, %		
		I	II	III
Control	100	68,0	30,0	2,0
Experimental I	104	68,3	26,9	4,8
Experimental II	103	77,7	22,3	-

CONCLUSIONS

The conducted experiments may explain the effect of propolis extract on mineral shell decontamination and increasing incubation indices, due to the ability of antimicrobial product used as a disinfectant.

There were recorded maximum indices of eggs, where was used as a disinfectant propolis extract in an amount of 2 ml/70 ml of water for the entire period of incubation, hatchability of fertile eggs was 93.7% or higher by 6.8% compared to the control group and by 4.9%, comparing to the same index in second experimental group. As well the embryos death during hatching period was lower in the experimental groups.

REFERENCES

Eremia N., 2014. Apicultura. Ed. Cargo – Print, Chisinau.

Majiene D., Trumbeckaite S., Grunoviene D., Ivanuaskas L., Gendroilis A., 2004. Investigation of chemical composition of propolis extract. Medicina (Kaunas), 40(8): 771-774.

Shahein E.H.A., Eman K. Seedeek, 2014. Role of spraying hatching eggs with natural disinfectants on hatching characteristics and eggshell bacterial counts. Egypt Poultry Science, 34(1):213-230.

Viela C.O.,Vargus G.D., Fisher S., Ladiera S., R.O. de Faria., Nunes C. F., M. de Lima, Huber S.O., Lur P., Osorio L.O., Anciuti M.A., 2012. Propolis: a natural product as an alternative for disinfection of embryonated eggs for incubation. Arg. Inst. Biol. Sao Paulo, 79(2): 161-167.

Бессарабов Б.Ф., 1983. Ветеринарно-санитарные мероприятия по профилактике болезней птиц. Россельхозиздат, Москва.

Шакирева Г.И., 1997. Эффективность применения янтарной кислоты и других стимуляторов роста для предпосевной обработки семян. Янтарная кислота в медицине, пищевой промышленности, сельском хозяйстве.Пущино,11(1):256-259.

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STUDY REGARDING RABBIT WELFARE INTENSIVELY BRED

Nicolae STANCIU, Elena POPESCU-MICLOȘANU, Minodora TUDORACHE

University of Agronomic Sciences and Veterinary Medicine from Bucharest, Blvd. Mărăști, no. 59, sector 1, 011464, Bucharest, Romania, Phone: +4021.318.25.64, Fax: + 4021.318.25.67

Corresponding author e-mail: stanciu_nicu77@yahoo.com

Abstract

This paper aims to disseminate the wider application of knowledge on the welfare of rabbits, considering the majority of the factors that contribute to it. Its development was based on scientific literature, the European legislation on the protection and welfare of animals and the Code of good practice for growing rabbits. To ensure the welfare of animals a number of factors that contribute to the comfort of their condition should be considered, such as nutrition and optimum microclimate factors, selection of parent lines for calm temperament and maternal skills, specific accommodation conditions operating system used, appropriate sanitation, eliminating stress caused by improper handling, noise etc. According to these data, harboring rabbits should be made in cages of welded wire mesh whose floor has to be flat, not thinner than 2.032 mm, mesh size 19x19 mm in case of square mesh and 75x12,5 mm, if rectangular. The floor made of mesh wire with too thin mesh and too big or rough welds can cause feet problems. It is recommended that for a normal position of the animal in the cage so that they can keep their ears erect and make install, the height of the cage should be 45 cm and the temperature of the shelter between 10-20°C depending on the category of animal. Knowing and applying of all welfare conditions in addition to eliminating the discomfort and stress also influences the quantity and quality of rabbit products. Given the literature is still quite poor and fragmented, it is necessary to continue research into breeding and exploitation of rabbits, their handling during transport and slaughter, and improving their welfare standards.

Key words: *Rabbit; welfare; intensive breeding, Code of good practice.*

INTRODUCTION

House rabbit can meet the basic requirements of human existence, namely: food through its flesh and clothing through the fur and wool by which it provides. It also produces agricultural fertilizers and it is particularly useful as laboratory animal and in scientific experiments. He is also the subject of sporting passions in exhibitions - contest (Georgeoni et al., 1984). If in 1975 the world production of rabbit meat was estimated at about 120,000 tones, in 1992 it was about 300,000 tones. In 1994 world production of rabbit meat stood at approx. 1.597 million tons, out of which 637,000 t was achieved in the traditional system, 528,000 t in intermediate or semi-intensive system and 435,000 t was obtained in intensive or commercial systems (Lebas and Colin, 1994). In 2012 world production of rabbit meat was estimated at 1.83 million tons, increasing by 14.2% the last 5 years (FAOSTAT, 2014). In Romania, in 1990, rabbit meat production was 10,625 t, then it decreased rapidly, so in 2000 it was by 71.77% lower, and in 2007 with 97.5% lower compared with production in 1990 (after FAO data, 2009). Until year 1999 our country exported rabbit meat mainly to Italy, France, Germany, and starting 2003 the market demand for this product was provided mainly by imports.

The rabbit, due to its extraordinary capacity of production and reproduction, can make an important contribution in the fight for increased of animal protein production worldwide (Baselga and Garcia, 2002). Research regarding rabbits welfare are still fragmented and were the preoccupation of a small number of researchers, who often did not take into account the commercial production aspects involved. In addition, many existing data, unlike other species of domestic animals, are obtained through observations on wild rabbit and no on the domestic ones.

MATERIALS AND METHOD

In preparing the paper, a series of bibliographical material consisting of books and journals, specific research papers and specialized websites have been studied.

RESULTS AND DISCUSSIONS

The most important aspects which compete in ensuring the welfare of intensively bred rabbits are the ones regarding choice of the breed, meeting the requirements of their housing and nutrition.

1. Breeds of rabbits raised in intensive system

In the world so far are cataloged about 200 existing breeds of rabbits (Popescu Micloşanu, 2011). They are classified as follows:

Depending on body weight in adulthood:

- Large breeds (over 5 kg: Flemish Giant, German Giant, French Lop, White or Termond Giant, German Checkered Giant);
- Medium Breeds (3-5 kg: Chinchilla, Viennese Blue, Silver French, Californian, New Zealand);
- Small breeds (2-3 kg: English Spot, Havana, Dutch);
- Dwarf breeds (less than 2 kg: Hermelin, Miniature Lop).

Depending on hair length:

- short-haired breeds of rabbits (Rex);
- middle-haired breeds of rabbits (most breeds);
- breeds of rabbits with long hair (Angora).

According to the main production there are breeds for meat, fur and mixed.

For meat production especially large and medium breeds are raised. Large breeds have the advantage of reaching in the adult stage weights exceeding 5 kg. Medium breeds are more precocious, they have high growth rate during youth, and a very good feed valorization.

In general, at birth, rabbits weigh between 50 and 70 g. At a month old they already have between 400 and 700 g. In intensive raising rabbits weaning occurs at the age of 28-35 days. Depending on breed, rabbit slaughter for meat production is made at 3-4 months of age (Iancu, www.crescătoriede iepuri.ro).

2. Requirements for rabbits housing

Rabbit cage size in industrial system raising should ensure the minimum area shown in Table 1 (after Code of Practice for the Welfare of Animals, Australia, 2003).

Table 1. The minimum surface required for growing rabbits (Code of Practice for the Welfare of Animals, Australia, 2003)

Breeding in cages	Minimum surface
Female rabbit with nestling up to 5 weeks	0.56 sqm total surface
Female rabbit with nestling up to 8 weeks	0.74 sqm total surface
Rabbits between 5 – 12 weeks	0.07 sqm per capita
Rabbits above 12 weeks	0.18 sqm per capita
Female rabbits and male rabbits for reproduction	0.56 sqm per capita

In intensive raising rabbits are maintained in closed housing, in welded wire mesh cages whose floor must be smooth and appropriate mesh size in accordance with animals size (according to animal protection law).

In case of square wire meshes, their side shall not exceed 19 x 19 mm and for rectangular mesh size shall not exceed 75 x 12.5 mm.

Cages for rabbits older than 12 weeks will have a height of at least 45 cm, or one that allows rabbits to stand in upright position, with erect ears.

The diameter of the wire used to manufacture the net shall be not less than 2,032 mm. The wire mesh will be smooth; all protrusions resulting from the manufacturing process are removed, so as not to cause feet problems. When it is necessary, because of the weight, that rabbits to be raised on continuous floor, a clean litter will be ensured.

Farrowing nest must have a minimum length of 30 cm and a minimum floor surface of 0.08 m^2.

Drop drinkers have to be located approximately 25 cm from the floor of the cage, so rabbits can have easy access to them.

In order to prevent uncontrolled growth of the incisors, which can lead to self-infliction, pieces of wood for stimulation of gnawing will be made available.

The recommended temperature in intensive raising is between 14-20ºC depending on the category of animals. Thus, it is recommended to have 18-20ºC in maternity and for youth raising 14-18ºC.

The limits of variation of temperature should be between 10-25ºC, otherwise reproduction is affected (Popescu-Micloşanu, 1998).

Verga (2007) states that the ideal temperature in rabbits raising shelter is 16-21°C for female and

12 -16 °C for males rabbits and relative air humidity should be 60%.
Rabbits are very sensitive to high temperatures. Thermal stress leads to reduced feed consumption (Morrow-Tesch et al., 1994), increased susceptibility to disease (Kamwanja et al., 1994) or affects the efficiency of breeding activity (Marrai and Rashwan, 2004).
In intensive - industrial system, rabbits are raised most frequently in cages batteries installed on one level and in tiered batteries partially or completely overlapped.
The batteries on one level (horizontal or flat-deck) are most suitable for breeding animals. They are used for fattening as well, especially in colony, in bigger cages. They provide great comfort for the animals and for the caregiver for the supervision and handling of animals, it does not require complicated ventilation and they have a higher durability. Disadvantages can be mentioned: low density of animals per built unit area, which raise the investment, although cages have quite low price.
Vertical batteries (all overlapping or compact) have cages placed on 2-4 levels. It allows the installation of more cages within a given area, low investment cost per animal, but it requires more complicated ventilation of the housing because of the high density. Light distribution is uneven for breeding female rabbits; access to cages is difficult on some levels. As a result, the rabbits from lower or higher levels are not as well cared for, watched, like the rabbits on more accessible levels, handling of animals is more difficult, respiratory diseases problems and cleanliness in the farm are increasing, a faster wear of materials is encountered.
Using floored batteries is less used worldwide (Popescu Micloşanu, 2011).
Because rabbits spend much time on the cage floor, this is a very important factor in the welfare of rabbits (Szendro and Luzi, 2006; Verga et al., 2006). Drescher (1992) mentions that rabbits spend less time on the floor of wire mesh compared to other types of floor. On the other hand, Trocino A. et al. (2004) demonstrated in an experiment that no significant differences can be found in terms of live weight between rabbits reared on wire mesh floors and on galvanized steel bars floor. A preference test showed that rabbit's bunnies prefer rubber floor, but once they age they prefer plastic floor (Matics et al., 2007). On the other hand, the plastic floor, due to feces and high humidity can lead to increased risk of coccidiosis (Princz et al., 2009).
In an experiment conducted in weeks of growth 4-11 on a group of 24 white New Zealand rabbits breed, Abdelfattah et al. (2010) found that the use of plastic or rubber affects the drinking and agonistic behavior without affecting other behaviors or growth performance in the last three weeks of the experiment, but it also reduces the incidence of eyes and ears injuries of rabbits and it reduces chronic stress measured by total and differential white blood cell number in rabbits.
The authors concluded that the use of plastic or rubber material as cage floor has advantages in terms of animal welfare.

3. Contention and proper handling of rabbits

Contention and handling of rabbits is done by grasping the elastic range skin from the neck with one hand and with the other supports hindquarters, to avoid sudden movements that can cause injuries. It is not recommended to content rabbit by the ears, which is very painful and may cause ear problems.
One of the key success factors in the intensive - industrial growth is the rational nutrition. This should be done on the basis of complete mixed feed and watering should be done at discretion, through automated systems.

4. Features regarding feeding rabbits reared in intensive system

Ration structure should allow supplying the required quantity and quality of nutrients for maintenance and production of various types of rabbits.
Physiological nutritional requirements are not sufficiently studied and there are large differences between different authors regarding the recommendations on the features that combined fodders should have (Stoica, 2001).
Lebas (quoted by Frăţilă et al., 1985) makes the recommendations on feed requirements for rabbits from Table 2.

Table 2. The minimum requirements for rabbits feed (Lebas, quoted by Frăţilă et al., 1985)

Specification	MU	Youth 4-12 weeks	Lactating females	Pregnant and reproducing females	Unique fodder
Metabolizable energy	Kcal/kg	2400	2600	2600	2400
Crude protein	%	15	18	15	17
Lysine	%	0.60	0.75	-	0.70
Methionine + cysteine	%	0.50	0.60	-	0.60
Crude fiber	%	14	12	14	14
Fats	%	3	5	3	3
Calcium	%	0.50	1.10	0.80	1.10
Phosphorus	%	0.30	0.80	0.50	0.80
Vitamin A	UI/100 g	600	1200	1200	1000
Vitamin D	UI/ 100 g	35	35	35	35
Vitamin E	ppm	50	50	50	50

In the intensive growth system feed technology can use in a farm one, two, three or four fodder recipes (Popescu - Micloşanu, 1998).

Eating with fodder produced after a single recipe (with unique fodder) uses the same recipe as for maternity and for young stock for breeding and fattening.

This system is practiced in order to avoid confusion that can cause accidents in case of reversal of the feed administration on the other categories of animals. It is recommended for small farms with less than 200 breeding females.

Two types of forage diet generally use a recipe for breeding females (type lactating female rabbits) and one for the other categories (young raising). It is recommended for farms with more than 300 female rabbits.

Three types of fodder diet use a broiler starter feed, fodder for broiler growth and fodder for adult animals.

In large farms with over 1.000-1500 breeding females 4 types of fodder can be used (young growing, female lactating rabbits, pregnant female rabbits, male and no pregnant females).

Front feeding line for broilers is 15 cm to 10 animals or 7-8 cm length of gutter when nourisher is used on both sides.

Given the industrial growth of rabbits, granulation of combined feed is a prerequisite because the rabbit presents an increased susceptibility to respiratory airways for powders, by granulation waste is removed and homogeneity of the mixture is stored. It is recommended that the diameter of the grain is 3-5 mm, and the maximum length of 10 mm.

In libitum feeding an adult rabbit consumes 4-5 g combined fodder in about 30 rounds per 24 hours. Eating is more frequently at night rather than day (Stoica, 2001).

Food management considers food ad libitum in feeding of females with nestlings, broiler rabbits and young replacement animals up to 8 weeks. Female rabbits with nestlings in intensive breeding, consume 350-380 g/mixed feed /day and broiler rabbits 110-130 g/day. Other categories have restricted feeding. Thus, to maintain the condition of breeding, males in service receive 120-140 g/day of fodder, females without nestlings up to 120 g/day, youth replacement animals after 8-12 weeks of age maximum 140-150 g/day, pregnant female rabbits maximum 140 -150 g/day.

CONCLUSIONS

Raising rabbits in industrial system requires a unified technological system that ensures favorable conditions for expressing their productive potential. The aim is to obtain high yields of meat (number of products per female, per sqm. of shelter, per value of the investment) and good economic efficiency relative to total expenditure (Stoica, 2003).

In the intensive rearing of rabbits especially medium breeds and hybrids are recommended, which have the advantage of having a high growth rate and very good food recovery rate.

The minimum surface on which lactating female rabbits with nestlings are maintained should be approximately 0.56 m^2 at the age of 5 weeks. Between 5-12 weeks of age a surface of 0.07 m^2 per rabbit should be provided, and after the age of 12 weeks 0.18 m^2 per rabbit.

The batteries on one level (horizontal or flat-deck) are recommended especially for breeding animals. Compared to completely floored batteries, they provide greater comfort for the supervision and handling of animals, it does not require complicated ventilation and their durability is large. Their most important disadvantage is the low density of animals per built unit area, which raises investment unit.

Rabbit feeding must ensure the animal requirements on its quality and quantity, for

intensive raising appropriate granulated mixed fodder is recommended.

Since the literature on the welfare of domestic rabbits is still quite poor and fragmented, it is necessary to continue research into breeding and exploitation of rabbits, their handling during transport and slaughter, as well as improving their welfare standards.

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REFERENCES

Abdelfattah E., Karousa M., Mahmoud E., El-Laithy S., E-Gendi G., Eissa N., 2010. Effect of cage floor type on behavior and performance of growing rabbits, J. Vet. Adv. 2013, 3 (2) : 34 – 42.

Australian Animal Welfare Standards and Guidelines (Model Codes of Practice), 2014. http://www.agriculture.gov.au/animal/welfare/standards-guidelines

Bura M., 2008. Cerinţele de bunăstare a iepurilor de casă, Rev. Ferma nr. 3(58).

Colin M., Lebas F., 1994. Production et consommation de viande la lapin dans le monde. Une tentative de synthèse. 6èmes Journ. Rech. Cunicole en France. La Rochelle, 6-7 décembre 1994, 449-465.

Drescher B., 1992. Housing of rabbits with respect to animal welfare, J. Appl. Rabbit. Res. 15, 678 – 683.

Fayez M.M., Rashwan A.A., 2003. Rabbits behavior under modern commercial production conditions – a review, Arch. Tierz., Dummerstorf 46, 4, 357-376.

Frăţilă N. et al., 1985. Creşterea industrială a iepurilor, Ed. Ceres, Bucureşti.

Garcia M.L., Baselga M., 2002. Genetic response to selection for reproductive performance in a maternal line of rabbits, Departamento de Ciencia Animal, Universidad Politecnica de Valencia, Valencia, Spain, World Rabbits Science, vol. 10 (2), 71-76.

Georgoni Al. et al., 1984. Iepurele de casă - creştere şi valorificare. Editura RECOOP Bucureşti.

Matics Z., Nagy I., Birone Nemeth E., Radani I., Gerencser Z., Gyovai P., Szendro Z., 2010. Examination of free choice of rabbits bucks among different cage-cage floors, Proc. 19th Hungarian Conference on Rabbit production, Kaposvar, Hungary, 83-87.

Morrow-Tesch J., McGlone J.J., Salak-Johnson J.L., 1994. Heat and social stress effects on pig immune measures, Journal of Animal Science, 72, 2599-2609.

Popescu – Micloşanu, Elena, 1998. Creşterea iepurilor şi animalelor de blană, Ed. Tehnică Agricolă, Bucureşti, ISBN 973-9305-01-6, 356 pag.

Popescu-Micloşanu Elena, Minodora Tudorache, 2011. Cunicultură, animale de blană şi vânat, Lucrări practice, Ed. a III-a, AMD - USAMV Bucureşti.

Princz Z., Dalle Zotte A., Metzger Sz., Radnai I., Biro-Nemeth E., Otrova Z., Szendro Z., 2009. Response of fattening rabbits reared under different housing conditions. Live performance and health status. Livestock Science, 121, 86-91.

Stoica I., 2001. Bazele nutriţiei şi alimentaţiei animalelor, Ed. Coral Sanivet, Bucureşti.

Szendro Z., Luzi F., 2006. Group size and stocking density. Recent Advances in Rabbits, Rabbit Science, ILVO, 121-127.

Trocino A., Xiccato G., Queaque P., Sartori A., 2004. Group housing of growing rabbits: effect of stocking density and cage cage floor on performance, welfare and meat quality, World Rabbit Sci., 13, 138-139.

Verga M, Luzi F., Carenzi C., 2007. Effects of husbandry and management systems on physiology and bahaviour of farmed and laboratory rabbits. Hormones and Behaviour, 52, 122-129.

Verga M., Luzi F., Szendro Z., 2006. Behavior of growing rabbits. Recent Advances in Rabbits in Rabbit Science, ILVO, 91-97.

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THE EFFECT OF CONCENTRATIONS OF LIME JUICE (*Citrus aurantifolia*) TOWARD ACCEPTABILITY AND STORAGE LIFE OF CULLED LAYER HENS MEAT

Kusmajadi Suradi, Eka Wulandari, Indah Permatasari

Faculty of Animal Husbandry, Padjadjaran University, Bandung Indonesia

Corresponding author email: kusmajadi@gmail.com

Abstract

Acids, essential oils, saponins and flavonoids compounds in lime juice is expected to improve the storage life of culled layer hens meat. The research's aim is to obtain a correct concentration of lime juice as marinade of culled layer hens meat that can extend storage life with an acceptable acidity. Research carried out experiments in the laboratory using a completely randomized design with 5 treatments. It were 4 treatments of concentration of lime juice as a marinade of the meat (10, 20, 30, and 40%), and 1 treatment without marinade of lime juice. Each treatment had 5 times replication, so there were 20 treatments. The measured variables are early decay, total bacteria, pH, and acceptability of culled layer hens meat which had various treatments of lime juice concentration and without the treatment of lime juice. The results showed that concentrations of lime juice significantly affect extended early decay and total bacteria, but not significantly affect on pH of culled layer hens meat. Increasing concentrations of lime juice is followed by an increasing in pH, increasing in the storage life and decreasing the amount of bacteria.The meat accidity up to 30% lime juice (Citrus aurantifolia) marinade can still be accepted by the panelists.

Key words*: chicken, meat, lime juice, acidity, storage.*

INTRODUCTION

Culled layer hens meat has loamy texture and because of that it less prefered by costumers. Nevertheless culled layer hens meat still has its potential as food because the meat has high nutrition, but the meat also a good media for microorganism to grow which make the meat decay.

Meat decay can be delayed by natural preservative such as lime juice. It is because lime juice has big proportion of organic acid (ascorbic acid and citric acid). Beside it, lime juice contain saponin compound and flavonoid such as hesperidin, tangeretin, naringin, eriocitrin (Dewi Maharani, 2009). Lime juice contain flavonoid compound which has function as bacteriside and bacteriostatic. This compound can obstruct metabolism process on bacteria which can also obstruct bacteria growth (Cowan, 1999).

Birk et al. (2010) said that marinated meat with organic acid such as acetate acid, citric acid, tartaric acid, lactic acid or malic acid can decrease pH of the meat which can also decrease growth of bacteria *Campylobacter jejuni* for 25 days storage at 4°C. Aritonang and Mihrani (2008) reported that marinated local chicken in acetate acid for 15 minutes with concentration until 12% can decrease pH and bacteria number and extend meat storage.

According to the research of Hantoro (2012) that organic acid in lime juice such as citric acid, malic acid, lactic acid and small amount of tartaric acid with low concentration is able to penetrate the cell wall of salmonella bacteria. It is the same with the research of Razak et al. (2013), that lime juice had an obstruct effect toward the growth of bacteria *Staphylococcus aureus.*

The effectivenes of lime juice as preservative affected by the level of solution concentration as marinade because it affects amount of acid component which is diffused inside the meat and in the end will affect durability, pH and total amount of bacteria in culled layer hen's meat, but high concentration of lime juice will decrease acceptability. Therefore, the aim of this research is toget a perfect lime juice concentration which can extend the storage life and acceptable by panelist.

MATERIALS AND METHODS

This research used 20 culled layer hens strain Isa brown age 24 month, and 40 kg limes *Citrus aurantium, subspes. Aurantifolia, var.fusca*. To make solution concentration of lime juice, the lime should be washed and peeled and squeezed until 16.000 ml (assumed that 1 kg lime makes ± 400 ml lime juice) (Geugeut, 2010). The result of lime juice solution for various concentration treatment listed on Table 3. Then carcass of culled layer hen soaked in each lime juice concentration.

Table 1. Manufacture of various concentration of lime juice solution

Concentration (%)	Lime Juice (ml)	Aquades (ml)
Without Lime Juice	0	40000
10	400	3600
20	800	3200
30	1200	2800
40	1600	2400

The research was done by experiment with Completely Randomized Design with 5 treatments there are soaking without lime juice (P1), soaking with 10% lime juice concentration (P2), soaking with 20% lime juice concentration (P3), soaking with 30% lime juice concentration (P4) and soaking with 40% lime juice concentration (P4), each treatment were soaked for 30 minutes. Each treatments repeated 4 times.

The measured variables are pH, total early decay of bacteria, and acceptibility toward meat acid level.

RESULTS AND DISCUSSIONS

The effect of concentration of lime juice toward durable power and acceptability of culled layer hen meat acid is listed in Table 2.

Table 2. Result of statistical test of the treatment effect toward acceptability and storage life of culled layer hens meat

Variable	Lime Juice (%)				
	0	10	20	30	40
Early Decay (minute)	678 (a)	746 (b)	933 (c)	1001 (d)	1098 (e)
Total Count ($x10^6$ cfu/g)	87.32 (a)	43.85 (b)	24.38 (c)	10.68 (d)	2.75 (e)
Acidity (pH)	5.63 (a)	5.46 (a)	5.29 (a)	5.02 (a)	4.91 (a)
Acceptability (acid/no acid):					
Taste	No Acid	No Acid	No Acid	No Acid	Acid
Smell	No Acid	No Acid	No Acid	No Acid	Acid
Flavor	No Acid	No Acid	No Acid	No Acid	Acid

Description: Value which is followed by the same letter to the line show no significant effect

Data on Tabel 2 showed that increasing concentration of lime juice will be followed by increasing of early decay and decreasing of amount of each bacteria significantly different ($P<0.05$). This is because increasing of concentration of lime juice followed by increasing of meat acidity so that microorganism growth inhibited, specially not acid resistant microorganism. This is in line with opinion of Buckle et al. (2009) that only small microorganism founded and can damage food ingredient which is pickled, beside that lime juice contain flavonoid compound which has function as anti bacteria and has bacteriostatic character. This compound inhibit metabolism process on bacteria and caused the growth of bacteria inhibited (Cowan, 1999). The more increasing of lime juice concentration which is used in marinating, the more flavonoid content in lime juice which cause inhibiting bacteria activity, so that storage life of culled layer hen meat can be extended.

Inhibited mecanism of microbe growth by antimicrobe compound such as (1) cell wall devastation, which caused lisis or inhibit forming the cell wall growth, (2) change permeability sitoplasm membran which caused nutrition leaked inside the cell, (3) protein denaturation, (4) devastation of metabolism system inside the cell with inhibiting intracelullar enzyme process (Pelczar et al., 2005).

Soaking treatment of culled layer hen meat on concentration 10%, 20%, 30%, 40% and soaking without lime juice (0%) don't give significant effect to pH of culled layer hen meat. This is because adding lime juice as meat

marinade from concentration 0% untill 40% produce pH between 5,63 – 4,91. This showed that pH of culled layer hen meat is in the same acid group, which include not acid until medium acid ingredient food. According to Buckle et al. (2009) that food ingredient between pH 5.63 – 4.91 grouped into not acid until medium acid food ingredients. Decreasing of pH not only caused by lime juice soaking but also the stop of blood circulation and oxygen supply after the animal being slaughtered which is caused glycolysis, a breakage of glycogen into lactic acid which make decreasing of pH of culled layer hen meat.

Lowest meat acidity (pH 4.91), lowest amount bacteria (2,75 x 10^6 CFU/g) and longest early decay (18 hours 18 minutes) is on treatment concentration of 40% lime juice but for acceptability (taste, smell and flavor) on that concentration, panelist had taste the acidity on the meat. This is because of an increasing of lime juice concentration will be followed by increasing of acidity of the culled layer hen meat, and it showed in meat pH test which is showed decreasing of pH with increasing of lime juice concentration. This showed that in acceptability, optimum limit of using lime juice as mariate of culled layer hen meat is on concentration 30%.

CONCLUSIONS

1. Concentration of lime juice as marinade of culled layer hen meat affect early decay and total amount of bacteria, but do not affect pH.
2. Increasing concentrations of lime juice is followed by an increasing in pH, increasing in the storage life and decreasing the amount of bacteria.The meat accidity up to 30% lime juice (*Citrus aurantifolia*) marinade can still be accepted by the panelists.

REFERENCES

Abdul Razak, Azis Djamal, Gusti Revilla. 2013. Uji Daya Hambat Air Perasan Buah Jeruk Nipis (Citrus aurantifolia s.) Terhadap Pertumbuhan Bakteri Staphylococcus aureus Secara In Vitro. Jurnal Kesehatan Andalas 2(1) : 5-8

Agustinus Hantoro, 2012. Efektivitas Jeruk Nipis Dalam Menurunkan Bakteri Salmonella dan Escherichia coli Pada Dada Karkas Ayam Broiler. IJAS. 2(3) : 91-94

Aritonang S.N., Mihrani, 2008. Pengaruh pencucian dengan larutan asam asetat terhadap nilai pH, kadar protein, jumlah koloni bakteri dan daya simpan daging ayam kampung pada penyimpanan suhu ruang. J. Agrisistem. 4(1) : 19 – 25.

Birk T., A.C., Grondlund, B.B. Christensen, S. Knochel, K. Lohse, M. Rosenquist, 2010. Effect of organic acids and marination ingredients on the survival of *Campylobacter jejuni* on meat. J. Food Protect. 73(2): 258– 265.

Buckle K.A, R.A. Edwards, G.H. Fleet, M.Wotton, 2009. Ilmu Pangan. Diterjemahkan oleh Hari Purnomo dan Adiono. Universitas Indonesia Press. Jakarta. 42, 133, 249-252.

Cowan M.M., 1999. Plant Products as Antimicrobial Agents. Clinical Microbiology Reviews, 12

Dewi Maharani, 2009. Potensi Jeruk Nipis (Citrus aurantifolia) Untuk Pencegahan dan Pengobatan Infeksi Bakteri Pada Lele Dumbo (Clariassp). Skripsi. Fakultas Perikanan, Ilmu Kelautan. Institut Pertanian Bogor.

Geugeut Isfany Haq, Anna Permanasari, Hayat Sholihin, 2010. Efektifitas Penggunaan Sari Buah Jeruk Nipis Terhadap Ketahanan Nasi. Jurnal Sains dan Teknologi Kimia. 1(1) : 44-58

Pelczar M.J., E.C.S. Chan, 2005. Dasar - Dasar Mikrobiologi 2. Jakarta, UI Press, 893

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STUDY ABOUT CHICKEN CARCASS PROTEIN CONTENT

Minodora TUDORACHE[1], Ioan CUSTURA[1], Ilie VAN[1], Maria Antoaneta POPA[2]

[1]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, Email: minodoratudorache@yahoo.com
[2]APIA Covasna, Sfântu Gheorghe, Str. Lt. Păiş David, no. 7, cod 520077.
Phone/Fax 0267-311.075 0267-311.148 0267-312.255, Email: apia.covasna@apia.org.ro.

Corresponding author email: minodoratudorache@yahoo.com

Abstract

Meat quality and control of meat quality in poultry production are requiring a good management through whole production chain aiming both performances improvement with the raise of profitability and production of products at required quality standard.
This study was performed with the aim to observe a poultry meat quality index compulsory inside the European Union because of the free food products trade inside the Union.
Experiments were performed inside Avicola Crevedia during two years and with two industrial chicken hybrids (ROSS 308 and COBB 500) with the aim of finding the influence of both hybrid and production season on total chicken meat protein content.
During firs year of experiment total protein content of ROSS 308 hybrids had values between 17.4272 g (season 1) and 17.4688 g (season 2) with difference non-significant statistically. COBB 500 hybrids had total chicken meat protein content between 18.3196 g and 18.3960 g (NS). Differences between average values of the two tested hybrids were highly significant statistically both in first and second season (respectively $t=4.9332^{***}$ and $t=5.2795^{***}$).
Results were similar during the second year of the experiment with carcass protein content values between 17.4472 g and 17.4032 g in ROSS 308 hybrids and between 18.5392 g and between 18.6212 g in COB 500 hybrids (NS). Like in the previous year, differences between the two hybrids for the analyzed parameter were highly significant statistically which allows us to affirm the superiority of COBB 500 hybrid.

Key words: *protein, carcasses, broiler.*

INTRODUCTION

Quality is paramount in promoting the product „hen broiler meat" (currently known as „broiler meat") on the market.
As nowadays concurrence is fierce in any business quality is both the main assurance factor of business sustainability and the main management tool used by the enterprises.
Poultry meat quality might be evaluated base on several criteria: anatomical-histological, zoo-technical, economical, commercial, shelf life and fitness for human consumption (Georgescu, and col., 2000).
Many authors have described meat quality as a combination of objective and measurable characters (features, indices and criteria) generally classifiable in four components: flavors, nutritional characters, technological characters and hygienic characters.

MATERIALS AND METHODS

Study was performing in a poultry production farm (Avicola Crevedia) during two years, using two genetic types of industrial broiler hybrids (industrial hybrids ROSS 308 and COBB 500).
Influence of genetic types and season on carcass protein content was evaluated.
Groups of 100 birds (50-50) were formed and raised in uniform conditions and in extended captivity, in upgraded houses, by sticking to standard technologies of both hybrids; feeding and watering were performed „ad libitum" and slaughtering was performed at 42 days of age.
Experimental groups were raised in two seasons (season 1 – warm: April-September; season 2 – cold: October-March), and the experimental schedule was repeated next year.
Poultry were feed with combined feeds processed according to nutritional requirements

of studied hybrids by three research phases: starter 1-10 days, production 11-25 days and finishing 26-42 days (table 1).

Table 1. Mixed feed used in experiment

SPECIFICATION	STARTER (1-10 DAYS)	PRODUCTION (11-25 DAYS)	FINISHING (26-42 DAYS)
TOTAL	100.00	100.00	100.00
ME, KCAL/KG	3055	3178	3228
CP%	24.00	22.00	20.00
CF%	5.82	7.66	7.89
CE%	3.63	3.75	3.90
CA%	1.00	0.90	0.85
AVAILABLE P %	0.50	0.45	0.42
NA%	0.16	0.16	0.16
CL%	0.22	0.22	0.22
LYSINE%	1.44	1.25	1.05
DIGESTIBLE LYSINE POULTRY%	1.32	1.13	0.94
METHIONINE%	0.96	0.85	0.71
DIGESTIBLE METHIONINE POULTRY %	0.93	0.83	0.69
METHIONINE+CYSTINE%	1.36	1.22	1.04
METHIONINE + CYSTINE POULTRY %	1.27	1.13	0.96
TREONINE%	0.93	0.82	0.75
TRIPTOPHAN%	0.26	0.25	0.23

The combined feeds contained several types of raw materials: cereals, vegetal and animal protein, minerals, premixes and synthesis feeds. Total carcass protein content was evaluated after slaughtering by using the total carcass nitrogen content by multiplying this with a factor of 6.25 and the statistical significance of noticed differences between averages of the two genetic types and also between the two seasons of each year was tested.

RESULTS AND DISCUSSIONS

Carcass protein content is an important quality sign because physiologically linked water is closely related with it. Analyze was performed based on carcass nitrogen content by multiplying this with a factor of 6.25 according to methodology described in chapter about working material and method.

We are going to show average value of feature analyzed for the two studied groups and the statistical significance of differences noticed between averages to emphasize a likely influence of genetic type carcass protein content as other possible influence factor were as much as possible eliminated especially by leveling the environment conditions and procedures of group designing.

In table 2 and figure 1 we are showing values found for "carcass protein content" of the two groups used in experiment in year I and seasons 1 and 2.

COBB 500 hybrids are having the best average value of feature analyzed in year I and season 1 – 4.87% higher than average value of ROSS 308 hybrids. Testing significance of noticed differences between averages of the two experimental groups showed estimated values of Student test higher than table values ($t = 4.9332^{***}$) which are illustrating that there are some highly statistically significant differences between them.

This proves the superiority of COBB 500 hybrids in this matter and differences noticed are related to genetic type as consequence of conditions in which experiment was performed.

Table 2. Influence of genetic type on carcass protein content, first year, first and second season

Genetic type	n	$\bar{X} \pm s_{\bar{x}}$	s	c.v.%
First year, first seson				
ROSS 308	25	17.4272 ± 0.1477	0.7384	4.2372
COBB 500	25	18.3196 ± 0.1045	0.5223	2.8510
Differences significance	$t = 4.9332^{***}$ $t_{48;0.05} = 2.01$; $t_{48;0.01} = 2.68$; $t_{48;0.001} = 3.51$			
First year, second seson				
ROSS 308	25	17.4688 ± 0.1532	0.7662	4.3863
COBB 500	25	18.3960 ± 0.0858	0.4289	2.3316
Differences significance	$t = 5.2795^{***}$ $t_{48;0.05} = 2.01$; $t_{48;0.01} = 2.68$; $t_{48;0.001} = 3.51$			

Hierarchy about average value of carcass protein content has been the same in year I and season 2 and best average performance has been noticed in COBB 500 hybrids with 5.04% higher than value noticed in chickens ROSS 308. Average performances for carcass protein content are actually the same in the two analyzed seasons. Noticed differences between averages of carcass protein content in carcasses from the two genetic types are highly significant statistically according to Student test ($t = 5.2795^{***}$) and so we are able to sustain further the superiority of COBB 500 hybrid at

least in house we analyzed and in the environmental conditions in which the experiment was performed.

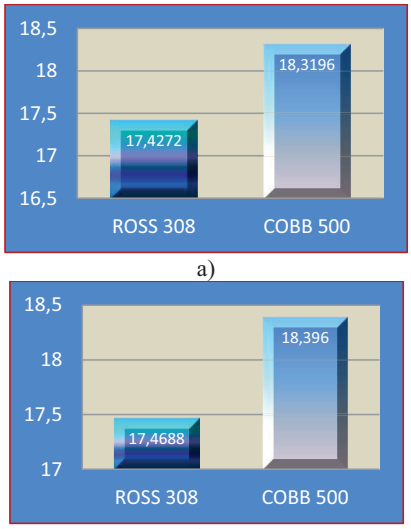

Figure 1. Average carcass protein content (g) at both hybrids, first year, first season (a) and second season (b)

In table 3 and figure 2 we are showing values found for "carcass protein content" of the two groups used in experiment in year II and seasons 1 and 2.

In year II and season 1 birds of group COBB 500 have showing the best average value of carcass protein content with 5.89% higher than value noticed in birds of ROSS 308 hybrid. Testing significance of noticed differences between averages of the two experimental groups showed estimated values of Student test higher than table values ($t = 6.6759^{***}$) which are illustrating that there are some highly statistically significant differences between them and so there is a superiority of COBB 500 hybrids.

Hierarchy is not changing in the second season and best average performance has been noticed in birds of COBB 500 hybrid (18.6212 ± 0.0836 percentage) which is a value 6.54% higher than in chickens ROSS 308 (17.4032 ± 0.1261 percentage). Noticed differences between averages of carcass protein content in carcasses from the two genetic types are highly significant statistically according to Student test ($t = 8.0522^{***}$) and so we are able to sustain further the superiority of COBB 500 hybrid in year II at least in house we analyzed and in the environmental conditions in which the experiment was performed.

Table 3. Influence of genetic type on carcass protein content, second year, first and second season

Genetic type	n	$\bar{X} \pm s_{\bar{x}}$	s	c.v.%
Second year, first seson				
ROSS 308	25	17.4472 ± 0.1345	0.6727	3.8555
COBB 500	25	18,5392 ± 0,0930	0.4652	2.5093
Differences significance	$t = 6.6759^{***}$ $t_{48;0,05} = 2.01$; $t_{48;0,01} = 2.68$; $t_{48;0.001} = 3.51$			
Second year, second seson				
ROSS 308	25	17.4032 ± 0.1261	0.6304	3.6224
COBB 500	25	18.6212 ± 0.0836	0.4178	2.2439
Differences significance	$t = 8.0522^{***}$ $t_{48;0,05} = 2.01$; $t_{48;0,01} = 2.68$; $t_{48;0,001} = 3.51$			

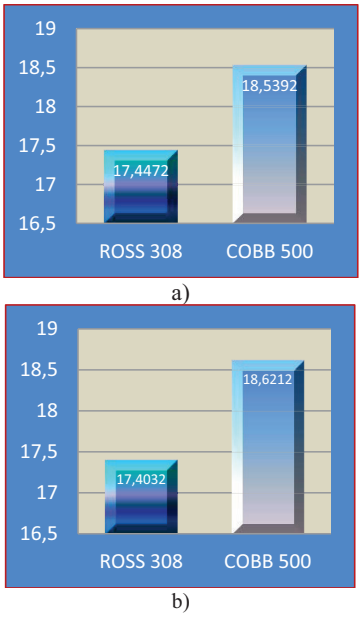

Figure 2. Average carcass protein content (g) at hybrids, second year, first (a) and second (b) season

The measure in which analyzed quality indexes are the same in year II in production house in which we performed the study is statistically analyzed by testing the significance of noticed differences between feature averages by year and season.

In tables 4 and 5 we are showing values found for Student test and their statistical significance.

Table 4. Testing of differences significance between years, first and second season, ROSS hybrid

Specification	Student value	Student critical value
First season ROSS		
Carcass weight	0.1020^{NS}	$t_{48;0,05} = 2,01$ $t_{48;0,01} = 2,68$ $t_{48;0,001} = 3,51$
Carcass protein content (b%)	0.1001^{NS}	
Second season ROSS		
Carcass weight	2.0699^{*}	$t_{48;0,05} = 2,01$ $t_{48;0,01} = 2,68$ $t_{48;0,001} = 3,51$
Carcass protein content (b%)	0.3306^{NS}	

Table 5. Testing of differences significance between years, first and second season, COBB hybrid

Specification	Student value	Student critical value
First season COBB		
Carcass weight	0.8660^{NS}	$t_{48;0,05} = 2,01$ $t_{48;0,01} = 2,68$ $t_{48;0,001} = 3,51$
Carcass protein content (b%)	1.5698^{NS}	
Second season COBB		
Carcass weight	0.0688^{NS}	$t_{48;0,05} = 2,01$ $t_{48;0,01} = 2,68$ $t_{48;0,001} = 3,51$
Carcass protein content (b%)	1.8804^{NS}	

Analyze of results is revealing that there are no statistical significant differences between averages of chickens of ROSS 308 hybrid between the two analyzed years excepting carcass weight in season 2. So it is recognized that there were no differences about the technologies of production, feeding and assurance of quality standard between previous and next year inside the house in which study was performed. The significant differences about carcass weight in season 2 might attributable to some trial errors.

Concerning results obtained for COBB hybrid (table 5) it is noticed that differences found between averages of the two analyzed years are not statistically significant.

CONCLUSIONS

Average carcass protein content had different values for the two analyzed hybrids. Value found in season I and year I has been 17.4272 ± 0.1477 percent in ROSS 308 and 18.3196 ± 0.1045 percent in COBB 500 and differences noticed between the two hybrids are highly significant statistically. Value found in season 2 has been 17.4688 ± 0.1532 percent in ROSS 308 and 18.3960 ± 0.0858 percent in COBB 500 and differences are highly significant statistically.

In year II and season 1 it was found a value of 17.4472 ± 0.1345 percent in ROSS 308 and 18.5392 ± 0.0930 percent in COBB 500 and differences noticed between the two hybrids are highly significant statistically. In season 2 it was found a value of 17.4032 ± 0.1261 percent in ROSS 308 and 18.6212 ± 0,0836 percent in COBB 500 and differences have been very significant from a statistical point of view.

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REFERENCES TNR

Sandu Gh., 1995. Modele experimentale in zootehnie. Ed. Coral Sanivet, Bucureşti.

Tudorache Minodora, I. Van, I. Custură, Elena Popescu-Micloşanu, Antoaneta Maria Popa, 2012. Study on unit cost of certificate-type broilers. Scientific Papers - University of Agronomic Sciences and Veterinary Medicine of Bucharest, Faculty of Animal Science, Seria D, Vol. LV, p. 250-255, ISSN 2285-5750, ISSN – L 2285-5750.

Van I. et al., 2010. Creşterea and industrializarea puilor de carne. Ed. Total Publishing, Bucureşti.

***Watt Poultry Statistical Yearbook.

*** Directiva CE 543/2008.

*** www.en.aviagen.com

*** www.cobb-vantress.com.

*** www.thepoultrysh. com, Farmes Weekly Interactive.

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HUSBANDRY SYSTEM EFFECTS ON EGG QUALITY PARAMETERS

Guler YENICE[1], Ozgur KAYNAR[1], Feryaz HIRA[2], Sayda Yadira Armenta PEREZ[3], Mustafa ILERITURK[1], Havva Nur IBRAHIMAGAOGLU[1], Armagan HAYIRLI[1]

[1]Faculty of Veterinary Medicine, Ataturk University, Erzurum 25240, Turkey
[2]Hiraogullari Poultry and Feed Mill Co., Erzincan 24100, Turkey
[3]Faculty of Science, Ataturk University, Erzurum 25240, Turkey

Corresponding author email: gulerata2003@hotmail.com

Abstract

This experiment was carried out to compare morphological egg quality parameters of brown eggs laid by chickens reared in different production systems: conventional, free-range, and family type production systems. A total of 270 brown eggs were obtained from commercial poultry companies raising Lohmann Brown in a multi-tier cage system and free-range unit as well as families possessing hens in their yards. Differences in egg quality parameters among the production systems were attained using the LSD option at $P < 0.05$. All egg quality parameters differed by the husbandry system, except for albumen index. Eggs from the free-range system had characteristics similar to those from the conventional system. Quality of eggs from the family type system had high coefficient variation. In conclusion, high variability in quality of eggs from the family type system could be related to variations in breed, age, and diet, which are uncontrollable and undeterminable. The most strikingly different egg quality parameter in eggs from the family type production, in comparison with other is yolk colour.

Key words*: egg quality, production system, welfare, organic egg.*

INTRODUCTION

The egg is one of the most consummate foods for human nutrition. Egg protein is of high quality and is easy to digest. Because it contains almost all vitamins an important vehicle to complement the essential vitamin supply to the human. It is also a good source of minerals (Sparks, 2006). The external and internal qualities of eggs are very important for the consumers. The egg production and quality are related with the type of husbandry system (Radu-Rusu et al., 2014). Thus, Ozbey and Esen (2007) reported that the different breeding systems have essential effects on egg production and egg quality characteristics. Similarly, Meng et al. (2014) reported that the hens in the large furnished cages treatment had lower productivity and higher egg quality than those in the small furnished and conventional cages treatments.

Different husbandry systems are available in laying hens breeding such as Free-range, organic, yard, battery cage and furnished cage. Battery cages type is one of the husbandry systems which controversial subject for among advocates animal welfare, animal rights and industrial producers. Thus the European Union banned battery husbandry of chickens from January 2012 for welfare reasons (Leenstra et al., 2014). We aimed to compare morphological egg quality parameters of brown eggs laid by chickens reared in different production systems: conventional, free-range and family type production systems.

MATERIALS AND METHODS

A total of 270 brown eggs were obtained from commercial poultry companies raising Lohmann Brown in a multi-tier cage system and free-range units as well as families possessing hens in their yards.

The egg weight was measured with an electronic balance to the nearest 0.01 g. The egg shape index (%) was determined by equipment developed by Rauch and egg shell strength (kg/cm^2) was measured by special equipment. Egg yolk diameter, albumen length, albumen width (mm) were measured with

digital caliper. The albumen and yolk height (mm) were measure using tripod micrometer.

The yolk (YI) and albumen (AI) indexes were calculated using the following formula as described by Doyon et al (1986):

YI = (yolk height/yolk diameter) x 100

AI = (albumen height/(albumen length + albumen width)/2)) x 100

Individual HU score was calculated using the egg weight and albumen height (Haugh, 1937).

$HU = 100 \times \log (H + 7.5 - 1.7W^{0.37})$, where: H = Height of the albumen (mm), W = Weight of egg (g).

Eggshell thickness was measured after removing the internal membranes of the eggshell. A precision micrometer was used to the nearest 0.01 mm. Measurements were taken at the three regions of the shell and the means were calculated. Egg yolk colour was determined according to Roche yolk colour fan. The all data were analyzed by using PROC GLM procedure of statistical analysis software (SAS v9.4) (SAS, 2013). Differences in egg quality parameters among the production systems were attained using the LSD option at $P < 0.05$.

RESULTS AND DISCUSSIONS

The effects of different housing systems on external and internal quality of egg are presented in Table 1. All egg quality parameters differed by the husbandry system, except for albumen index. The deepest yolk colour is observed in family type system as expected. Shell breaking strength, eggshell thickness, shape index, yolk colour in conventional and free-range systems were similar but significantly ($P<0.05$) differ that of the family type system. Similiarly, Sekeroglu et al. (2010) reported that there was no difference among free range and cage systems.

Egg weight was the highest (62.53 g) in conventional system, intermediate (58.14 g) in free-range system and the lowest (54.02 g) in family type. Similarly stated by Pištěková et al. (2006), we observed significant differences for egg weights among the husbandry systems in the present study ($P<0.05$). Conversely Sekeroglu et al. (2008) reported that weights of eggs were not affected by housing system in a similar study.

Eggshell quality (breaking strength, weight and thickness) could be related to Ca level of shell. It is generally accepted that dietary Ca supplementation should play an important role in maintaining good eggshell quality (Arpášová et al., 2010). High variability in quality of eggshell from the family type system could be related to diet, which is uncontrollable.

Table 1. The quality parameters of eggs obtained from different production systems.

	Production System*		
Parameter	Conventional	Free-range	Family type
Egg weight, g	62.53±0.51a	58.14±0.39b	54.02±0.81c
Eggshell weight, g	7.66±0.07a	7.44±0.09a	6.62±0.11b
Eggshell weight, %	12.28±0.22b	12.81±0.14a	12.31±0.16b
Shell breaking strength, kg/cm^2	2.70±0.12a	2.85±0.10a	2.13±0.12b
Eggshell thickness, mm	0.39±0.002a	0.39±0.003a	0.35±0.04b
Shape index, %	77.74±0.24a	78.01±0.25a	74.55±0.40b
Yolk colour	10.36±0.09b	10.42±0.07b	11.85±0.21a
Yolk index, %	4.19±0.03a	4.08±0.02b	4.06±0.04b
Albumen index, %	0.60±0.02	0.57±0.02	0.60±0.02
Haugh unit	70.10±0.89a	67.81±0.99ab	66.65±1.48b

*Different superscripts among columns differ ($P < 0.05$).

CONCLUSIONS

Eggs from the free-range system had similar characteristics to those from the conventional system. Quality of eggs from the family type system had high coefficient variation. High variability in quality of eggs from the family type system could be related to breed, age, and diet, which are uncontrollable. The most striking egg quality parameter in eggs is yolk colour.

REFERENCES

Arpášová H., Halaj M., Halaj P., 2010. Eggshell quality and calcium utilization in feed of hens in repeated laying cycles. Czech J. Anim. Sci., 55(2):66-74.

Doyon G., Bernier-Cardou M., Hamilton R.M.G., Castaigne F., Randall C.J., 1986. Egg quality 2. Albumen quality of eggs from five commercial strains of white leghorn hens during one year of lay. Poultry Science, 65(1): 63-66.

Haugh R.R., 1937. The haugh unit for measuring egg quality. US Egg Poultry Magazine, 43: 552-555.

Leenstra F.R., Maurer V., Galea F., Bestman M.W.P., Amsler Z., Visscher J., Vermeij, I., Krimpen van M.M., 2014. Laying hen performance in different production systems; why do they differ and how to close the gap? Results of discussions with groups of farmers in The Netherlands, Switzerland and France, benchmarking and model calculations. Archiv für Geflügelkunde, 78(3):1-10.

Meng F., Chen D., Li X., Li J., Bao J., 2014. Effects of large or small furnished cages on performance, welfare and egg quality of laying hens. Animal Production Science.

Özbey O., Esen F., 2007. The Effects of Different Breeding Systems on Egg Productivity and Egg Quality Characteristics of Rock Partridges. Poultry Science, 86:782–785.

Pištěková V., Hovorka M., Večerek V., Strakova E., Suchý P., 2006. The quality comparison of eggs laid by laying hens kept in battery cages and in a deep litter system. Czech J. Anim. Sci., 51, (7): 318–325.

Radu-Rusu R.M., Usturoi M.G., Leahu A., Amariei S., Radu-Rusu C.G., Vacaru-Opriş I., 2014. Chemical features, cholesterol and energy content of table hen eggs from conventional and alternative farming systems. S. Afr. J. Anim. Sci., 44(1): 33-42.

SAS: SAS® v9.4 User's Guide. SAS Inst., Inc., Cary, NC, 2013.

Sekeroglu A., Sarica M., Demir E., Ulutas Z., Tilki M., Saatci M., Omed H., 2010. Effects of Different Housing Systems on Some Performance Traits and Egg Qualities of Laying Hens. J. Anim. Vet. Adv, 9(12): 1739-1744.

Sekeroglu A., Sarica M., Demir E., Ulutas Z., Tilki M., Saatci M., 2008. The Effects of Housing System and Storage Length on the Quality of eggs Produced by Two Lines of Laying Hens. Arch.Geflügelk., 72(3):106-109.

Sparks N.H.C., 2006. The hen's egg - is its role in human nutrition changing? World's Poultry Science Journal, 62:308-315.

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CASE STUDY REGARDING BEEKEEPERS DYNAMIC FROM THE NORTH EASTERN REGION OF ROMANIA THAT ACCEDED TO ORGANIC AGRICULTURE SYSTEM

Daniela BĂDESCU[1], Dumitru DRĂGOTOIU[2]

[1]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, Email: daniela_badescu18@yahoo.com
[2]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 Email: dumitrudragotoiu@yahoo.com

Corresponding author email: daniela_badescu18@yahoo.com

Abstract

In Romania there is a tradition regarding beekeeping and in last years the focus was on organic beekeeping. This type of activity is quite easy to achieve in Romania because of a large number of areas favourable to organic beekeeping, especially hills areas, where pollution has failed to penetrate and destroy the natural environment. The present paper aimed to highlight the dynamics of beekeepers which are producing ecological honey during the period 2012-2014. To achieve the objective, the case study was carried out on beekeepers who are part of Beekeepers Association from Romania, Bacău subsidiary, association who has members from one of the areas of maximum performance in this domain. The study followed two directions: both number dynamic of beekeepers who acceded to organic farming system for each of the studied years, and the evolution of the beehives number in each exploitation from one year to another. As a conclusion, the results of this case study show growth trends for the number of beekeepers who produce organic honey, while the evolution of beehives number in the apiary is weather dependent and also dependent on the efficiency of preventive treatments applied.

Key words*: beekeeping, honey, organic agriculture, Romania.*

INTRODUCTION

Organic farming is an activity practiced since antiquity which led to the development of the great civilizations. Even if in those times the current word which refers to the organic system was not known, the agriculture was based on biodynamic principles kindly with the environment, very well known and forwarded from generation to generation.

"Agricultura ecologică" is a protected term assigned to Romania by the European Union in order to define a new direction of the nowadays agriculture, namely sustainable agriculture. This term is similar to "organic agriculture" or "biological agriculture" used by other Member States.

In Romania, organic farming is a dynamic sector which knew an ascendant evolution in the last period, both in vegetal and animal sectors. This thing is due to the intensive promotion of this concept, sustained first of all by the European Union through Ministry of Agriculture and Rural Development, on one hand and to the increasing awareness by the consumers of the organic products' quality on the other hand (Pocol, 2011).

Organic beekeeping is a branch of the organic farming and it represents an activity which in Romania can be easy performed due to the proper natural environment (Toncea, 2002).

In this manner, to convert a conventional apiary to an organic one it must be browsed through a conversion period by minimum 1 year, according to the legislation in force. In this period the existent frames should be replaced with new frames and the wax from these frames should come from organic certified apiary (Popescu, 2013). The main condition for the conversion of the apiary is the bees to have consistent sources of pollen in order to fill all the honeycomb existent in

the apiary (conventional honeycomb) and the new added frames to comply with the legislation in force regarding the organic farming.
In order to be considered finished the conversion period it will be presented proves certifying both origin of the wax used for the new frames and the purchased quantity.
In this context, the present article represents a case study which wants to highlight the evolution of the number of beekeepers from North-East of Romania which are members of Romania Beekeepers Association, Bacău subsidiary and which acceded to the organic farming system in the period 2012-2014 and, also to highlight the apiary evolution year by year.

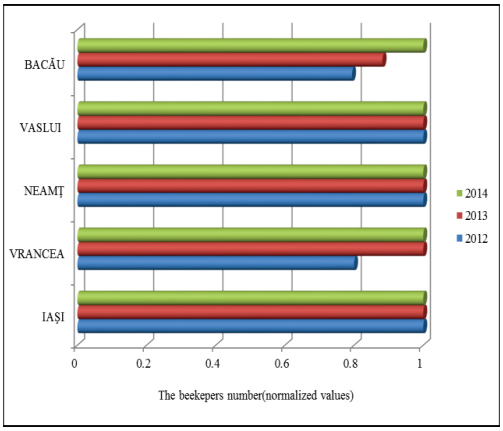
MATERIALS AND METHODS

In order to analyze the dynamics of beekeepers which make the subject of this case study, there were used the following indicators: the number of beekeepers registered to the County Agricultural Directions for the geographical area taken for the study, the number of bee families and the quantity of honey obtained.
The period analyzed in this study was 2012-2014.
The data presented in this article comes from personal monitoring performed to onsite through annually and seasonally apiary visits, in the months in which the picking honey was performed: April, May and September.
The data obtained by personal monitoring was completed by information taken over from the Ministry of Agriculture and Rural Development and from SC Apicola Bacau SRL, they were statistically processed and interpreted, in this manner being able to highlight trends in this area for years to come.

RESULTS AND DISCUSSIONS

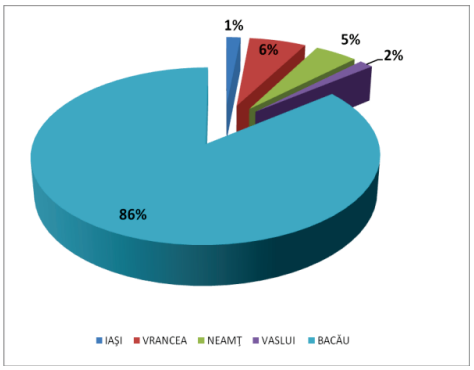
During this case study we monitored several aspects so that we can obtained a more realistic picture to the organic farming concept evolution of the number of beekeepers from North-East of Romania.
The first indicator analyzed in this case study is the distribution by county of beekeepers which acceded to the organic farming for the period 2012-2014, and it was realized following two directions: the evolution of beekeepers number from each county for the three years and, also the evolution of beekeepers number from each county reported to the total number of beekeepers for each year took into study.
Thus, in figure 1 it can be observed the distribution by county evolution of beekeepers in the period 2012-2014. From this chart it can be observed that in all three years of study only in two counties the number of beekeepers is varying, namely Vrancea and Bacău, while for the other three counties the number of beekeepers is constant from year to year.

Figure 1. The evolution of beekeepers' distribution by counties of in the period 2012-2014



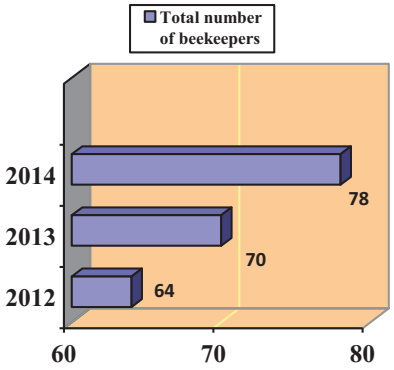
Regarding the percentage distribution by counties of beekeepers from North-East of Romania which make subject of this case study, it can be observed a linear evolution for the three years took in study.
Thus, the most operators are from Bacău county, having a percentage of 86.2, followed by Vrancea county with an average of 6.63%, by Neamţ county with an average percentage of 4.3 and by Vaslui and Iaşi counties with the same average percentage of 1.43 (figure 2).

Figure 2. Distribution by counties of beekeepers



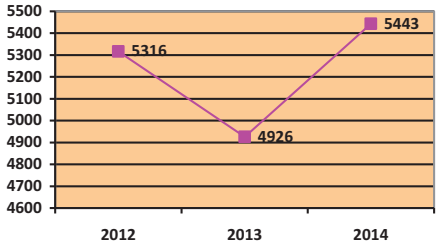
Another indicator analysed in this case study is the evolution of beekeepers total number and it can be observed an obvious growing trend for the period 2012-2014, like it results from figure 3. Thus, it was recorded an increase with 9.5% of beekeepers number in 2013 against 2012 and an increase of 11.5% in 2014 against 2013, fact which confirms the becoming greater interest of beekeepers from Romania to produce organic honey.

Figure 3. The evolution of total number of beekeepers for period 2012-2014



For studying the evolution of bee families' number, from the three years lists of beekeepers we selected only the beekeepers which remained in the organic farming system the entire period and we calculated the total number of organic or in conversion bee families (figure 4).

Figure 4. The evolution of total number of bee families



The values in above figure represent the total number of bee families for a number of 57 beekeepers.

In figure 4 we can observe that in 2013 the total number of bee families decreased against 2012 with about 7%, this decreasing of apiaries being due to the winter 2012-2013 unpropitious weather conditions when the bee families' mortality increased. Another cause is represented by bee diseases with the parasite *varroa destructor*, when the preventative treatments accepted by the legislation for organic farming wasn't successfully.

Regarding also the evolution of bee families' number it can be observed a significant increasing, with about 10% in 2014 against 2013, fact which leads to a growth trend of apiaries when the environmental conditions are friendly and the treatments prevents the apiaries diseases.

For create a more clearly image regarding the evolution of beekeeping in organic farming system, apart from the indicators presented till now, we monitored also the quantity of honey obtained for period 2012-2014 for the following categories: acacia honey, linden honey and polyfloral honey (table 1).

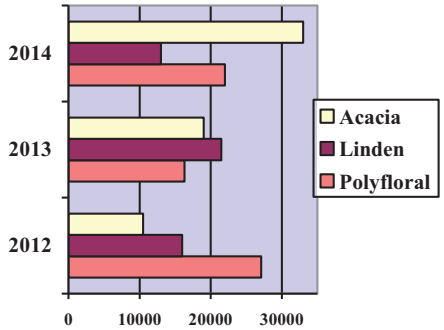
Table 1. The honey production assortments

Assortment (kg)/year	2012	2013	2014
Acacia	10500	19000	33000
Linden	16000	21500	13000
Polyfloral	27100	16300	22000

The honey production is presented graphically in figure 5 where we can observe a growing

of the acacia honey production from a year to another. It is known that acacia honey is the most appreciated assortment by the consumers due to the fact that the crystallisation phenomena is slower and due to its sensorial properties. In the same time, this assortment is the most expensive one, fact which is stimulating the beekeepers to produce this organic honey assortment in order to obtain a higher profitability.

Figure 5. The honey production for period 2012-2014



In addition, we can tell that the honey production is also influenced by the specificity of the area, meaning the fact that the North-East area from Romania is a hilly area with a lot of acacia forests, in which the linden honey areas and the wild grassland are found in smaller proportion, being an area exploited for vine crops, orchards and cereals crops.

CONCLUSIONS

Organic beekeeping is an important sector which contributes to the development of the organic farming concept in Romania. Thus, following the case study made in this paper we can observe a growing trend of number of beekeepers which wants to produce organic honey in North-East of Romania from year to year.

Also, in favourable weather conditions and because, over time due to the organic treatments the bees acquire its own immunity which help them to resist pests and diseases, we can observe a significant increasing of apiaries dimensions over the years, too.

Regarding the quantity of organic honey obtained from the organic certified apiaries, we can say that the production of acacia honey increased very much (from 10500 kg in 2012 to 33000 kg in 2014). The beekeepers are stimulated to produce this assortment of honey due to the very high consumers' request, due to its best quality and, being the most expensive one, the beekeepers profitability is greater.

For the other two assortments of organic honey, linden honey and polyfloral honey, there is a significant production, but it doesn't know the same evolution like the acacia honey production.

As a final conclusion, the organic beekeeping represents a dynamic sector, having an increasing evolution, trend which seems to be kept for the coming years, mainly due to the publicizing and promoting of a healthy life style. Also, the organic farming is sustained by supporting programs with European funds.

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REFERENCES

Pocol C.B., 2011. Perception study regarding organic beekiping in the North-West region of Romania, Lucrări Ştiinţifice, seria Agronomie, 54 (2):445-449.

Popescu A., 2013. Study concerning the trends in Romania's honey market. Lucrări Ştiinţifice – Seria Zootehnie, vol 59, 153-158.

Regulamentul (CE) nr. 834 al Consiliului din 28 iunie 2007 privind producţia ecologică şi etichetarea produselor ecologice, precum şi de abrogare a Regulamentului (CEE) nr. 2092/91, Jurnalul Oficial al Uniunii Europene, L 189/1/2007.

Regulamentul (CE) nr. 889 al Comisiei din 5 septembrie 2008 de stabilire a normelor de aplicare a Regulamentului (CE) nr. 834/2007 al Consiliului în ceea ce priveşte producţia ecologică, etichetarea şi controlul, Jurnalul Oficial al Uniunii Europene, L 250/1/2008.

Toncea I., 2002. Ghid practic de agricultură ecologică, Editura Academicpres, Cluj Napoca.

http://www.madr.ro.

http://ec.europa.eu/agriculture/organic/home_ro

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CHARACTERIZATION OF MYOFIBRILLAR PROTEINS OBTAINED FROM FRESH ABRAMIS BRAMA (COMMON BREAM) MEAT

Floricel CERCEL*, Mariana STROIU, Petru ALEXE

Department: Food Science, Food Engineering And Biotechnology Applied,
Faculty of Food Science and Engineering "Dunarea de Jos" University of Galati,
111, Street: 800201, Galati, Romania

Corresponding author email: fcercel@ugal.ro

Abstract

It must be pointed out that fish protein ingredients production has a growing trend all around the world because their low-cost, high nutritive quality and more concentrated protein levels. The current study refers to miofibrilar protein extraction and their characterization, from fresh carp meat. Acid pH dissolution, followed by precipitation at isoelectric pH was used as extraction method. The myofibrillar protein chemical characterization was made by taking into account the functional and rheological properties. Spray-dryer method for myofibrillar proteins solubilized at alkaline pH and acid pH and scanning electron microscopy (SEM) was used for dry. The solubility of the muscle proteins, constituent components of protein derivatives, is a critical property that controls the other functional characteristics of the protein (emulsifying capacity, foaming and gel formation). The protein concentrates/isolates, studied by their functional properties, protein solubility and gelling characteristics, can be suitable raw materials for protein films and biodegradable coatings generation.

Key words*: abramis brama, common bream, gelling characteristics of proteins, myofibrillar protein, solubility of the muscle proteins.*

INTRODUCTION

Fish myofibrillar protein concentrate is an important under utilised commodity in Romania even though they are known to be good source of protein and other nutrients. A variety of raw materials that come from aquaculture and marine are used for obtain fish protein concentrate. Abramis Brama (Common Bream)is in volume terms by far the most important aquaculture species in world fisheries. We focused on that food sources because they are most available to them.

Production of fish protein ingredients such as fish myofibrillar protein concentrate is growing throughout the world. It ingredient is a low volume but high value and relatively low prices and it can serve as active ingredients in functional foods, in edibles film and other health related products.

The pH of the protein environment are one the most important factor affecting protein solubility, conformation and functional properties, such as emulsification and foaming ability.

Protein is, after water, the most important constituent of animal bodies.

Myofibrillar proteins are located in myofibrils, contribute in the filamentous organization of the muscle and directly participate in the mechanochemical process of muscle contraction and stiffness. Structural proteins are the most abundant protein fraction of muscle tissue (54-70% of total muscle protein).

Myofibrillar proteins, from technological point of view, contribute to meat tenderness, determine the capacity of water retention and hydration of meat, fat emulsifying and gelling capacity. Myofibrillar proteins by high intake of essential aminoacids, contribute about 70% to the nutritional value of meat. (Ionescu et al., 2009).

Myofibrillar proteins have intermediate solubility between sarcoplasmic and stromal proteins (insoluble in water, but soluble in saline solutions with ionic strength higher than 0.3 or in solutions with controlled pH). They are fibrillar proteins that associate with each other to form complex parallel structures (Cuq et al., 1995).

Meat proteins have important functional properties, such as: water holding capacity, gelation and emulsification.

The factors which influence heat-induced gelation properties were studied for different myofibrillar proteins, in particular, beef, pork, poultry, fish and rabbit myosin (Fretheim et al., 1986; Smith et al., 1988; Hennigar et al., 1989; Chan et al., 1992; Xiong, 1992; Lan et al., 1995a, 1995b; Boyer et al., 1996a, 1996b). Gelation of muscle proteins involves partial denaturation followed by irreversible aggregation of the ends of myosin by the formation of disulfide bonds and the transition of the molecule's body from the helix form to spiral which results in a three-dimensional network structure (Samejima et al., 1981; Smith et al., 1988; Sharp et al., 1992; Stone et al., 1992). During gelation, myosin and other salt soluble proteins undergo complex changes of the rheological properties, depending on the temperature and pH at which they are exposed (Egelandsdal et al., 1986; Xiong, 1993).

MATERIALS AND METHODS

1. Raw materials

Fresh Abramis Brama (Common Bream) specimens (≈1000 g) were obtained from a commercial supplier. The samples were transported to the laboratory in ice. Immediately on reaching the lab, the fishes were thoroughly washed with cold water to remove blood, slime, dirt, etc. After decapitation and evisceration, fishes were de-skinned and filleted. The fillets were immediately used for myofibrillar proteins preparation.

2. Myofibrillar proteins extraction.

Myofibrillar proteins were extracted from fish muscle according to following methods: alkaline pH method; acid pH method and KCl + EDTA. The resulting concentrates myofibrillar proteins were used immediately for determination of their physicochemical properties and for protein recovery, also. The physicochemical properties of fish protein concentrateswere determined according to Association of Official Methods of Analysis (AOAC, 1990; Ionescu et al., 1992). Crude protein contentwas calculated by multiplying N by 6.25. The results were expressed as g/100g protein concentrate. Nitrogen (N) was calculated using the Kjeldahl method (AOAC, 1990; Ionescu et al., 1992) (Raypa, Spain). Crude lipid contentwas determined according to Soxhlet method described in (AOAC, 1990; Ionescu et al., 1992) (Solvent extractor VELP Scientifica SER148, USA). The results were expressed as g/100g protein concentrate. Crude ash contentwas obtained by heating the protein concentrates in a furnace at 550°C for 24 h. Crude ash was determined according to method. The results were expressed as g/100g protein concentrate. Protein recovery (% yield) of the washed mince from different washing methods was determined according to the method of Kim and al. The recovery of protein was expressed as % in DM (dry matter).

The pH was measured potentiometric, using the pH meter type "Hanna" using protein dispersions with a concentration of 10% (G/V)), at a temperature of 22 ± 1^0C.

All chemical analyzes were carried out in duplicate.

3. Myofibrillar proteins characterization.

Finally, after finding the bestmyofibrillar proteinsextraction methods for fresh fish, the functional properties of myofibrillar proteins obtained by these methods were determined. Functional properties such as foaming capacity, emulsion capacity and solubility, are important factors if fish proteins are to be incorporated into a food or dish as additives during preparation.The solubility of protein obtained from best method of extraction was measured according to the method of Choi and Park with slight modifications. The protein solubility was calculated on the basis of 100% solubility of the protein.The emulsion capacity was calculated by dividing the emulsion volume after centrifugation by the original emulsion volume and then multiplying by 100. Emulsifying stability was determined by the same procedure except that, before centrifugation, the emulsion was heated at 90°C for 30 min followed by cooling in tap water for 10 min.The method of Miller and Groninger wasused to determine foaming properties. The foam was calculated as the volume of mixture after blending compared to the original volume. The foaming stability was the ratio of the foam capacity after time

observation divided by the original foam capacity.
The determination was performed in duplicate.
4. Gelling properties
The gelation properties were determined by dynamic rheological measurements at oscillations of small amplitude, performed by a voltage-controlled rheometer (AR 2000, TA Instruments, New Castle, DE), attached to a control software computer (Rheology Advantage Data Analysis Program, TA , New Castle, DE). The temperature was monitored using a Peltier temperature control system. All rheological measurements were made using a cone plate geometry of 40 mm with an angle of 2° and a gap of 2000 μM. For each test, about 2 g of protein suspension was placed at the base of the rheometer plate. To prevent dehydration low viscosity silicone was added around the edges of the plate. The measurements were made at a constant angular frequency of 0.3142 rad / min (0.05 Hz frequency) by scanning the temperature ranges 4.3 – 74.9° C and 31-80°C. Changes in storage modulus (G') and phase angle or deformation (δ) were recorded depending on the temperature. The heating rate was programmed to 1°C/min. For all samples the linear viscoelasticity domain was established at a constant temperature of 20°C and at a frequency of 0.10 Hz. For each test, the sample was kept for 5 minutes for temperature equilibration. Samples were running in duplicate.

RESULTS AND DISCUSSIONS TNR 12

1.Determining the approximate chemical omposition

Table 1. Proximate chemical composition of Abramis Brama (Common Bream) meatand a protein concentrated obtain.

Sample nature	Water (%)	Proteins %	Fat %	Ash %	Other
MHAB	76.52	17.21	4.28	1.26	0.71
CPMAB 1	82.05	16.42	0.52	0.06	0.93
CPMAB 2	84.38	13.84	0.59	0.05	1.12
CPMAB 3	85.82	12.75	0.56	0.09	0.75

MHAB - Muscle homogenate of Abramis Brama
CPMAB 1 -Protein concentrate – Alkaline extraction
CPMAB 2 - Protein concentrate – Acid extraction
CPMAB 3 - Protein concentrate - KCl and EDTA extraction
Depending on the method of extraction is higher protein concentrate (more pure) the method of extraction by acid leaching at pH compared with other methods, as shown in the (Table 1).
2.Protein solubility
The solubility characteristics of the myofibrillar proteins are interesting because of the relationship with other functional properties, particularly the gelling and water retention properties (Hultin et al., 1995). Muscle proteins are properly differentiated by their solubility.
To find the proper pH values for maximum solubilization and recovery of muscle protein, we constructed the solubility curves (protein concentration versus pH) for myofibrillar protein concentrates and isolates.
Protein solubility curves are shown in the Figure 1. Solubility profiles were similar for all analyzed protein paste.

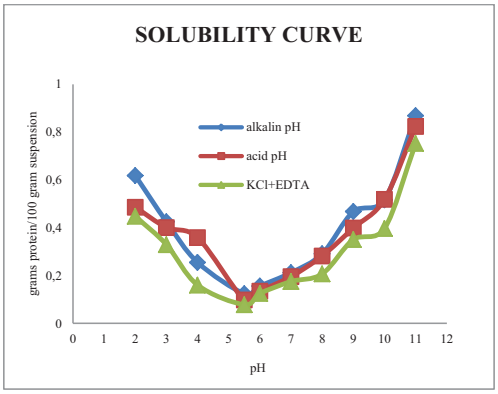

Figure 1. Myofibrillar protein solubility of Abramis Brama (Common Bream)

Fish concentrates showed minimum solubility in isoelectronic range with pH ranging from 5.5 to 6.0, characteristic for most muscle protein (Xiong, 1997), the lowest values of protein solubility being observed at pH 5.5. For protein concentrates / isolates obtained by alkaline and acid solubilisation, higher values of solubility at pH 5.5 were recorded (12.46 to 9.88 % per s.u.) than protein concentrates obtained by

washing the minced meat with water with or with different solutions (KCl and EDTA) (7.85 to 7.31% per s.u.). We explain this by the presence, in the composition of those concentrates, of sarcoplasmic proteins soluble in water and in solutions of low ionic strength and which represent 20-30% of the muscle proteins (Haard et al., 1994; Ionescu et al., 2009).

Lowering the pH to the isoelectric point resulted in a substantial increase in the protein solubility up to a pH of 2.0 where the proteins exhibited a solubility of more than 45% for all the samples we tested. The maximum solubility was reached at pH 2.0 (for the concentrate obtained by solubilization in alkaline pH and the one obtained in acidic pH).

Increasing the pH value relative to pI (isoelectric point) leads to increased solubility, suddenly up to 7, than we have a gentle slope to reach the maximum solubility at pH 11.

By changing the value of the pH of the protein solution, the protein gains a net negative or positive charge at which the moisture of the charged residues and electrostatic repulsion causes an increase in solubility (Damodaran et al., 1996). At pH values close to the isoelectric pH of the protein, the repulsion between the chains of the proteins is reduced and their association occurs. As a result, most of the proteins have minimum solubility at the isoelectric point (pI), since the lack of electrostatic repulsion promotes hydrophobic interactions (protein-protein) and aggregation of the protein molecules. Because of the protein aggregation under these conditions, they can be separated from the solution by means of an appropriate centrifugal force.

At pH below 5.5, the proteins become negatively charged resulting in electrostatic repulsion which facilitates protein to bind water and swell. Also, at pH higher than the isoelectric point, proteins gain positive net charge resulting in repulsion, hydration of the proteins and increase in their hydrodynamic size, viscosity of the protein solutions (Damodaran et al., 1996).

3.Gelling properties

Myofibrillar proteins are responsible for the textural properties of the processed meat products (Yasui et al., 1980; Asghar et al., 1985).

Among the myofibrillar proteins, myosin and actomyosin contribute most to the development of gel characteristics of salted meat processed products.

We studied the gelling properties of some carp homogenized muscle and wet protein concentrates obtained from Abramis Brama (Common Bream).

In our study, we followed the rheological behavior of protein suspensions by scanning a wide temperature range (4.3-74.8^{0}C or 31-80^{0}C) and monitoring parameters: elastic modulus and phase angle (delta). Rheological measurements were determined by dynamic rheological method at small deformation, non-destructive, conducted in the linear region of viscoelasticity, which enables the determination of the elasticity and viscous nature of the tested sample.

Elastic shearing modulus (storage or storage facilities, G') is a measure of the released energy per cycle of deformation per unit volume and the property which makes the correlation with the elastic nature of the material.

Phase or deformation angle (δ) is a measure of the prevalence of viscous properties (characteristic to the liquids) and elastic properties (characteristic to the solids) in the viscoelastic behavior of a material. The phase angle is related to the formation of bonds in the gel during the heating/deformation, mainly in temperature increase/oscillation frequency decrease.

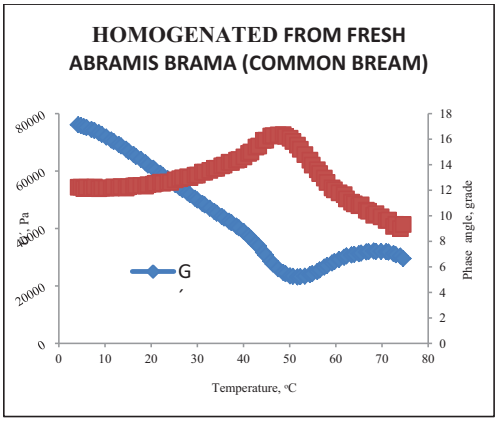

Figure 2. Shows the rheological behavior of Abramis Brama (Common Bream)

As can be seen, the values of the elastic modulus and phase angle (δ) of the homogenate

and the Abramis Brama (Common Bream) muscle protein derivatives have evolved differently depending on the temperature domain and the nature of the sample.

In the case of homogenated Abramis Brama (Common Bream) muscle (pH 6.3), elastic modulus had a moderate downward trend in the temperature domain between 4.3-35.9°C, characterized by high values of G ', 76140 Pa at 4.3°C and 43700 Pa at 35.8°C. This interval is followed by another temperature domain (35.9-51.7°C) characterized by a more significant reduction of this parameter to a minimum of 23150 Pa (51.7°C). In these temperature ranges, the reduction of storage module can be attributed to the complex structure of fish muscle proteins due to denaturation of certain protein fractions. Denaturation of the quaternary structure, tertiary and secondary when applying external stress (heating) possibly involved subunit dissociation of protein filaments, breaking of the disulfitic bonds (-S-S-), dipole-dipole non-covalent interactions between polar aminoacids and interactions between non-polar aminoacids in the side chains, as well as partial conversion of α-helix structures and ß-folded at the configuration of random twisted spiral.

The thermo-rheogram, shows below, a portion close to a plateau in the 50.7-59.7°C domain, possible characteristic to the denaturation and simultaneous aggregation of some protein fractions, given the complex nature of the system investigated. Our findings are in agreement with those reported by Westphalen, etc. (2005), who found the existence of the plateau in the range of 50-57°C, for myofibrillar protein samples with a 6.0 pH and lower concentration.

Starting with the inflection point of the curve (51.7^{o}C), elastic modulus values increased very slowly at first, then the increase was accelerated when the temperature was raised above 59.7^{o}C to the finalization of the heating process at 74.6°C. This rheological behavior is typical for the thermal gelation of Abramis Brama (Common Bream) muscle proteins and for the increase of the formed gel strength. The gel formation involves irreversible aggregation of denatured proteins to form new disulfitic bonds, in particular, between the globular myosin ends and the transition of the helical spiral the myosin molecule rod to a three-dimensional network structure (Stone et al., 1992; Sharp, et al, 1992; Samejima et al., 1981). Changes in rheological characteristics depending on the temperature of the Abramis Brama (Common Bream) homogenate are confirmed by the evolution of the phase angle. The thermo-rheogram of the phase angle indicates a reverse trend relative to the elastic modulus. Low values of the phase angle, between 8.998-16.34 grade, across all the temperature domain of 4.3-74.6°C are specific to the viscoelastic bodies at which elastic component was permanently predominant relatively to the viscose component. The base zone of the elastic modulus in the thermo-rheogram corresponds to the highest value of phase angle,> 12.0°.

Below are presented the thermo-rheograms of the elastic modulus and phase angle for wet protein concentrates, extracted from Abramis Brama (Common Bream) muscle by the alkaline and acid procedure and extracted by washing with KCl and EDTA. The thermo-rheogram profile of the protein concentrates was similar with the one of the Abramis Brama (Common Bream) muscle homogenate except for the elastic modulus values which were different, being much higher for the muscle homogenate (see the table).

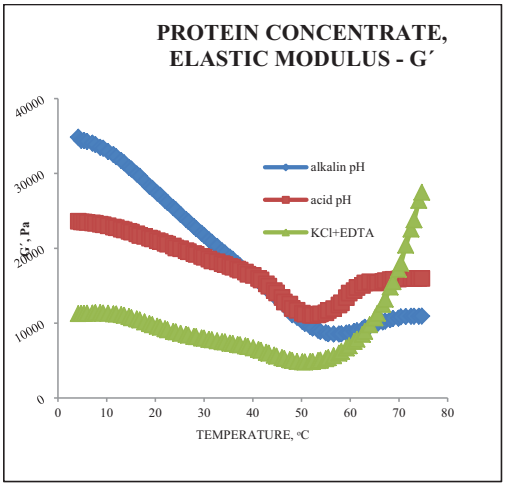

Figure 3. The elastic modulus change depending on temperature and changing of the phase angle depending temperature

If we compare the three types of protein concentrates (acid, alkalin, and KCl) it can be observed that the values of G' were higher for the alkaline protein concentrate compared with the acid one and the one extraced by washing with KCl. For the three types of protein concentrates, the transition temperature from ground to gel was the same (50.8°C), slightly lower than the one registered for the muscle homogenate (51.9°C).

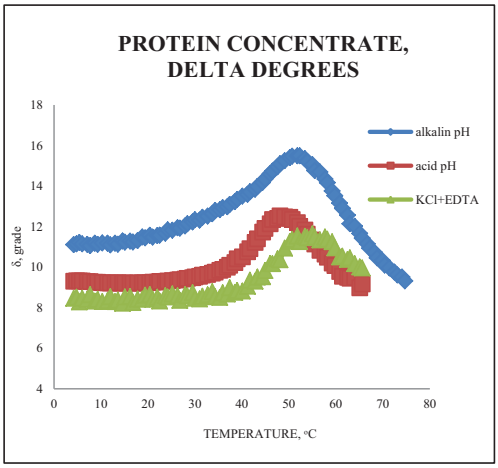

Figure 4. The influence of temperature on the phase angle values

The modifications of the rheological properties on heating of the Abramis Brama (Common Bream) protein concentrates compared to the Abramis Brama (Common Bream) muscle homogenate we ascribe on the greater complexity of the homogenate, differences in protein content and characteristic pH values and potential denaturing changes in the protein system during extraction treatments (Yongsawatdigul and Park, 2004).

Table 2. The temperature dependence of the elastic modulus and the method of extraction of muscle proteins

Sample nature	Elastic modulus (G´), Pa			
	4.2°C	50.7°C	51.7°C	74.6°C
MHAB	76140	-	23150	29560
CPMAB 1	34840	9975	-	10940
CPMAB 2	23620	11210	-	15990
CPMAB 3	11280	4809		27490

MHAB - Muscle homogenate of Abramis Brama

CPMAB 1 -Protein concentrate – Alkaline extraction

CPMAB 2 - Protein concentrate – Acid extraction

CPMAB 3 - Protein concentrate - KCl and EDTA extraction

Protein concentration and pH are very important parameters in thermal gelation of meat protein. In addition, it is a well-known fact that during the extraction of muscle proteins by the acid procedure, due to the high concentration of hydrochloric acid suffers modifications which influence the functional and rheological properties.

Reduced capacity to form gels of acid treated protein, when compared to those treated under alkaline conditions may be attributed to conformational changes (partial loss of myosin heavy chain) or due to the unfavorable conformation of the protein during the acid treatment (several hydrophobic groups leading to larger aggregates and to a less ordered gel). Another explanation could be that related to the presence of denatured sarcoplasmic protein that are retained in the acid process, but not in the alkaline one (Ingadottir, 2004).

CONCLUSIONS

The protein content of protein derivatives was conditioned by the extraction technique applied.

Solubilization of muscle proteins, in a strongly alkaline medium, followed by their precipitation in the solution at the pH of isoelectric point (pI) also ensures the recovery of sarcoplasmic proteins which precipitate at 5.5 pH.

The solubility of muscle proteins, components of protein derivatives, is a critical property it controls the other functional characteristics of the protein (emulsifying, foaming and gels formation capacity).

The variation of the elastic modulus (G') and phase angle (δ) during thermal treatment of protein suspensions reflects profound changes in the protein system (denaturation, dissociation and reassociation) depending on the temperature.

All concentrates/isolates of muscle protein behaved, from rheological point of view, as viscoelastic systems with high elastic

component, but variable depending on the temperature, source of proteins, extraction method and drying process through lyophilization.

Elastic modulus values were directly proportional to the protein concentration from proteic suspension. The correlation coefficients between protein concentration and elastic modulus during heating (30-71.9^{o}C) showed values above 0.930, values slightly higher at lower protein concentrations.

The analyzed protein concentrates/isolates have functional capabilities suitable for use in various systems based on meat, bringing products added nutritional value through their protein component but their production is only justified economically for species of inutilisable fish, inferior quality meats and some organs.

REFERENCES

Ankur Ajmera, Regina Scherließ, 2014. Stabilisation of proteins via mixtures of amino acids duringspray drying. *International Journal of Pharmaceutics* 463, 98– 107

AOAC, 1990. Moisture in Meat. *Official Methods of Analysis* 950, 46, 11, 931.

AOAC, 1990. Crude protein in Meat. *Offcial Methods of Analysis* 981, 10, 11, 937.

Asghar A., Samejima K., Yasui T., 1985. Functionality of muscle proteins in gelation mechanisms of structured meat products. CRC *Crit. Rev. Food Sci., Nutr*., 22, 27-106.

Boyer C., Joandel S., Ouali A., Culioli J., 1996b. Ionic strength effects onheat-induced gelation of myofibrils and myosin from fast- and slow-twitch rabbit muscles. *J. Food Sci*., 61, 1143-1148

Boyer C., Joandel S., Roussilhes V., Culioli J., Ouali A., 1996a. Heat-induced gelation of myofibrillar proteins and myosin from fast- and slow-twich rabbit muscles. *J. Food Sci*., 61, 138-143.

Chan J.K., Gill T.A., Paulson A.T., 1992. Cross-linking of myosin heavy chains from cod, herring and silver hake during thermal setting. *J. Food Sci*., 57, 906-912.

Cuq B., Aymard C., Cuq J.L., Guilbert, S., 1995. Edible packaging films based on fish myofibrillar proteins: formulation and functional properties. *Journal of Food Science*, 60, 1369-1374.

Damodaran S., 1996. Amino Acids, Peptides and Proteins. In *Food Chemistry*; O. R.

Egelandsdal B., Fretheim K., Samejima K., 1986. Dynamic rheological measurements on heat-induced myosin gels, effect of ionic strength, protein concentration and addition of adenosine triphosphate or pyrophosphate. *J. Sci. Food Agric*., 37, 915-926.

Fretheim K., Samejima K., Egelandsdal B., 1986. Myosins from red and white bovine muscles: Part 1 – Gel strength (elasticity) and water-holding capacity of heat-induced gels. *Food Chemistry*, 22, 107-121.

Haard N.F., Simpson B.K., Sun Pan B., 1994. Sarcoplasmic Proteins and Other Nitrogenous Compounds. In *Seafood Proteins*; London, 13-39.

Hennigar C.J., Buck E.M., HultIn H.O., Peleg M., Vareltzis K., 1989. Mechanical properties of fish and beef gels prepared with and without washing and sodium chloride. *J. Food Qual*., 12, 155-156.

Hultin H.O., Feng Y.M., Stanley D.W.A., 1995. Re-Examination of Muscle Protein Solubility. *Journal of Muscle Foods*,6, 91-107.

Ingadottir B., 2004. The use of acid and alkali-aided protein solubilization and precipitation methods to produce functional protein ingredients from Tilapia. A Thesis presented to the graduate school of the University of Florida in partial fulfillment of the requirements for the degree of Master of Science, 105p.

Ionescu A., Berza M., Banu C., 1992. Metode şi tehnici pentru controlul peştelui şi produselor din peşte. Editura Universităţii din Galaţi, 238p.

Ionescu A., Aprodu I., Alexe P., 2009. *Tehnologii generale – Tehnologie şi control în industria* cărnii, Galaţi University Press, 123 p.

Lan Y.H., Novakofski J., McCusker R.H., Brewer M.S., Carr T.R., McKeith F.K., 1995a. Thermal gelation of pork, beef, fish, chicken, and turkey muscles as affected by heating rate and pH. *J. Food Sci*., 60, 936-940, 945.

Lan Y.H., Novakofski J., McCusker R.H., Brewer M.S., Carr T.R., McKeith F.K., 1995b. Thermal gelation properties of protein fractions from pork and chicken breast muscles. *J. Food Sci*., 60, 742-747.

Samejima K., Ishioroshi M., & Yasui T., 1981. Relative roles of the head and tail portions of the molecule in heat-induced gelation of myosin. *Journal of Food Science*, 46, 1412-1418.

Sharp A., Offer G., 1992. The mechanism of formation of gels from myosin molecules. *J. Sci. Food Agric*., 58, 63-73.

Smith D.M., Alvarez V.B., Morgan R.G., 1988. A generalized model for predicting heat-induced chicken myofibrillar protein gel strength. *J. Food Sci*., 53, 359-362.

Stone D.W., Stanley A.P., 1992. Mechanisms of fish muscle gelation. *Food Res. Int*., 25, 381-388.

Westphalen A.D., Briggs J.L., & Lonergan S. M., 2005. Influence of pH on rheological properties of porcine myofibrillar protein during heat induced gelation. *Meat Science*, 70, 293-299.

Xiong Y.L., 1997 Structure-Function Relationship of Muscle Proteins. In *Food Proteins and Their Applications*; S. Damodaran and A. Paraf, Eds.; Marcel Dekker: New York, 341-392.

Xiong YL., 1993. A comparison of the rheological characteristics of different fractions of chicken myofibrillar proteins. *J. Food Biochem*., 16, 217-227.

Xiong Y.L., 1992. Thermally induced interactions and gelation of combined myofibrillar proteins from

white and red broiler muscles. *J. Food Sci*., 57, 581-585.

Yasui T., Ishiroshi M., and Samejima K., 1980. Heat-induced gelation of myosin in the presence of actin. *J. Food Biochem*., 4, 61-78.

Yongsawatdigul J.; Park J.W., 2004. Effects of Alkali and Acid Solubilization on Gelation Characteristics of Rockfish Muscle Proteins. *Journal of Food Science*, 69, 499-505.

Zadow J.G., 1992. Whey and Lactose Processing. *Elsevier Applied Science*, London, 449-460.

Zayas F.J., 1997: *Functionality of proteins in Food*: ed. Springer, 373p.

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ANALYSIS OF THE IMPACT OF RUSSIAN EMBARGO ON THE EU AGRICULTURAL AND FOOD SECTOR

Vasile DREVE[1], Ion CĂLIN[1], Bogdan BAZGĂ[2]

[1]Faculty of Animal Sciences, 011464 Bucharest 59 Mărăşti Boulevard, District 1, Romania, Phone: +4021.318.25.64, Fax: + 4021.318.25.67, e-mail: drevevasile@gmail.com, icalin63@yahoo.com
[2]Institute of National Economy, 050711 Bucharest 13 September 13 Boulevard, District 5, Romania, Phone: +4021.318.24.67, e-mail: bogdan.bazga@gmail.com

Corresponding author e-mail:drevevasile@gmail.com,

Abstract

The Russian government adopted a list of products that are banned for a period of one year from the EU, United States, Norway, Canada and Australia. These products cover almost all milk and dairy products, meat products fruits and vegetables, as well as fish and crustaceans. These restrictions put a serious pressure on the European agri-food sector because of the temporary loss of a significant commercial market and because of possible cascade effects leading to oversupply on the internal market given the volumes involved and the quantity of perishable products banned in full harvesting season. Some sectors and Member States are more heavily affected - i.e. 31% of EU milk products export, 29% of fruits and vegetables export. The overall temporary restrictions currently applied by Russia potentially jeopardize 5 billion EUR worth of trade and affects the income of 9.5 million people in the EU working on the holdings most concerned.

Key words: *embargo, agri-food sector, oversupply, European Union, market.*

The global economy has entered into a new stage of geopolitical instability after the financial and economic crisis from 2007/2008, a stage with intensified economic uncertainty. The sanctions imposed on Russia by the European Union and also by the United States, Australia and Norway, have led the Russian authorities to impose their own restrictions in return.

On 7th August, The Russian Federation imposed an embargo on agricultural products from Europe, the United States, Canada, Australia and Norway as an answer to the sanctions in the context of the situation in Ukraine. The restrictions have been issued for a period of one year and cover almost all meat products (beef, pigmeat, poultry, and certain sausages), milk and dairy products, fruits and vegetables, as well as fish and crustaceans. Some processed agricultural products are also banned (Cenusa et al., 2014).

Exceptions are introduced for goods destined to baby food, certain animal products (fat, meat offal) and live animals, as well as preparations in the fruit and vegetables sector (such as fruit juices, canned fruit or prepared vegetables). Since end of August, lactose-free milk and milk products, seed potatoes, onion sets, hybrid sweet corn and nutritional supplements are also lifted from the ban.

Although the latest bans add to a long list of import restrictions already in place, the scope of the bans, involving a large range of products from the main exporters to the Russian market raised concerns that supplies of key commodities to the Russian market would be further constrained, with negative implications for Russian consumers across all income levels, at least in the short run.

Russia is one of the world's largest markets for agricultural and food products, being the fifth largest importer after EU, US, China and Japan. As reported by FAO, Russia represents the second most important destination for EU agri-food exports (after the USA) and according to the European Institute for Statistics it absorbs 10% of EU's agricultural and food exports. The agri-food products recently banned by The Russian Federation represent a total value of 5.06 billion Euros (i.e. 43% of EU agri-food

exports to Russia), while agricultural and food exports from the EU to Russia annually reach about 11.8 billion Euros (Table 1).
The EU agriculture and food sector is put under serious pressure because of the temporary loss of a significant commercial market in main agricultural sectors (almost all meat products, milk and dairy products, fruits and vegetables).An oversupply on the internal market is likely to happen, given the volume involved and the quantity of perishable products banned.

Table 1.Agri-food exports of the main member state towards Russia in 2013 (million Euros)

	Agri-food exports	**Products under embargo**
Lithuania	1374	922
Poland	1267	840
Germany	1649	589
The Netherlands	1551	503
Denmark	627	341
Spain	572	326
Belgium	558	281
Finland	464	273
France	756	229
Italy	705	163
Greece	158	114
Austria	247	103
Hungary	266	77
Ireland	216	70
Estonia	228	60
EU-28	**11864**	**5064**

Source: www.ec.europa.eu

In the case of sudden drop in demand – or oversupply, markets will react and this could lead to a price decrease that might exceed the level at which prices tend to stabilise after the shock is absorbed. The current theory is applicable for perishable products, that are non-storable, and also for those that are at the early stage of the marketing year, when prices are at the highest under normal conditions.
An immediate negative impact on prices is already felt in some sectors and this is both related to oversupply situations (e.g. rerouting ofseasonal products and perishable towards the EU markets, combined with difficulties in identifying alternative markets) and to "psychological" effects of the announcement of the ban.
According to several analysis made so far by different bodies it is shown that not only countries which were traditionally exporting substantial quantities to Russia are affected, and that also oversupply may spread into the internal market.
A significant external trade disruption will take time and may imply costs for private operators and producers on short or medium term.
The current restrictions are affecting in the first place the perishable products sector. Taking into account that Russia is considered an important destination for EU fruit and vegetables and that for many products the harvest was ongoing or about to start in the EU, this effect is immediate.
The EU fruit and vegetables sector represents about 17% of the total agricultural output value, of which 10% corresponds to vegetables and the remaining 7% to fruits. In most of the southern Member States this sector has a higher importance and it represents between one third and one quarter of their total agricultural output (on average for the period 2011-2013, more than 30% in Greece, Cyprus, Malta and Portugal, and between 25% and 30% in Spain, Italy and Romania).
Only 15% of the total fruit production and 7% of the vegetables production is exported in third countries, while most of the EU's production of fresh fruit and vegetables is consumed internally. Russia absorbs only 5% of total EU fruit production and 2% of the vegetable production (about 9% of the production of pears and kiwis, 6-7% of apples and nectarines and 3-4% of the production of peaches and mushrooms).
The Russian market represents however one of the main export markets for the EU's fruit and vegetables sector, currently purchasing about 34% of our fresh fruit exports (1.225 million EUR) and 26% of the fresh vegetable exports (734 million EUR). This market was the top destination for products like: cherries, mushrooms and cabbages (70% of extra-EU exports, in quantities), pears, peaches, eggplants, tomatoes and carrots (60-64%), nectarines, strawberries, apples and cucumbers (around 50%), and apricots, potatoes and sweet peppers (40%).Also 30% of lemons and table grapes exports are sent to Russia.
Poland, Spain, Greece, Italy and Belgium are the main EU fruits suppliers and the Netherlands, Poland, Spain and Belgium are the

main vegetables suppliers (Table 2). Lithuania is considered an important channel for re-exports to Russia of fresh fruit and vegetables imported from other EU producers such as Spain, the Netherlands and Germany; Poland and Belgium play a similar role for specific products.

According to latest rumours Russia intends to purchase vegetables and fruit in particular from Turkey, Serbia, Azerbaijan and Uzbekistan.

Table 2. Production, total exports extra-EU and exports to Russia of fresh fruit and vegetables – average 2011-2013

Quantities	Production		Exports extra EU		Exports to Russia			
	1000 tonnes	% of EU-28	1000 tonnes	% of production	1000 tonnes	% of EU-28	% of production	% of exports extra_EU
FRESH FRUIT								
Belgium	545.3	1.6	197.9	36.3	171.8	12.9	31.5	86.8
Greece	3076.0	8.9	313.3	10.2	92.9	7.0	3.0	29.6
Spain	8511.0	24.6	415.0	4.9	133.4	10.0	1.6	32.1
France	2871.0	8.3	267.4	9.3	29.0	2.2	1.0	10.8
Italy	9766.0	28.2	497.4	5.1	85.1	6.4	0.9	17.1
Lithuania	59.6	0.2	206.1	346.1	167.0	12.6	280.4	81.0
Netherlands	681.0	2.0	134.9	18.9	55.1	4.1	8.1	40.9
Poland	3284.0	9.5	860.8	26.2	549.7	41.3	16.7	63.9
Romania	1379.0	4.0	2.6	0.2	1.1	0.1	0.1	41.2
FRESH VEGETABLES								
Belgium	4300.6	4.4	80.3	1.9	56.3	7.3	1.3	70.1
Germany	12642.6	12.8	94.2	0.7	33.5	4.3	0.3	35.5
Spain	9871.4	10.0	134.5	1.4	41.9	5.4	0.4	31.1
France	9435.4	9.6	76.8	0.8	17.4	2.3	0.2	22.7
Italy	13075.8	13.3	26.1	0.2	2.1	0.3	0.0	8
Lithuania	733	0.7	211.1	28.8	192.2	24.8	26.2	91
Netherlands	10997.3	11.2	1227	11.2	261.3	33.8	2.4	21.3
Poland	12316.4	12.5	215.5	1.7	124.7	16.1	1.0	57.9
Romania	5168.4	5.3	3.1	0.1	0.3	0	0.0	10.3
U.K.	6760.2	6.9	13.4	0.2	5.4	0.7	0.1	39.8

Source:www.ec.europa.eu

The EU milk production is around 20% of the world production, approximately 153 million tonnes of milk were produced in 2013. This sector represents 15% of the total EU agricultural output with a value of production close to 55 billion EUR. Germany and France are the main producers covering around 40% of EU's production and they are followed by United Kingdom, Poland, the Netherlands and Italy.

Only 11% (in milk equivalent) from the EU's dairy production is exported while the rest is consumed domestically. Cheese is the main dairy product obtained, using 50% of EU's milk production and only 8% of the EU cheese production is exported. The main exported milk products are powders (50%), butter (6%) and fresh dairy products. The Netherlands, France, Germany, Belgium, Poland and Denmark are exporting more than a million tonne of milk equivalent each and they gather more than 70% of the EU exports (Table 3).Russia's main suppliers are the EU followed by Belarus, both supplying 80% of the Russian dairy imports.

EU exports to Russia only account for 1.4% of the EU cow's milk production in milk equivalent, which is a significant share of the total 11% of exported EU milk production. The Baltic countries have the highest export share: Finland (22%), Lithuania (14%), Estonia (8%) and Latvia (5%)[1]. It is to be noted that the share is much higher in the case of cheese 32% and butter 24%. Finland, Germany, the Netherlands, Lithuania, Poland and France being the main EU exporters to Russia.

Table 3. Production, total exports extra-EU and exports to Russia of milk and milk products

MILK	Average 2011-2013 Quantities							
	Production		Exports extra-EU		Exports to Russia			
	million tonnes	% of EU-28	million t. in milk eq.	% of production	million t. in milk eq.	% of EU-28	% of production	% of exports extra EU
EU-28	**152.4**	**100**	**16.5**	**10.8**	**2.1**	**100**	**1.4**	**12.8**
Germany	30.8	20.2	2.1	6.7	0.4	19.3	1.3	19.7
Estonia	0.7	0.5	0.1	9.9	0.1	2.7	7.9	80.1
France	24.7	16.2	3.1	12.4	0.2	7.2	0.6	4.9
Italy	11.4	7.5	0.5	4.4	0	1.7	0.3	7.1
Lithuania	1.8	1.2	0.4	20.5	0.2	11.7	14	68.6
Netherlands	12	7.9	3.2	26.8	0.3	14	2.5	9.1
Poland	12.6	8.3	1.2	9.7	0.2	8.3	1.4	14.5
Finland	2.3	1.5	0.8	32.7	0.5	24	22	67.3
U.K.	14	9.2	0.5	3.3	0	0.4	0.1	1.8

Source: www.ec.europa.eu

Cheese is the most affected product by the ban and the exports to Russia accounted for close to one third of extra-EU exports of cheese. Since 2011, EU exports of cheese to Russia have increased by 24%, more rapidly than the total EU cheese exports. For Finland and the Baltic countries Russia is an exclusive trading partner with regard to cheese(about or over 85% of these countries' cheese exports), which also represents for each of these countries around or

[1] Some of these exports can originate from other EU Member States.

above 20% of the national cheese production. Other main cheese exporters to Russia are represented by: Netherlands with 42% (about 8% of national production), Germany with 38% (about 2% of national production) and Poland with 43% (corresponding to less than 4% of national production).

Regarding the butter production approximately 6.1% of was exported outside the EU and 1.5% to Russia (31.6 thousand tonnes). Half of the butter exported to Russia comes from Finland (17 000 tonnes covering 95% of Finland's butter exports). Close to 50% of the powder production was exported outside the EU and only 1% to Russia (19 000 tonnes).Germany, France and the Netherlands are covering 53% of the total EU production. Although Russia is a minor destination of the EU powder exports, the prices have decreased significantly since the implementation of the embargo (by 19% for SMP and 15% for WMP).

Since early 2014, a downward pressure had been registered on prices for dairy products, due to increased supply both in the Union and in the main milk producing regions of the world. European average prices remain above intervention levels. At Member States' level, the situation is variable: those Member States who are the primary suppliers of dairy products to Russia undergo deeper price drops.

Almost all the dairy products designed for the Russian market have to find their way on the internal market, increasing pressure on European prices. As an additional burden to this immediate impact on the internal market, while looking for alternative outlets, some of the volume of milk that would have been used for cheese production will have to be channelled to butter and powder production, increasing the risk of unbalancing those markets.

At world level, EU is the third largest producer of beef/veal (12%) after the USA (17%) and Brazil (13%) and before China (10%). The share of EU in world beef/veal trade amounts to 2% both in exported quantities and value while it represent 5% in imported quantities and 9% in value. Due to a weak internal demand and a declining production, the beef sector has been facing structural difficulties. Over the last ten years EU beef production decreased by more than 1 million tonnes due to the combined effect of the impact of animal diseases (Bovine Spongiform Encephalopathy - BSE, Food and Mouth Disease - FMD), higher feed costs and policy changes, and structural decrease of the dairy herd associated to increasing milk yields. After a period of two years with high prices in 2012 and 2013, average prices for adult male bovine have decreased in the first six months of 2014. Beef represents 8% of the total EU-28 agricultural output value.

The main producing Member States are France, Germany, Italy, the United Kingdom, Spain and Ireland (producing 5.6 million tonnes, approx. 72% of the EU-28 beef production and 75% of the value of beef production). With a very limited domestic production (around 40 000 tonnes) Lithuania is an important channel for re-exporting beef of different EU origins. (Table 4)

Table 4.Production, total exports extra-EU and exports to Russia of beef

Beef	Average 2011 -2013 - Quantities					
	Production		Exports extra_EU		Exports to Russia	
	1000 tonnes	% of EU-28	1000 tonnes	% of production	1000 tonnes	% of production
EU-28	7716.4	100.0	232.8	3.0	67.6	100.0
Denmark	128.9	1.7	8.6	6.7	5.4	7.9
Germany	1145.7	14.8	41.6	3.6	11.3	16.7
Estonia	10.2	0.1	0.1	0.7	0.0	0.0
Ireland	519.9	6.7	11.2	2.1	5.0	7.3
Spain	592.1	7.7	15.6	2.6	7.2	10.6
France	1494.9	19.4	17.0	1.1	1.2	1.8
Italy	948.5	12.3	18.0	1.9	7.0	10.3
Latvia	17.2	0.2	0.1	0.2	0.0	0.0
Lithuania	40.2	0.5	16.7	41.6	14.9	22.0
Poland	371.6	4.8	61.6	16.6	11.8	17.5
Finland	82.0	1.1	0.1	0.1	0.0	0.0
U.K.	888.6	11.5	5.8	0.7	0.0	0.0

Source: www.ec.europa.eu

Even before the introduction of the current ban the EU meat sector has been confronted with sanitary related trade restrictions from Russia. The immediate impact of the current ban was limited due to relatively high producer prices for all meats, moderate feed costs and good export demand from other markets than Russia.

The first destination of EU exports for beef meat is Russia, rising to 25% of all EU beef meat exports. Breeding animals and low value products (offal, trimmings and fats) are EU's most relevant beef products for the Russian market. France, Spain, Ireland and Italy made up for around 30% of total EU exports to

Russia, despite being major beef producers. British beef has been banned on BSE grounds since 1986. The products affected directly by the ban represent less than 1% of EU production. The ban affects the export of fresh, chilled and frozen beef but not the exports of live animals, offal or fats. In order to avoid significant drops in prices, which could generate an additional drop in European production, it is essential to search for alternative export markets. Asia can be an alternative market for EU beef exports and needs to be encouraged.

The EU is the main world pig meat exporter and the world's second producer of pig meat with a share of 20%from the global production, after China (with a share of 47%).The EU exports around 3.2 million tonnes of pig meat (fresh, frozen, salted meat, offal, fats and preparations) to the extra EU countries with main destinations as Russia (24% of pig meat exports) followed by China (17%), Hong-Kong (14%), Japan (8%) and South Korea (5%).The pig sector accounts for approximately 9.5% in total EU-28 agricultural output value. Germany, Spain, France, Italy, Poland, Denmark and the Netherlands are the EU's main producer countries, representing altogether around 76% of EU-28 value of pig production at producer prices. These countries altogether are supplying the Russian market with 80% of EU pig meat exports to Russia.

Since February 2014 EU pig meat exports to Russia are banned after the discovery of a few cases of African swine fever (ASF) in the wild boar population in Lithuania and Poland. The Commission started a WTO procedure against this unjustified measure. Therefore, pig prices did not show any reaction after the announcement of the new Russian embargo. Products affected directly by the ban represent 1.9% of EU production. Most exported products to Russia are the frozen meat (43%), lard (32%) and offal (15%).

Through increased exports to Japan, South Korea and the Philippines operators have been able to partially absorb the drop of exports to Russia. The Russian ban now also affects other pig meat exporters on the world market (USA and Canada) that are now looking for new outlets.

The least affected of the meats sector is poultry meat, being a well-integrated sector with a short cycle of production (less than 2 months for broilers).In the last years, the poultry meat sector has been growing as demand is increasing worldwide. The European Union is a large producer of poultry meat (around 12% of world production), after the USA (19%) and China (17%) and a net exporter of poultry products with a self-sufficiency rate at 104%. France, the UK, Poland, Germany, Spain and Italy are the leading producers, altogether producing 70% of total EU poultry meat. Russia ranks on fifth place in EU export destinations with 8%, mainly fresh and frozen and exports amount to 0.7% of EU. The EU broiler price even increased after the Russian ban to 194 €/100 kg.

CONCLUSIONS

According to the European Parliament, the impact on GDP is supposed to be modest for almost all EU-countries, despite considerable values affected by the food ban, as agriculture accounts for a decreasing and low part of the EU's GDP (1.7 % in the EU 27, OECD 2012).

The risk of a negative macroeconomic impact is highest for Lithuania (2.6 % share of exports to Russia in GDP 2013) followed by Estonia (0.4 %) and Latvia (0.3 %); assuming that no alternative markets can be found. For seven further EU-countries, the share of exports in GDP was 0.1 % in 2013 (Belgium, Finland, Greece, Hungary, Ireland, Netherlands and Cyprus).

Even if the national overall share in national GDP is zero, in countries showing a considerable absolute value of food exports to Russia, certain regions with a high concentration of agriculture may be affected as well as the related exporters and transport services.

Within the one-year period for which the food embargo has been imposed short-term losses could be considerable and a full compensation is not very probable.

Additionally to the loss of external markets, producers are suffering even more from income losses due to falling prices on the internal market as banned (perishable) agricultural products are being offered for domestic use. According to Copa-Cogeca prices in the EU

fruit and vegetable and dairy sectors have decreased by over 50 % in some Member States in early September, and milk prices were down by up to 30 %.

Several countries consider job losses as probable, while there is, so far, no evidence to corroborate this. For example: in Belgium, the Meat association estimates 500/5000 job losses due to the Russian embargo; for Greece, economists state that the food ban will hit Greek farmers hard and that seasonal workers will need alternative employment.

The status-quo can be explained trough a number of factors which have implications for the future monitoring of changes in the employment situation due to the Russian food embargo:

- In EU-28 most farm work is carried out by the farmers and members of their family, mainly spouses (92.2 %), whilst many farmers combine farm work as minor activity with work as employee.
- Even if the loss of the Russian market will cause job losses in the future, numbers may be too small to be reflected in national aggregate labor market statistics, as the share of agriculture in GDP is low in most countries. Agricultural and food products only count for 1.7 % of GDP, 6.6 % of all EU exports and 4.5 % of employment (2012). According to initial estimates by ING Group NV from 22 August 2014, the Russian food ban could cost the EU 130,000 jobs corresponding to an increased unemployment rate of approximately 0.1 %.

The sanctions directed at the financial sector by the EU, together with Russia's weak economic development, have tightened the financial situation in Russia and are particularly impacting sales of the EU's technology industry's investment goods in Russia. In addition, the uncertainty increased by the Ukraine crisis is weakening the investment climate in Russia. Decline in demand for investment products in Russia will impact EU's economy and exports more extensively than the import restrictions imposed by Russia on the food sector.

Overall, the review of accessible European and national information within the scope of this article shows that a targeted in-depth analysis of affected sectors and regions would be necessary to detect the real impact of the Russian food ban on the employment situation, which might be limited to certain regions and specialized exporters.

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REFERENCES

Cenusa D., Emerson M., Kovziridse T., Movchan V., 2014. Russia's Punitive Trade Policy Measures towards Ukraine, Moldova and Georgia, Centre for European Policy Studies, Brussels.

http://ec.europa.eu/agriculture/russian-import-ban/pdf/info-note-03-09_en.pdf- Information note on the Russian ban on agri-food products from the EU

http://www.fao.org/3/a-i4055e.pdf- Russia's restrictions on imports of agricultural and food products: An initial assessment

http://ec.europa.eu/agriculture/russian-import-ban/pdf/dairy-production_en.pdf - Analysis of the EU dairy sector: EU production and exports to Russia (2011-2013)

http://ec.europa.eu/agriculture/russian-import-ban/pdf/fv-production_en.pdf - Analysis of the EU fruit and vegetables sector: EU production and exports to Russia

http://www.europarl.europa.eu/RegData/etudes/BRIE/2014/536291/IPOL_BRI%282014%29536291_EN.pdf- The Russian Embargo: Impact on the Economic and Employment Situation in the EU

http://www.momagri.org/UK/focus-on-issues/The-Russian-Embargo-direct-and-indirect-impact-for-French-agriculture_1498.html- The Russian Embargo: direct and indirect impact for French agriculture

http://www.chambres-agriculture.fr/fileadmin/user_upload/Revue/Article/Revue_1036/1036_Embargo_Russe.pdf- Impacts directs et indirects pour l'agriculture Française

http://www.coface.com/Economic-Studies-and-Country-Risks/Russian-Federation

http://valtioneuvosto.fi/tiedostot/julkinen/pdf/2014/budjettineuvottelut-2014/eu-venaja-pakotteet/en.pdf- Effects of the Russian embargo on the Finish economy.

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RESISTANCE OF *Lactobacillus sp.*ISOLATED FROM BOVINE COLOSTRUM THAT ENCAPSULATED ON VARIOUS FORMULA TOWARDS ACIDIC CONDITIONS AND BILE SALTS AS PROBIOTIC CANDIDATE

Khusnul KHOTIMAH[1], Karina Restu FITRIANA[2], Ratu SAFITRI[2], Mia MIRANTI[2], Lobo Balia ROOSTITA[3], Hartati CHAIRUNNISA[3]

[1]Agriculture and Animals Husbandry Faculty, Muhammadiyah Malang University, Malang
[2]Biology Department, Mathematics and Science Faculty, Padjadjaran University, Jatinangor
[3]Animals Husbandry Faculty, Padjadjaran University, Jatinangor

Corresponding author email: thuthul17@yahoo.com

Abstract

Research about characterization of probiotic Lactobacillus sp. origin from bovine colostrum in some types of formula encapsulation has been done. The research method was carried out experimentally using a factorial Completly Randomized Design with two factor: a x b, the value of a and b depend on the level of each treatment, which a was bacteria that encapsulated in some types of formula and b was characterization of probiotic test, with three times repetition. The data were analyzed using analysis of variance with a level of 95%, followed by Duncan's Multiple Range Test. The results showed that L. paracasei in alginate tapioca and L. curvatus in alginate skim had high viability during storage of 4 weeks with each of the bacterial population were 2,98 x 1010 CFU/ml and 2,25 x 1010 CFU/ml. Bacteria L. curvatus encapsulated in alginate skim had the highest resistant to the acidic environment of pH 2 and 4 with each of the bacterial population were 1,02 x 103 CFU/ml and 3,69 x 105 CFU/ml and resistant to bile salt concentration of 0.3% and 0 , 5% with each of the bacterial population were 3,5 x 1010 CFU/ml and 2,4 x 1010 CFU/ml. The supernatant of L. curvatus in alginate skim also had the highest antimicrobial activity against pathogenic bacteria Escherichia coli and Salmonella typhimurium with each inhibitory diameter was 20.83 mm and 12.67 mm. Encapsulation formula, especially the combination of alginate and skim milk, can protect the probiotic during processed, storage, and applied in digestive tract.

Key words: *encapsulation, Lactobacillus, probiotik iv.*

INTRODUCTION

Bovine colostrum is pre-milk fluid produced by cows in the first 24-48 hours after birth and several types of microorganisms that have been widely used as probiotics found on it (Brandano, et al., 2004; Lindner, et al., 2011). Probiotics are living microorganisms that are beneficial to humans, especially in maintaining health and preventing disease. They can live in digestive tract to control the balance of gut microbial and has characteristics such as non-pathogenic, resistant to stomach acid conditions, resistant to bile salt concentration in the intestine, producing organic acids, and also has antimicrobial properties against pathogens digestion (Salminen and von Wright, 2004). One of the probiotic bacteria found in bovine colostrum is *Lactobacillus sp.* (Anal and Singh, 2007). The bacteria have the ability to attach to host cells, remove or reduce pathogenic bacteria, produce acid, hydrogen peroxide and bacteriocins which able to inhibit the growth of pathogenic bacteria (Rizqihati, et al., 2009).

Due to environmental factors that are less conducive for probiotic bacteria to survive, viability of probiotic bacteria in product should reach 10^7-10^9 CFU/ g. Low pH of 1.5 to 2.0 for an empty stomach or pH of 4.5 to 5.0 for filled stomach and presence of bile salts in the small intestine which must pass by the bacteria would decrease the viability while in the digestive tract or even during storage. Bile salt concentration equivalent to physiological concentrations of bile salts in the duodenum is 0.5% (Puspawati, 2010). While all microbial that could live in 0.3% bile salt was resistant to bile salts in the small intestine (Wijayanto, 2009).

Encapsulation is a process of core material coating, in this research the probiotic bacteria. Specific encapsulation material used to maintain viability and protect probiotics bacteria from damage due to the environmental conditions (Rizqihati et al., 2009). The encapsulation

material that often used is alginate. Alginate used to protect or wrap core material from unfavorable environmental factors (Desmond, et al., 2002). The advantages of alginate use is easy to form gel matrix overlying bacteria, safe to consumed, easy to obtain, and can release the trapped cells. However, alginate also has the disadvantage that vulnerable in an acidic environment. Therefore, in order to optimize the alginate encapsulation process, combination of alginate with a variety of other polymer compounds needed. The addition of starch, skim milk, gelatin, and chitosan combined with alginate can provide better resistance on encapsulation (Chavarri, et al., 2013).

In this study, resilience of probiotic bacteria *Lactobacillus sp.*, which encapsulated with various encapsulation formula tested to acidic conditions and high bile salts to determine the best encapsulation material formula that can be select for encapsulation process on probiotic bacteria.

MATERIALS AND METHODS

The tools used in this study are centrifuge Hermle Z 300, syringe, filter, and vortex. Materials used are alginate acid (sodium salt) from Sigma, acetic acid, *Lactobacillus paracasei* and *Lactobacillus curvatus* isolated from cow colostrum, bile salt (Oxoid), phosphate buffer, 0.1 M $CaCl_2$, Nutrient Agar (NA), Nutrient Broth (NB), skim milk and tapioca.

Biomass Production (Puspawati, 2010)

Lactobacillus sp. biomass production obtained by sub-culture the isolate on NB for 24 h at 37°C. Sterilized NB inoculated by the 10% culture and then incubated at 37°C for 18-20 hours. Culture were centrifuged at a speed of 5000rpm for 10 minutes. Supernatant separated with the filtrate to obtained biomass.

Encapsulation with Alginate (Purwandhani, et al., 2007)

Bacterial biomass suspended in physiological NaCl at MacFarland turbidity three then counted by Total Plate Count (TPC) to determine the number of bacteria before encapsulated. Three ml cultured *Lactobacillus paracasei* and *Lactobacillus curvatus* re-suspended in 10 ml of sterilized NaCl then 60 ml of Sodium Alginate 3% (w/v) were added. After mixing dropped into 200 ml of $CaCl_2$ 0.1 while stirring with a magnetic stirrer then washed with sterilized 0.8% NaCl and dried.

Encapsulation with Skim Alginate (Purwandhani, et al., 2007)

Isolates of *Lactobacillus paracasei* and *Lactobacillus curvatus* subcultured in 2.5 ml NB incubated for 9 hours, then re-subcultured in consortium twice at 25 ml and 250 ml of skim milk and incubated for 9 hours. The inoculum of bacteria in skim milk centrifuged at 5000 rpm for 10 min at 4°C. Biomass obtained suspended in 100 ml distilled water then mixed with 100 ml of 3% alginate as carrier material. The mixture then put into a syringe and dropped into $CaCl_2.2H_2O$ 0.1 mol/l with 10 cm distance from syringe tip and the surface of CaCl2.$2H_2O$. Hold for an hour so that the grains of the encapsulation yields harden while kept on a shaker. The encapsulation pellets yields rinsed with sterilized 0.8% NaCl and sterilized distilled water, then dried.

Encapsulation with Tapioca Alginate (Wijayanti, 2010)

Sterilized sodium alginate as much as 1% (w / v) dissolved into 90 ml of distilled water followed by addition of 3% (w / v) tapioca powder. Ten percent biomass consortium aseptically inoculated into 90 ml of coating material formula. The mixture of biomass consortium and coating material dropped into 0.1 M $CaCl_2.2H_2O$ and then washed twice using sterilized distilled water.

Ca-alginate capsule that washed inoculated into NB and incubated for 24-30 h in a rotary shaker with speed 150 rpm at room temperature for secondary multiplication. Washed twice using sterilized distilled water and then dried.

Acid Tolerant Test (Lian, et al., 2003)

One gram encapsulated isolates of *Lactobacillus paracasei* and *Lactobacillus curvatus* grown at 9 ml NB with pH 2, pH 4, pH 6 and control. Vortexed and incubated at 37°C for 6 hours then centrifuge at 5000× g for 10 min at 4°C and washed twice with phosphate buffer. Pellet diluted in 10 ml of sterilized distilled water then poured into NA and incubated at 37°C for 48 hours then the number of colonies counted.

Bile Salt Tolerant Test (Lian, et al., 2003)

One gram encapsulated isolates inoculated into 9 ml NB that bile salt added (0.3% and 0.5%) and

control. Vortexed and incubated at 37 ° C for 6 hours, centrifuged at 5000 × g for 10 min at 4°C then washed twice with phosphate buffer. Pellet diluted in 10 ml of sterilized distilled water then poured into NA and incubated at 37°C for 48 hours then the number of colonies counted.

Data Analysis

Data analyzed using analysis of variance with the level of 95% and treatment effect will analyzed by Duncan's Multiple Range Test (DMRT).

RESULTS AND DISCUSSIONS

Probiotic candidate isolated from bovine colostrum

Two species of bacteria of *L. paracasei* and *L. curvatus* isolated from bovine colostrum on preliminary research. Both have proved as type of probiotic bacteria naturally found in the human digestive tract because their ability to survive in acidic stomach conditions and high concentration of bile in the intestine (Anal and Singh, 2007). Isolates of *L. paracasei* and *L. curvatus* encapsulated to maintain their probiotic characteristics while in the digestive tract.

Probiotic encapsulation

Encapsulation of probiotics is the process of wrapping probiotic bacterial cells using protective materials. Protective material used in this study is alginate, skim, and tapioca. Alginate used as a protective material because the ability to wrap core material, in order to protect from unfavorable factors (Desmond, et al., 2002). The use of alginate combined with another polymer compound such skim milk and tapioca. Skim milk was a good source of nutrients for microbes, especially sugars and proteins (Triana, et al., 2006). Tapioca also a good source of nutrients for microbes due to high carbohydrate content (Vidhyalakshmi, et al., 2009). The formulations used as encapsulation material in this study is alginate, alginate skim, and alginate tapioca that will form gel encapsulation containing bacterial biomass (Figure 1).

Gambar 1. The encapsulan gels of *Lactobacillus curvatus* and *Lactobacillus paracasei* in alginate formula, skim alginate and tapioca alginate

Before encapsulation process *L. paracasei* biomass as much as 1.55 x 10^{10} CFU / ml and *L. curvatus* as much as 1.21 x 10^{10} CFU / ml. Bacterial biomass produced mixed into the formula protective material. Mixture dropped into $CaCl_2$ solution to form gel encapsulation. Gel encapsulation formed by the reaction of sodium alginate with calcium chloride ($CaCl_2$) which causing gelatinization of calcium alginate gel-matrix occured. Encapsulan tested for acidic conditions and various bile salt levels resistance.

Encapsulated Probiotic Tolerance towards Acidic Environment

Test done to determine the encapsulated probiotic tolerance towards environment pH of 2, 4, and 6. Based on the analysis of variance, it known that there was an interaction between pH and the probiotic bacteria inside the encapsulation formula. Test continued with DMRT at 5% significance level and the results shown at Table 1.

Table 1. DMRT at 5% of encapsulated probiotic tolerance towards acidic environment
Description: The mean treatment followed by the same capital letter (vertical direction) and lowercase letters the same

pH	Bacteria (CFU/ml)					
	e_1	e_2	e_3	e_4	e_5	e_6
p_1	5,05 x 10^2 C b	4,33 x 10^2 c C B	6,12 x 10^2 c C ab	1,81 x 10^2 c C c	1,02 x 10^2 c a	1,08 x 10^2 c d
p_2	7,53 x 10^4 B C	2,84 x 10^5 b B ab	1,86 x 10^5 b b	2,17 x 10^5 b ab	3,69 x 10^5 b a	2,08 x 10^5 b b
p_3	4,03 x 10^8 A A	3,93 x 10^8 a A A	4,57 x 10^8 a a	3,26 x 10^8 a A	3,71 x 10^8 a a	3,86 x 10^8 a A a

(horizontal direction) are not significantly different according to DMRT 0.05

Table 1 showed that higher pH value gave higher number of probiotics colonies bacteria in the encapsulation material. Probiotic bacteria *L. paracasei* and *L. curvatus* that already encapsulated by the formula were optimally grew at pH 6. It is because *Lactobacillus* bacteria are optimally grew at the pH 5.5 to 6.2. Some can survive at low pH of 3.2, high pH of 9.6 and several of them only grows in a narrow pH range (4.0 to 4.5) (Wijayanto, et al., 2009).

Probiotic bacteria *L. curvatus* in skim alginate formula has high resistance towards acidic environment which shown by lower number of decreased bacterial colonies than another encapsulation formula, such as 5.556 cycles log CFU / ml. *Lactobacillus curvatus* in tapioca alginate formulas have highest colonies decreased among other encapsulated formula, namely 6.605 cycles log CFU / ml. The number of bacteria colonies declined because of low pH values (pH 2 and 4) which destruct the bacterial cell. Denaturing effect of enzymes that exist on the surface of cells, damage lipoposaccharide, outer membrane and cytoplasmic pH through increased membrane permeability causing bacteria cell destruction (Puspawati, 2010). Skim addition as protective material able to increased *L. curvatus* tolerance towards acidic environments than other materials. Skim milk composed by variety of complex materials such as lactose, casein, citrate and phosphate which able to act as buffer so that could protect the bacteria from acid and bile salts exposure (Puspawati, 2010).

Decreased of total bacteria was occured after 5 hours incubation in acidic media (Puspawati, 2010). Addition of skim milk, lactose and maltodextrin as protective material could increased tolerance of *L.brevis* A17 towards low pH (each declined of 5.37 log CFU/ml; 4.78 log CFU/ml; 5.13 log CFU/ml). While addition of all kinds of protective material on *L. rhamnosus* R21 could increased the tolerance towards low pH with the amount of decreased of 3.95 log CFU/ml in sucrose; 3.21 log CFU/ml in skim milk; 5.09 log CFU/ml on lactose; and 1.38 log CFU/ml in maltodextrin (Puspawati, 2010).

Encapsulated Probiotic Tolerance towards Various Bile Salt Concentrations

Test conducted to determine the probiotic bacteria tolerance in an environment that has a high concentration of bile salts (0.3% and 0.5%). Analysis of variance results showed that there were no interaction between concentration of bile salts towards encapsulated probiotic bacteria *L. paracasei* and *L. curvatus*. Various concentration of bile salts did not gave significant effect towards probiotic bacteria colonies number, so that DMRT was not carried out.

The results showed that the encapsulation formula has a high resistance to 0.3% and 0.5% bile salts after 6 hours incubation. Probiotic bacteria colonies in the encapsulation material shown approximately 10^{10} CFU / ml. *Lacto-bacillus sp.*could tolerates high concentrations of bile salts because the bacteria own bile salt hydrolase (BSH) enzyme. The enzyme was able to change physico-chemical abilities possessed bile salts so that not harmed the lactic acid bacteria (Puspawati, 2010). Bacteria tolerance towards bile salts thought to be caused by the role of polysaccharides as constituent of the cell wall

of gram-positive bacteria (Surono, 2000). In addition, the presence of lipid components in the membrane of gram-positive bacteria were also an important part to maintained the structure of membrane because fatty acids has role in lowered cell leakage caused by bile salts (Kimoto, et al., 2002).

Based on the results of encapsulated probiotic bacteria tolerance towards bile salt concentration of 0.3% and 0.5% (Table 2), *L. curvatus* in skim alginate formula has the highest average colony. Although the results did not have significantly different with other encapsulation materials, but it can concluded that the combination of skim and alginate was good formula in protecting bacteria against high concentration of bile salts. Skim milk contains complex nutrients that can act as buffer that protects the bacteria from high bile salts environmental conditions (Puspawati, 2010).

Table 2. Average colony of encapsulated *Lactobacillus paracasei* and *Lactobacillus curvatus* towards various bile salt concentration

Probiotic	Formula	Bile Salt	CFU/ml	Average
L. paracasei	Alginate	0.3%	3.50×10^{10}	2.97×10^{10}
			1.99×10^{10}	
			3.42×10^{10}	
		0.5%	3.74×10^{10}	3.10×10^{10}
			3.59×10^{10}	
			1.98×10^{10}	
	Skim-Alginate	0.3%	2.75×10^{10}	3.11×10^{10}
			3.30×10^{10}	
			3.16×10^{10}	
		0.5%	2.90×10^{10}	3.99×10^{10}
			3.18×10^{10}	
			3.35×10^{10}	
	Tapioca-Alginate	0.3%	4.36×10^{10}	3.11×10^{10}
			3.90×10^{10}	
			1.07×10^{10}	
		0.5%	3.60×10^{10}	3.14×10^{10}
			4.40×10^{10}	
			3.97×10^{10}	
L. curvatus	Alginate	0.3%	1.40×10^{10}	2.73×10^{10}
			3.89×10^{10}	
			2.90×10^{10}	
		0.5%	3.78×10^{10}	3.10×10^{10}
			3.30×10^{10}	
			3.84×10^{10}	
	Skim-Alginate	0.3%	2.55×10^{10}	4.42×10^{10}
			5.14×10^{10}	
			5.57×10^{10}	
		0.5%	4.68×10^{10}	4.43×10^{10}
			5.21×10^{10}	
			3.40×10^{10}	
	Tapioca-Alginate	0.3%	4.04×10^{10}	3.80×10^{10}
			3.57×10^{10}	
			3.80×10^{10}	
		0.5%	4.18×10^{10}	3.12×10^{10}
			0.85×10^{10}	
			4.34×10^{10}	

CONCLUSIONS AND SUGGESTIONS

Conclusions

Lactobacillus paracasei and *Lactobacillus curvatus* colostrum origin that encapsulated in various formulas could tolerates acidic conditions and high levels of bile salts. Skim-alginate encapsulation formula was the best coating material that could maintained viability of bacteria towards acidic conditions and high levels of bile salts

Suggestions

To produce encapsulan gels that have high tolerance towards low pH and bile salts advisable to combine alginate with other polymer compounds such as skim, tapioca and others. The presence of second coating will protect bacteria better from unfavorable environmental conditions.

REFERENCES

Brandano P. S. P. G. Rassu. and A. Lanzu. 2004. Feeding dairy lambs. In: G. Pulina dan R. Bencini (Ed). Dairy Sheep Nutrition. CABI Publishing : Wallingford.

Lindner J. Marcela S. Caroline T.Y. Carlos R.S. and Erasmo N. 2011. Recovery and identification of bovine colostrum microflora using traditional and molecular approaches. Food Technol. Biotechnol. : 49 (3) 364–368.

Salminen. S and Atte von Wright. 2004. Lactic Acid Bacteria : Microbiology And Functional. 2nd Edition. Revised and Expanded. Marcel Dekker. inc : New York.

Anal. A.K. and Singh. H. 2007. Recent advances in microencapsulation of probiotics for industrial applications and targeted delivery. Trends in Food Science & Technology:18:240-251.

Rizqiati H. B.S.L. Jenie. N Nurhidayat. dan C.C. Nurwitri. 2009. Karakteristik mikrokapsul probiotik *Lactobacillus plantarum* yang dienkapsulasi dengan susu skim dan gum arab. J.Indon.Trop.Anim.Agric. : 34 (2).

Wijayanto. U. 2009. Analisis in vitro Toleransi Isolat Bakteri Asam Laktat Asal Daging Sapi Terhadap pH Lambung. pH Usus. dan Garam Empedu Sebagai Kandidat Probiotik bentuk. Bogor: Fakultas Peternakan IPB.

Puspawati. N.N. 2010. Penggunaan Berbagai Jenis Bahan Pelindung Untuk Mempertahankan Viabilitas Bakteri Asam Laktat Yang Diisolasi Dari Air Susu Ibu Pada Proses Pengeringan Beku. J. teknol dan Industri Pangan: 21(1).

Desmond C.C.S. G.F.K. Collin. and R.P. Ross. 2002. Improved survival of *Lactobacillus paracasei* NFBC 338 in spray dried powders containing gum acacia. J of Appl Microbiol.; 93:1003-1012.

Chavarri M. Izaskun M. and María C.V. 2013. Encapsulation Technology to Protect Probiotic Bacteria. Chapter 23. Intech Open Science.

Purwandhani S.N. Made S. dan Endang S.R. 2007. Stabilitas thermal agensia probiotik l. Acidophilus snp 2 terenkapsulasi metode ekstrusi dan emulsi. Seminar Nasional Teknologi, ISSN : 1978 – 9777.

Wijayanti G. 2010. Viabilitas Azospirillum brasilense pada Enkapsulasi Menggunakan Campuran Natrium Alginat dan Tepung Tapioka . Semarang: Universitas Diponegoro.

Lian W.C. Hsio H.C. and Chou C.C. 2003. Viability of microencapsulated bifidobacteria in simulated gastric juice and bile solution. Int J Food Microbial: 86. 293-301

Gomez. K.A. dan Gomez A.A. 1995. Prosedur Statistik untuk Penelitian Pertanian. Edisi Kedua. Jakarta : UI – Press.

Triana E. Eko Y. dan Novik. N. 2006. Uji viabilitas *Lactobacillus* sp. mar 8 terenkapsulasi. Biodiversitas : 7(2) : 114-117

Vidhyalakshmi R. R. Bhakyaraj. and S. Subhasree. 2009. Encapsulation "the future of probiotics"-a review. Advances in Biological Research : 3 (3-4): 96-103.

Kimoto H. Ohmomo S. Okamoto T. 2002. Enhancement of bile tolerance in *Lactococci* by Tween 80. J. of Applied Microbiology ; 92: 41-46.

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BAT-BORNE ZOONOTIC VIRUSES RISK OF EMERGENCE IN EUROPE: HENDRA VIRUS, MENANGLE VIRUS AND NIPAH VIRUS

Stelian BARAITAREANU, Marius Alexandru CIOCANAU, Maria Rodica GURAU, Georgeta STEFAN, Doina DANES

University of Agronomic Sciences and Veterinary Medicine of Bucharest, Veterinary Medicine Campus, 105 Splaiul Independentei, District 5, Bucharest, Romania, Phone: +4021.411.11.22, Fax: + 4021.401.11.22, Email: danes.doina@gmail.com

Corresponding author email: doruvet@gmail.com

Abstract

The livestock's surveillance of bat-borne zoonoses, as the Paramyxovirus infections with Hendra virus, Menangle virus and Nipah virus, it's a new concern of national veterinary authorities all over the world. The high volume of commercial trade and the human travel between European countries and the countries were bat-borne zoonoses are endemic make a risk of virus introduction into the European continent. In this paper, we review the factors associated with bat-borne zoonoses risk of emergence in European free-countries in relation to the introduction of the ParamixovirusesHendra, Menangle and Nipah. Hendra virus proved to be pathogen for horses and humans, while Menangle and Nipah viruses for swine and humans. Until now, Hendra and Menangle viruses' transmission to the humans have been only after a close contact with infected animals. Transmission of Nipahvirus can be done by contact with human patients and infected animals (human-human transmission was proved). In the light of this data the Hendra, Menangle and Nipah viruses have a limited potential of introduction if the national authorities applies an efficient border control of all live animals imported from endemic areas and evaluate all suspect cases of human diseases. The natural reservoir of all three viruses are fruit-bat species (genus Pteropus), and the bat migration may be another rout of viruses introduction into Europe. The routes of Pteropus bats migration from the endemic territories to European countries have not been investigated, and are slight indication of major migration pathways into Europe. Also, fruit-bat species classified as host reservoir for specific zoonotic Paramyxovirus could transmit the virus to another bat species (e.g. Rousettus aegyptiacus may overlap with some migratory European bat species).
In conclusion, the risk of emergence of Hendra, Menangle and Nipah viruses into the European countries seems to be low, but the risk cannot be excluded until the completely investigation of the route of migration for all fruit-bat species with history of infection.

Key words*: epidemiology, infection, transmission, risk factors, surveillance.*

INTRODUCTION

The bat-borne zoonosis and the livestock's surveillance in this respect must be the new concern of the national veterinary authorities. It is already known that bats are reservoir hosts of several emerging viruses (Calisher et al., 2006), some of them have been already reported in Europe and other possess high potential for introduction into the EU (Simons et al., 2014).
The bats host a broad spectrum of viruses belonging to family *Arenaviridae* (Tacaribe virus), family *Bunyaviridae* (genus *Bunyavirus*, Catu virus, Guama virus, Nepuyo virus); genus *Hantavirus*, Hantaan virus; genus *Phlebovirus*, Rift Valley fever virus, Toscana virus; unassigned genus, KaengKhoi virus, Bangui virus), family *Coronaviridae* (SARS coronavirus, MERScoronavirus), family *Flaviviridae* (genus *Flavivirus*, Bukalasa bat virus, Carey Island virus, Central European encephalitis virus, Dakar bat virus, Entebbe bat virus, Japanese encephalitis virus, Jugra virus, Kyasanur Forest disease virus, Montana myotisleucoencephalitis virus, Phnom-Penh bat virus, Rio Bravo virus, St. Louis encephalitis virus, Saboya virus, Sokuluk virus, Tamana bat virus, Uganda S virus, Yokose virus),family Filoviridae (genus Ebolavirus, Bundibugyoebolavirus, Reston ebolavirus, Sudan ebolavirus, Taï Forest ebolavirus, Zaire ebolavirus; genus Marburgvirus, Marburg marburgvirus), family *Herpesviridae* (Agua Preta virus, cytomegalovirus of *Myotislucifugus*, Parixa virus), family *Orthomyxoviridae* (genus *Influenzavirus A*,

influenza A virus), family *Paramyxoviridae* (genus *Henipavirus*, Hendra virus, Nipah virus; genus *Rubulavirus*, Mapuera virus, Menangle virus, Tioman virus; undetermined genus, parainfluenzavirus of *Rousettus leschenaultia*), family *Picornaviridae* (Juruaca virus), family *Reoviridae* (genus *Orthoreovirus*, Nelson Bay virus, Pulau virus, Broome virus; genus *Orbivirus*, Ife virus, Japanaut virus, Fomede virus), family *Rhabdoviridae* (genus *Lyssavirus*, Aravan virus, Australian bat lyssavirus, Duvenhage virus, European bat lyssavirus 1, European bat lyssavirus 2, Irkut virus, Khujand virus, Lagos bat virus, Rabies virus; unclassified genus, Gossas virus, Kern Canyon virus, Mount Elgon bat virus, Oita 296 virus),family *Togaviridae* (genus *Alphavirus*, Chikungunya virus, Sindbis virus, Venezuelan equine encephalitis virus), Issyk-kul (Keterah virus), Mojui dos Campos virus, Yogue virus, Kasokero virus (Halpinet et al., 2000; Badrane and Tordo, 2001; Chua et al., 2001; Childs, 2004; Li et al., 2005; Leroy et al., 2005; Calisher et al., 2006; Kurth et al., 2012; Luby, 2013; Memish et al., 2013; Ithete et al., 2013; Simons et al., 2014).

Despite the large number of human pathogenic viruses which were isolated from bats, is quite possible that several undiscovered viruses are still hosted in bats, many of them in a silent way. Moreover, it has been established that bat-borne zoonotic viruses are significantly more than rodent-borne zoonotic viruses (Luis et al., 2013).

On the list of emerging zoonoses are some bat-borne paramyxoviruses: Hendra virus, Menangle virus and Nipah virus.This viruses have been identified in various bat species (fruit bats of the genus *Pteropus*) in Africa, Australia, South America or Asia. Also, three paramyxoviruses have been identified in insectivorous bats in Europe, but they are not phylogenetic related with *Pteropus*–paramyxoviruses (Kurth et al., 2012). Although to date has not been reported *Pteropus* – paramyxovirusesin free-ranging European bats, the inter-speciespilloverof viruses should be considered. The high volume of goods traded and the human travel frequency between European countries and countries were bat-borne zoonoses are endemic, rise a risk of virus introduction into the European continent.

In this paper, we review the factors associated with bat-borne zoonoses and their risk of emergence in European free-countries in relation to the introduction of the Paramixoviruses Hendra, Menangle and Nipah.

MATERIALS AND METHODS

In this study we correlated the results of 31 scientific papers that present epidemiological features of paramyxovirus infections with Hendra, Menangle and Nipah viruses.

The method of analyses consist in the evaluation of the introduction routes of Hendra, Menangle and Nipah viruses to the EU by human travel, by trade, by bat migration, by accidental transport of bats and by the expansion of bats areal following ecological and climate changes.

RESULTS AND DISCUSSIONS

Hendra virus have been shown to be pathogenic for horses and humans, while Menangle and Nipah viruses are pathogenic for pigs and humans (Wong et al., 2007).

Human travel

Transmission of Nipahvirus can be done by contact with human patients and infected animals (human-human transmission was proved) (Homaira et al., 2007), while the human cases of Hendra and Menangle viruses infection were associated only with animal contact (Philbey et al., 1998; Field H., 2009; Young et al., 2011).Therefore, the travellers (tourism, business, or migrations) could be the main route for transmission of Nipah virus into the EU. Bangladesh, India, Malaysia and Singapore had registered Nipah virus infections outbreaks in humans (Homaira et al., 2007; Harit et al., 2006; Clayton et al., 2012; Paton et al., 1999).

In our opinion, national authorities should apply efficient border control and should evaluate all suspect cases of human diseases related to people coming from high risk area, where active outbreaks of Nipah virus infections were reported. The recommendation is supported by MERS-CoV cases of human infections, another bat-borne disease, when the

European outbreaks were related with human travel. (ECDC, 2015).

Trade

The pramyxoviruses could be introduced in Europe with vegetable food products or live-animals and animal products and sub-products imported. Until present, only for Nipah virus was proved transmission by palm sap drinking, and for this reason it was assumed that all unprotected fruit grown in the endemic regions could be contaminated with saliva or urine of *Pteropus* bats (Rahman et al., 2008). Because only horses and pigs were involved in human transmission of this paramyxoviruses, in the live-animals trade should be restricted at least these species when active outbreaks were reported. If the national authorities applies an efficient border control of all live animals imported from endemic areas then Hendra, Menangle and Nipah viruses will have limited potential of introduction.

Bat migration

The natural reservoir of Hendra, Menangle and Nipah virus are fruit-bat species (genus *Pteropus*), and the bat migration may be another way of introduction of viruses into Europe. The median travel distance of the migratory bats is 860 km (Tsoar et al., 2011) and in some circumstances bat species from endemic areas can be in contact with European bat species (e.g. *Rousettusaegyptiacus* may overlap with some migratory European bat species). However, the routes of *Pteropus* bats migration from the endemic territories to European countries have not been investigated, and are slight indication of major migration pathways into Europe (Fleming and Eby, 2003; Hutterer et al., 2005).

Accidental transport of bats

Pteropus bats are often involved in aircraft strikes (Parsons, 2009), and transcontinental flights could bring in Europe infected dead bats. Also, bats could be transported in bilge waters of transoceanic ships. In both cases, the European airports and ports should take measures to collect and destroy the bat carcass remains.

Areal expansion of bats by ecological and climate changes

The adaptation of some bat species in response to climate changes has been reported (Lundy et al., 2010).

Deforestation, fragmentation, and urbanization of *Pteropus*bats habitat conducted to Hendra and Menangleviruses' emergence (Philbey et al., 1998; Daszak et al., 2001).

CONCLUSIONS

The risk of emergence of Hendra, Menangle and Nipah viruses into the European countries seems to be low, but cannot be ignored. The risk associated with each route of virus introduction cannot be assessed for entire Europe into unitary manner. The volume of human travel and trade are quite different from one country to another. Until today, the routes of migration for all fruit-bat species with history of infection have not been entirely investigated. Ecological changes can create different opportunities for expansion of bats habitat.

REFERENCES

Badrane H., Tordo N., 2001. Host switching in *Lyssavirus* history from the Chiroptera to the Carnivora orders, J. Virol., 75:8096–8104.

Calisher C.H., Childs J.E., Field H.E., Holmes K.V., Schountz T., 2006. Bats: Important Reservoir Hosts of Emerging Viruses. Clinical Microbiology Reviews,19(3):531-545.

Childs J.E., 2004. Zoonotic viruses of wildlife: hither from yon. Arch. Virol. Suppl. 19:1–11.

Chua K.B., WangL.F., LamS.K., CrameriG., YuM., WiseT., BoyleD., HyattA. D., EatonB.T., 2001. Tioman virus, a novel paramyxovirus isolated from fruit bats in Malaysia, Virology, 283:215–229.

Clayton B.A., Middleton D.,Bergfeld J., Haining J.,Arkinstall R., Wang L., Marsh G.A., 2012, Transmission routes for Nipah virus from Malaysia and Bangladesh,Emerg. Infect. Dis., 18:1983–1993.

Clayton B.A., Wang L.F., Marsh G.A., 2013. *Henipaviruses*: An Updated Review Focusing on the Pteropid Reservoir and Features of Transmission. Zoonoses Public Health, 60:69–83.

Daszak P, Cunningham A, Hyatt A., 2001. Anthropogenic environmental change and the emergence of infectious diseases in wildlife,ActaTropica, 78:103-16.

ECDC, 2015. Updated rapid risk assessment: Severe respiratory disease associated with Middle East respiratory syndrome coronavirus (MERS-CoV). Eleventh update, 21 August 2014. Available online:

http://www.ecdc.europa.eu/en/publications/Publications/Middle-East-respiratory-syndrome-coronavirus-Saudi%20Arabia-Qatar-Jordan-Germany-United-Kingdom.pdf (accessed on 11 Mars 2015).

Field H., 2009. Hendra virus infection risks. Neurology Asia, 14:77-78.

Fleming T.H., Eby P., 2003. Ecology of bat migration, Bat Ecol., 4:156-208.

Halpin K., YoungP.L., FieldH.E., MackenzieJ.S., 2000. Isolation of Hendra virus from pteropid bats: a natural reservoir of Hendra virus, J. Gen. Virol., 81:1927–1932.

Harit A.K.,Ichhpujani R.L., Gupta S., Gill K.S.,Lal S.,Ganguly N.K., Agarwal S.P., 2006.Nipah/Hendra virus outbreak in Siliguri, West Bengal, India in 2001, Indian J. Med. Res., 123, 553-560.

Homaira N., Rahman M., Hossain M.J., Epstein J.H., Sultana R., Khan M.S.U.,Podder G.,Nahar K., Ahmed B., Gurley E.S., Daszak P., Lipkin W.I., Rollin P.E., Comer J.A., Ksiazek T.G., Luby S.P., 2010.Nipah virus outbreak with person-to-person transmission in a district of Bangladesh, 2007,Epidemiol. Infect. 138:1630-1636.

Hutterer R., Ivanova T., Meyer-Cord C., Rodrigues L., 2005. Bat Migrations in Europe: A Review of Banding Data and Literature; Naturschutz und BiologischeVielfalt., 28:1-172.

Ithete N.L., Stoffberg S., Corman V.M., Cottontail V.M., Richards L.R., Schoeman M.C., Drosten C., Drexler J.F., Preiser W., 2013, Close Relative of Human Middle East Respiratory Syndrome Coronavirus in Bat, South Africa. Emerg. Infect. Dis., 19, 1697–1699.

Kurth A., Kohl C.,Brinkmann A.,Ebinger A., Harper J.A., Wang L.F., Muhldorfer K., Wibbelt G., 2012. Novel Paramyxoviruses in Free-Ranging European Bats, PLoS One, 7, e38688.

Leroy E. M., Kumulungui B., Pourrut X., Rouquet P., Hassanin A., Yaba P., Delicat A., Paweska J.T., Gonzalez J. P., Swanepoel R., 2005. Fruit bats as reservoirs of Ebola virus, Nature, 438:575–576.

Li W., Shi Z., Yu M., Ren W., Smith C., Epstein J.H., Wang H., Crameri G., Hu Z., Zhang H., Zhang J., MacEachern J., Field H., Daszak P., Eaton B.T., Zhang S., Wang L.F., 2005. Bats are natural reservoirs of SARS-like coronaviruses, Science, 310:676–679.

Luby S.P., 2013. The pandemic potential of Nipah virus. Antivir. Res., 100, 38–43.

Luis A.D., Hayman D.T.S., O'Shea T.J., Cryan P.M., Gilbert A.T., Pulliam J.R.C., Mills J.N., TimoninM.E., Willis C.K.R., Cunningham A.A., Fooks A.R., Rupprecht C.E., Wood J.L., Webb C.T., 2013. A comparison of bats and rodents as reservoirs of zoonotic viruses: Are bats special?, Proc. R. Soc. B Biol. Sci., 280(1756):20122753.

Lundy M., Montgomery I., Russ J., 2010. Climate change-linked range expansion of Nathusius' pipistrelle bat, *Pipistrellus nathusii* (Keyserling&Blasius, 1839), J. Biogeogr, 37:2232-2242.

Memish Z.A., Mishra N., Olival K.J., Fagbo S.F., Kapoor V., Epstein J.H., Alhakeem R., Durosinloun A., Al Asmari M., Islam A., Kapoor A., Briese T., Daszak P., Rabeeah A.A., Lipkin W.I., 2013. Middle East respiratory syndrome coronavirus in bats, Saudi Arabia. Emerg. Infect. Dis., 19, 1819–1823.

Parsons J.G., Blair D., Luly J., Robson S.K.A., 2009. Bat Strikes in the Australian Aviation Industry. J. Wildl. Manag, 73:526–529.

Paton N.I., Leo Y.S.,Zaki S.R., Auchus A.P., Lee K.E., Ling A.E., Chew S.K.,Ang B., Rollin P.E.,Umapathi T., Sng I, Lee C.C., Lim E., Ksiazek T.G., 1999. Outbreak of Nipah-virus infection among abattoir workers in Singapore, Lancet, 354:1253-1256.

Philbey A.W., Kirkland P.D., Ross A.D., Davis R.J., Gleeson A.B., Love R.J., Daniels P.W., Gould A.R., Hyatt, A. D., 1998. An apparently new virus (family *Paramyxoviridae*) infectious for pigs, humans, and fruit bats. Emerging Infectious Diseases, 4(2), 269–271.

Rahman M.A., Hossain M.J., Sultana S., Homaira N.; Khan S.U., Rahman M., Gurley E.S., Rollin P.E., Lo M.K., Comer J.A., Lowe L., Rota P.A., Ksiazek T.G., Kenah E., Sharker Y., Luby S.P., 2012. Date Palm Sap Linked to Nipah Virus Outbreak in Bangladesh, 2008. Vector Borne Zoonotic Dis. 2012, 12, 65–72.

Simons R.R.L, Gale P., Horigan V., Snary E.L., Breed A.C., 2014. Potential for Introduction of Bat-Borne Zoonotic Viruses into the EU: A Review, Viruses, 6(5): 2084-2121.

Sulkin S.E., Allen R., 1974. Virus infections in bats. Monogr. Virol. 8:1–103.

Tsoar A., Nathan R., Bartan Y., Vyssotski A., Dell'Omo G., Ulanovsky N., 2011. Large-scale navigational map in a mammal. Proc. Natl. Acad. Sci. USA, 108:E718-E724.

Wong S., Lau S., Woo P., Yuen K.Y., 2007. Bats as a continuing source of emerging infections in humans, RevMedVirol, 17:67–91.

Young J.R., Selvey C.E., Symons R., 2011. Hendra virus. Med J Aust.;195:250–251.

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SYSTEM CYCLING STAGE ON AQUAPONIC SYSTEMS AS REQUIRED PREREQUISITE FOR SOILLESS AGRICULTURE

Ehud ELIA, Camelia HODOŞAN, Lucica NISTOR, Florentina DUMITRACHE, Nicoleta-Alina UDROIU

University of Agronomic Sciences and Veterinary Medicine of Bucharest,
59 Mărăşti Blvd 011464, Bucharest, Romania, Phone: +4021 318 2564, Fax: + 4021 318 2567,
e-mail: ehud@ynet.ro, cameliahodosan@yahoo.com, lucia_mamina@yahoo.com,
mihalache75@yahoo.com, udroiuna@yahoo.de

Corresponding author email: ehud@ynet.ro

Abstract

The goal of the project was to establish a self-sustaining herb production unit in a soilless environment, using an aquaponic system as a bionutrient source for the plants.
Aquaponic systems are closed-loop symbiotic systems of aquaculture and soilless agriculture which uses nutrient-rich water from the fish culture to irrigate and fertilize the plants, while the plants clear the water before being recirculate to the fish tank. In this process, the wastes generated by the fish (such as urine, ammonia and decomposed fish fodder) are converted by nitrifying bacteria into forms readily to be assimilated by plants. The process of building the nitrifying bacteria cultures is known as "cycling". On this process the daily values of temperature, nitrates, nitrites, ammonia and pH in the fish tank water are to be assessed and controlled toward the goal of having the bacteria cultures established as quick as possible. The methodology of choice for the assessment of nitrates, nitrites and ammonia was spectrophotometric determination.
After completion of cycling stage, a soilless grow bed for the plants was established. Different combinations of substrates and plants are to be tested on this stage of the project, in order to achieve the best combination of "pairs" of substrates and plants to be grown in an aquaponic setup.

Key words*: aquaponics, nitrification, nitrogen-fixing bacteria, spectrophotometric determination.*

INTRODUCTION

Worldwide, in our days millions of people are facing hunger. They are also affected by food security issues (FAO, IFAD and WFP, 2013). The causes may be diverse: rapid population growth (practically the global population has doubled in the last 50 years), local conflicts, over-exploitation of lands, pollution and so on. One of the ways to solve food security issues is the use of *aquaponics systems*, the combined culture of plants and fish in symbiotic systems in which fish wastes provide nutrition to the plants, which, in turn, purify the water for the fish (Diver, 2006). Basically, water is continuously recycled among the fish tanks to the plant grow beds, and then back to the fish tanks. This recirculation capitalizes on the mutually beneficial (symbiotic) relationships among three components: fish, beneficial bacteria, and plants (he most commonly species grown in aquaponics systems are lettuce as plant and tilapia as fish). So, fish are raised on healthy vegetarian feed, bacteria convert fish waste and non-eaten fish fodder into nutrients necessary for plants growth, while the plants serve as a biofilter, purifying the water before it is returned to the fish tanks (Rakocy et al., 2006; Pantanella, 2010) (Figure 1).

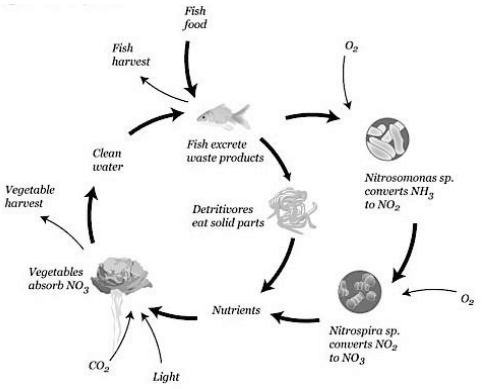

Figure 1. How aquaponic systems works

However, establishing a successful aquaponic systems is all about "growing" the "good bacteria" that perform those chemical reactions in the water to transform the compounds which are harmful for the fish and not usable by the plants into compounds harmless for the fish and usable for the plants, process known as "nitrification" (part of the global nitrogen cycle). The process of luring and establishing of "good" bacteria colonies is known as "system cycling".

In this context, the paper presents the cycling stage of an aquaponic system built out of a newly built non-cycled ornamental aquarium.

MATERIALS AND METHODS

In order to transform the new aquarium into an aquaponic system the following steps were taken (Elia et al., 2014; Connolly and Trebic, 2010):

- a grow bed was set up aside the fish tank and on top of a water collector tank;
- the grow bed was then provided with an overflow system in order to guide the excess water into the water collector tank;
- a layer of gravel on top of a layer of hydrotone was used as grow media;
- a lightning system was set up above the grow bed to provide light and heat to the plants;
- a piping system was set up between the fish tank and the grow bed, and between the collector tank back to the fish tank;
- a new biofilter was added to the system as a replacement for the power filter found in the aquarium;
- two water pumps controlled by an automatic "smart" control unit were added to the system;
- basil and parsley seedlings (grown from seeds aside the system) were used in the cycling stage.

In this build the water is recirculated in a closed loop from the aquarium and through the filter to the grow bed, and from the grow bed, back to the aquarium through the water collector tank (Figure 2).

Figure 2. The aquaponic system

The cycling stage of the system took place between December 2014 - January 2015. During that period water temperature, pH value and nitrogen concentration (NH_3/NH_4^+, NO_2^-, NO_3^-) were daily assessed (Hodoșan, 2012; Hodoșan, 2014). While water temperature and pH value were determined by direct observation (using a thermometer and a commercial test kit), nitrogen concentrations were determined by using spectrophotometric analysis of water probes (Hodoșan, 2014). This method requires measuring the intensity of light as a beam of light passes through the probe, knowing that each chemical compound absorbs or transmits light over a specific and known wavelength. The concentration values of nitrites and nitrates are then determined from the benchmark curves built out of the extinctions shown by spectrophotometer.

RESULTS AND DISCUSSIONS

The main sources of organic nitrogen in the water are fish and plants. Since an aquaponic system will not have aquatic plants planted in the fish tank the organic ammonia will mainly be the result of fish's respiratory and digestive systems activities and from decomposed fodder (Nicolae, 2007). An important step in the global nitrogen cycle starts when ammonia is oxidized to nitrates under the action of autotrophic bacteria, process known as "nitrification". Further, the final phase of nitrogen cycle take place, when nitrates are oxidized back to nitrogen by heterotrophic bacteria, process known as "denitrification". In context, autotrophic bacteria metabolize carbon out of CO_2 molecules, while heterotrophic bacteria metabolize carbon out of organic carbon compounds.

The process of interest in aquaculture systems is nitrification.

In the water ammonia is found at all times in both ionized and non-ionized forms, (ammonium and ammonia), the ratio between the two forms being related to water's temperature and pH level (more ammonia at higher temperatures and pH values).

$$NH_3 + H_2O <=> NH_4^+ + HO^-$$

Because ammonium is less toxic to fish than ammonia, relatively low temperature and pH values are to be preferred if possible. The sum of the two forms of ammonia (ionized and non-ionized) represents *Total Ammonia Nitrogen* (*TAN*). In the aquaculture systems the level of ammonia is empirically calculated based on the quantity of fish fodder (1 kg of fish fodder delivered to the fish ultimately produce 30 grams of ammonia). In our new system, right after the fish were added to the fish tank, ammonia started to build up. This stage represent both the beginning of System cycling and of nitrification process. Once ammonia was present in the system, the first colonies of nitrifying bacteria *Nitrosomonas* started to be established. Nitrosomonas, in order to build their own cells, need to gather elements present in the water (oxygen, nitrogen, phosphorus, carbon, potassium and calcium). To be able to use these elements and to run their own metabolism processes, they need energy. In order to get that energy, they drive chemical reactions that release energy as follows:

$$2\ NH_3 + 3\ O_2 => 2\ NO_2^- + 2\ H_2O + 2\ H^+$$

or

$$2\ NH_4^+ + 3\ O_2 => 4\ H^+ + 2\ NO_2^- + 2\ H_2O$$

This process converts ammonia into *nitrites* (NO_2^-), a compound even more toxic for the fish than ammonia (NH_3). *This point is the critical point of cycling. If not managed well, all fish can die as a result of the presence of both NH_3 and NO_2^- in the water*. Lured by the presence of NO_2^- in the water, the second nitrifying bacteria (*Nitrobacter*) start to colonize the system. Nitrobacter converts the nitrites to nitrates (NO_3^-), a nitrogen based compound *harmless to fish and an excellent food supply for plants*, as follows:

$$NO_2^- + 1/2\ O_2 =>\ NO_3^-$$

Considering both stages of system cycling as phases of a unique process, the total reaction of nitrifying bacteria is (Gujer and Boller, 1986):

$$NH_4^+ + 2\ HCO_3 + 1{,}9\ O_2 =>$$
$$=> NO_3^- + 2{,}9\ H_2O + 1{,}9\ CO_2 + 0{,}1\ CH_2O$$

where CH_2O is cellular biomass.

We can now assess that 1 g of NH_3 consumes during the nitrification process 4,34 g O_2 and 7,14 g alkalinity in order to produce 0,21 g cellular biomass and 4,43 g nitrates.

The nitrification process depends on ammonia level, pH value, alkalinity, temperature, solvate oxygen and light:

- any level above 2 mg/l of NH_3 stops nitrification;
- any value of pH between 6 and 9 is optimal for the nitrification, faster around value of 9 and slower around 6. Though, around the high values of pH, the ratio between ammonia and ammonium will favor ammonia, which is harmful to fish;
- alkalinity is a limiting factor also, since it is consumed during the process;
- there is no nitrification process above 38 ^OC and below - 5^OC

The nitrification process and can be identified by assessing the values of water parameters obtained during system cycling (Figure 3):

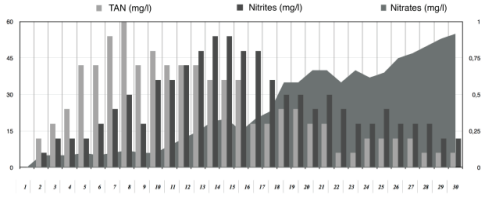

Figure 3. TAN, NO_2^- and NO_3^- values during system cycling

The daily outcome of the project was:

- day no. 1 - some random fishes were added to the system in order to provide ammonia;
- day no. 7 - NH_3 - 1 mg/l; NO_2^- - 0,5 mg/l; NO_3^- - 0,15 mg/l. Conclusion: Nitrosomonas started to colonize the system;
- day no. 7 - 50 litres of water were removed from the aquarium and replaced with 50 litres of nitrogen-free water;
- day no. 14 - NH_3 - 0,6 mg/l; NO_2^- - 0,8 mg/l; NO_3^- - 20 mg/l. Conclusion: Nitrosomonas colonies are established. Nitrobacter started to colonize the system;
- day no. 14 - 50 litres of water were removed from the aquarium and replaced with 50 litres of nitrogen-free water;
- day no. 25 - system cycling was concluded. The levels of NH_3 and NO_2^- dropped below 0,2 mg/l, with NO_3^- in excess of 40 mg/l. *The system is ready to receive fish and plants in order to start production.*

CONCLUSIONS

Food security poses a very real and serious threat in the world today. What makes aquaponic food production so attractive is its ability to address these issues of resource conservation and access to a reliable and quality food source (Mc Murtry et al., 1990). In addition to this, the simplicity of an aquaponic system makes it accessible and friendly use so it has the potential to help families who are most in need of it. The addition of some income through in the form of food has the ability to significantly impact the lives of families (Nelson, 2008). Furthermore, it can be a profitable endeavor and a lucrative vegetable and fish production for farmer or family that developed an aquaponic system. The potential is high for this type of agriculture and it will likely gain notoriety as global circumstances continue to necessitate an increasing amount of innovation, conservation, and consciousness.

REFERENCES

Connolly K. and Trebic T., 2010, Optimization of a backyard aquaponic food production system, Faculty of Agricultural and Environmental Sciences, Macdonald Campus, McGill University, BREE 495 Design 3, Bioresource Engineering.

Diver, S., 2006, *Aquaponics—Integration of Hydroponics with Aquaculture.* ATTRA-National Sustainable Agriculture Information Service (National Center for Appropriate Technology)

Elia E., Popa D. C., Nicolae C. G. 2014, STARTUP STAGES OF A LOW-TECH AQUAPONIC SYSTEM. Scientific Papers. Series D. Animal Science, Vol. LVII, ISSN 2285-5798, 263-269

FAO, IFAD and WFP, 2013. The State of Food Insecurity in the World 2013 - The multiple dimensions of food security. Rome, FAO, ISBN 978-92-5-107916-4

Gujer, W., Boller, M. (1986). Design of a nitrifying tertiary trickling filter based on theoretical concepts. Wat. Res. 20(11), 1353-1362

Hodoşan, C., 2012, Chimia apei şi a solului, Ed. PRINTECH - Bucureşti, pp. 115-120

Hodoşan, C., 2014, Chimie anorganică, Ed. PIM - Iaşi, pp. 78-85

Hodoşan, C., 2014, Chimie fizică şi coloidală, Ed. PIM - Iaşi, pp. 98-102

Mc Murtry, M.R., Nelson, P.V., Sanders, D.C., and Hodges, L., 1990, Sand culture of vegetables using recirculating aquaculture effluents. Applied Agricultural Research, Vol. 5, No. 4, pp. 280-284.

Nelson, R. L., 2008, *Aquaponics Food Production: Raising fish and plants for food and profit.* Montello: Nelson and Pade Inc, Copyright © 2010-2013 Nelson and Pade, Inc. PO Box 761, Montello, WI 53949, USA, ISBN 978-0-9779696-1-6.

Nicolae, C. G., 2007, Noţiuni generale de ihtiologie, Ed. Printech, Bucureşti, pp. 9-15

Pantanella, E., 2010, New aquaponics research in Italy, Aquaponics Journal, issue 56, pp. 25-27, www.aquaponicsjournal.com

Rakocy, J. E., Masser, M. P., & Losordo, T. M., 2006, Recirculating Aquaculture Tank Production Systems: Aquaponics - Integrating Fish and Plant Culture. Southern Regional Aquaculture Center, Publication No. 454, 1-16.

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LOW-TECH AQUAPONIC SYSTEM BASED ON AN ORNAMENTAL AQUARIUM

Carmen Georgeta NICOLAE, Dana Cătălina POPA, Adrian TUREK RAHOVEANU, Florentina DUMITRACHE, Dorina MOCUȚA, Ehud ELIA

University of Agronomic Sciences and Veterinary Medicine of Bucharest,
59 Mărăşti Blvd 011464, Bucharest, Romania, Phone: +4021 318 2564, Fax: + 4021 318 2567,
E-mails: carmennicolae19@yahoo.com, danasandulescu@yahoo.com,
mihalache75@yahoo.com, ehud@ynet.ro

Corresponding author email: carmennicolae19@yahoo.com

Abstract

The goal of the aquaponic system was to establish a self-sustaining herbs production in an applied research environment based on a home ornamental aquarium as a nutrient source for the plants. The study was conducted in two different stages. The first stage was to set up an aquarium with ornamental fish. This stage was conducted over a 40 days period (including water cycling). On this stage a 400 liters aquarium was set up then populated with Goldfish (Carassius auratus auratus) and Bronze Corydoras (Corydoras aeneus). During the time needed to set up the aquarium, basil, oregano and parsley seedlings were prepared aside. Upon completing this stage, the seedlings were moved into the grow bed and an aquaponic system was established. Thus, the NO_3^- rich water from the fish tank was directed to the grow bed. The working paper will present the steps to establish an ornamental aquarium, and how to turn it afterwards into a natural nutrients factory for a self-sustainable plant crop.

Key words*: aquaponics, biotechnologies, sustainable food system.*

INTRODUCTION

Basically, the aquaponic systems (or "aquaponics", or "aquaponic agriculture", depending on the system's scale) are food production units, based on a combination of aquaculture and hydroponics, tailored to provide healthy food productions (fish and plants). Aquaponics were borned in 1984 through the article published by Watten and Busch (Connolly and Trebic, 2010). Today, this new innovative agriculture technology is widely adopted in countries in America and Australia. Unfortunately, in Europe, the number of aquaponic implementations is still scarce.

In brief, an aquaponic system is a symbiotic closed-loop recycling fresh water system between fish and plants, where the wastes generated by fish (such as urine and ammonia) are converted by nitrifying bacteria into forms that plants can accept in their nourishment processes, thus acting as biofilters and cleaning the water before being sent back to fish (Figure 1). The most common cultivated plants are green leafy plants such as lettuce, basil, parsley and mint.

There also have been cultivated tomatoes, cucumbers, cabbage, kale, celery, eggplant and okra but the income obtained from the herbs is much higher and therefore those are preferred (Rakocy et al., 2006; Connolly and Trebic, 2010).

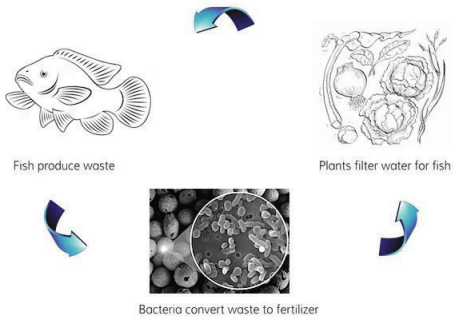

Figure 1. How aquaponic systems work?

Regarding the fish, the most common grown species is tilapia *(Oreochromis niloticus)*. However, any species of fresh water fish can be

suitable for an aquaponic system as long as a proper fish tank is prepared (dimension wise) and the required environmental conditions are met (Elia et al., 2014).
In this context, the paper present the steps to build an ornamental aquarium and to turn it afterwards into a fully fledged *reversed* aquaponic system used to grow healthy plants for consumption or use and also to reduce aquarium maintenance.

MATERIALS AND METHODS

The project required not only to turn an existing aquarium into an aquaponic system, but to build the aquarium also. *Not any aquarium, but an ornamental aquarium intended to be shown in public, placed one meter above the floor*.
This latter requirement hindered a "regular" implementation of an aquaponic system due to the fact that, in case of a "regular" implementation, the grow bed will be suspended out of reach, two meters above the floor. This has led to the emergence of novelty in implementation of such a system, which ultimately had to be configured with the grow bed placed *below* the level of the fish tank. This model of build is known as *reversed* aquaponic system.
To assess the water parameters, the following indicators were used: water temperature, pH value and nitrogen concentration (ammonia, nitrites and nitrates) (Nicolae, 2007). While water temperature was determined by direct observation (using a thermometer), nitrogen concentrations were determined using spectrophotometric analysis of water probes. pH value was assessed by using a commercial test kit. The water assessment was carried out between December 2014 - January 2015.
The aquarium was built out of tampered glass, using silicone to harden and seal the joints. A sturdy aquarium stand was also built out of metal bars to withstand a total weight of about 500 kg.

RESULTS AND DISCUSSIONS

The aquarium

To build the aquarium (Figure 2) were necessary: four 10 mm thick tempered glass sheets for the side walls; one 12 mm thick tempered glass sheet for the bottom; four 12 mm thick 50 mm wide tempered glass stripes for reinforcements and lid support; painter's tape; scissors and scraper; aquarium silicone and silicone gun. The thickness of the glass chosen for the build was based on the physical parameters of the glass.

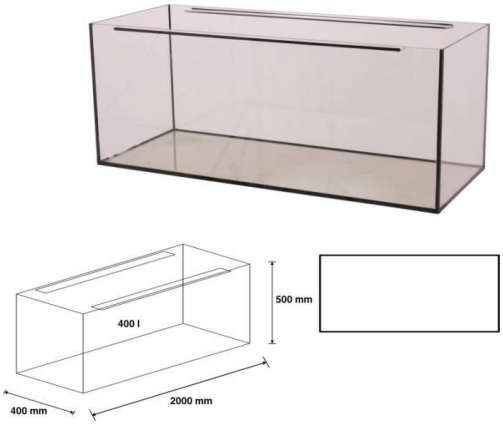

Figure 2. Aquarium blueprints

Based on the thickness and the dimensions, the weight of an empty aquarium can be calculated (Table 1). *This is a very important factor to be taken into account when designing the aquarium stand.*

Table 1. Physical parameters of tampered glass

Glass thickness (mm)	**6**	**8**	**10**	**12**	**15**
Weight (kg/m^2)	**15**	**20**	**25**	**30**	**37,5**
Light transmission (%)	88	87	86	84	82
Light reflection (%)	8	8	7	7	7
UV absorption (%)	38	43	46	52	56
Shadow reduction (%)	0,88	0,82	0,80	0,74	0,70

In order to build the aquarium, the aquarium elements were first lined out and checked one against the others, to make sure that every glass element was cut according to the blueprints.
Then, the edges of the aquarium sides were covered on both sides with painter's tape to prevent soiling with silicone.
The sides of the bottom were also covered with painter's tape except for the surfaces to be jointed with the sides. One by one, the ends of the bottom and the corresponding edges of the

side elements were covered with silicone and put together in position. The reinforcements were also covered with silicone and placed in position.
After all the edges of the side elements and the bottom were aligned and the silicone in excess removed, the painter's tape was removed. The silicone was left to cure for three days.
Meanwhile, the aquarium stand was placed in position and levelled so as the table surface to be perfectly horizontal. The spot where the aquarium stand was positioned was chosen according to the following rules:
- to be away from direct bright light in order to prevent excessive algae growth;
- to preserve a constant temperature;
- taking into consideration the ability of the floor to support the weight of the full loaded aquarium and grow beds (the stand should be as close as possible to floor crossbeams);
- close to a power outlet.
Prior to place the aquarium on its stand a shock absorbent 5 mm thick polystyrene sheet was placed and fixed to the table surface. Finally, the aquarium was filled with water in order to make sure the quantity of gluing was good and that there are no water leaks. The aquarium was then emptied and cleaned.
The next step was to install the power filter, water heating system and air pump. While the heating system and the air pump were placed into their final designated position (taken into consideration the future aquaponic system), the power filter role was only to hasten the Nitrogen cycling process. Later on the power filter was replaced by an experimental biofilter to deliver both mechanical and active water filtration. None of the electrical devices were turned on yet.
Chemically inert artificial gravel was chosen as substrate.
The gravel was rinsed in warm tap water before adding it to the aquarium (the less dust in the water, the faster it will clear when the filter is started up).
The substrate was slightly sloped upward toward the back of the aquarium. Some artificial plants and decorations were added also, and then the aquarium was filled with tap water (Figure 3).

Before that, a plate was placed on the substrate to prevent its dispersion when the water is added.

Figure 3. The aquarium build

Water dechlorinator was added to the water in order to remove the chlorine and chloramines.
According to the best practices on any aquarium build some facts are necessary to be taken into account:
- regarding the size of an aquarium, the rule "bigger is better" always apply. A bigger aquarium is easier to maintain due to its high inertia: thermal shocks and water imbalances are much less likely to occur;
- the heaters are to be plugged in only after the aquarium is filled with water and after the thermostat in the heater has adjusted to the water temperature;
- the *effective* water volume is not the geometric volume of the aquarium. It only represents the *real* volume of the water contained in the aquarium.
The number and adult size of the fish to live in the aquarium will always dictate the dimensions of the aquarium. A simple calculation model (based on the length of an adult fish) will show the needs in term of water of *one* adult fish. Knowing the total number of fishes meant to be in the aquarium in the production stage, the *effective* water volume may be calculated (Table 2).

Table 2. The needs in term of water of an adult fish

Fish length (cm)	**< 5**	**5 - 9**	**9 - 13**	**> 14**
Litres of water / cm	1,5	2	3	4

For example, based of the above formula, a 4 cm neon tetra (*Paracheirodon innesi)* will need 6

liters of water, while a 15 cm hoplo catfish (*Hoplosternum thoracatum)* will need 60 liters of water.

System cycling - *a process common to all aquarium setups*

After the water was allowed to sit for a few days in order to remove the Chlorine and to reach optimum temperature the aquarium was populated with only a few Goldfish *(Carassius auratus auratus)* and Bronze Corydoras *(Corydoras aeneus)*.

The fish, as a result of their respiratory and digestive processes, started to produce ammonia (NH_3), a Nitrogen based compound toxic to the fish. Some food in excess was also provided, in order to *increase* the ammonia level in the fish tank while decomposing.

Once ammonia was present in the system, the first nitrifying bacteria (*Nitrosomonas*) was lured into the system and started to colonize.

As a result of its presence, ammonia started to be converted to nitrites (NO_2^-), also a Nitrogen based compound, even more toxic for the fish than NH_3. *This point is the critical point in terms of fish welfare, when in the water are found high levels of both NH_3 and NO_2^-.* Fortunately, the presence of NO_2^- in the water lured the second nitrifying bacteria to the system (*Nitrobacter*), which converts the nitrites to nitrates (NO_3^-), a Nitrogen based compound harmless to fish *and* an excellent food supply for plants (Hodoșan, 2012).

The process of biological oxidation from ammonia to nitrates, carried out by autotrophic bacteria, is known as *nitrification* and can be identified by assessing the values of water parameters obtained during system cycling (Figure 4).

Nitrification is also the process that drives the aquaponic systems.

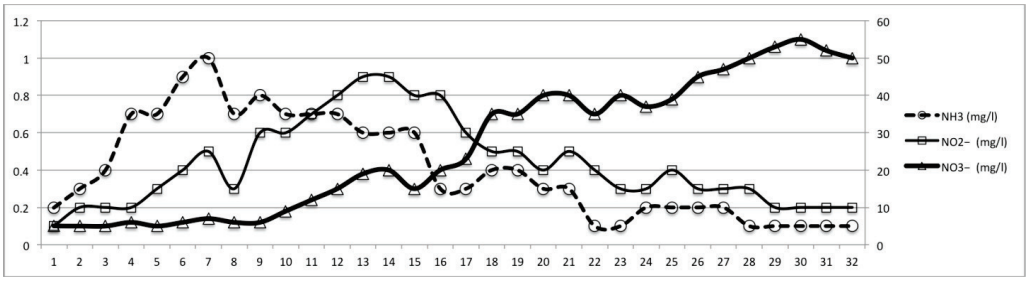

Figure 4. The nitrification process

System cycling is concluded once the levels of NH_3 and NO_2^- are below 0,3 mg/l, while NO_3^- is in excess of 30 mg/l. *At this point any fresh water aquarium can be turned into an aquaponic system*.

Thereby, along with the production of plants, the required periodical removal of a part of the water from the aquarium with the addition of clean water will no more be necessary: instead, the plants will do the cleaning of the water.

System conversion

In order to turn the aquarium into an aquaponic system were made some preparations:

- at the same time with system cycling process we started some basil and parsley seeds in perlite;
- a small grow bed was established, in order to support some seedlings to clear the water while cycling the system;
- a piping system was set up between the fish tank and the grow bed, and between the collector tank back to the fish tank;
- for basil we made sure that the plugs are not too wet, as the seed will rot;
- an experimental new biofilter was added to the system, aiming to replace later the actual power filter;
- a lightning system was also added to the grow bed.

The grow bed

Normally, the grow bed sits *above* the fish tank, and the water showers back to the fish tank, also providing natural aeration through water surface movement (Figure 5).

In addition, no additional pump is needed (apart from the pump which lift the water from the fish tank to the grow bed).

Figure 5. The grow bed

In our setup, the grow bed was placed on a table *below* the level of the fish tank. To make the system work, we provided the grow bed with an overflow (Figure 6) leading to a collector tank placed below the level of the grow bed.

Figure 6. The overflow

A 10 cm thick layer of Hydrotone was added at the bottom of the grow bed, followed by a 20 cm thick layer of aquarium gravel. For this stage, a germination tray was placed on top of the gravel.

How the system works?

The water is pumped from the aquarium to the biofilter (flood and drain system) (Figure 7). At this stage, the filter retains all suspended particles (Hodoșan, 2014).

Also, most of the nitrifying process occurs within the filter. Coming out from the filter water begins to fill the grow bed till the overflow level is reached. The water in excess flows into the collector tank.

Figure 7. The aquaponic system

When the water reaches a certain "High" level, a "smart" pump starts to move the water from the collector tank back to the aquarium. The drain pump only stops when a preset "Low" level is reached.

The plants

Parsley (*Petroselinum crispum*) is a common herb rich in calcium, iron and vitamins A and C. It also has a high market value. Even it can resist to 0^{o}C temperatures, the minimum temperature for growth is 8^{o}C. It enjoys sun for up to eight hours a day.

While in the first year the plant produce only leaves, in the second year the plant will begin sending up flowers for seed production. Harvesting begins when the individual stalks are 15 cm long.

Basil (*Ocimum basilicum*) has a high value and high demand in urban zones. Its seeds need a high and stable temperature (around 22^{o}C) to germinate. It grows in warm conditions with full exposure to the sun. When temperatures above 27^{o}C are reached, shading is needed.

Harvesting begins when the plant is 15 cm high and continues for the next 30 - 50 days. A few variations are also available: Italian Genovese basil (sweet basil), lemon basil and purple passion basil.

CONCLUSIONS

Any existing ornamental aquarium can be easily transformed into a self-sustaining herb production unit.

The costs of such a system are very low and no special skills or tools are required. Moreover, even an aquarium can be made out of low cost components. It is not required special skills and tools also.
This is a way for urban people to get closer to the nature, to enjoy the peaceful view of some beautiful fish, and, with virtually no production costs, to have in their kitchen fresh herbs straight from the grow bed all year round.

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REFERENCES

Connolly K., Trebic T., 2010. Optimization of a backyard aquaponic food production system, Faculty of Agricultural and Environmental Sciences, Macdonald Campus, McGill University, BREE 495 Design 3, Bioresource Engineering.

Elia E., Popa D.C., Nicolae C.G., 2014. Startup stages of a low-tech aquaponic system. Scientific Papers. Series D. Animal Science, Vol. LVII, ISSN 2285-5798, 263-269.

Hodoşan C., 2012. Chimia apei şi a solului, Editura Printech, Bucureşti, 115-120.

Hodoşan C., 2014. Chimie fizică şi coloidală, Editura Pim, Iaşi, 98-102.

Nicolae Carmen Georgeta, 2007. Noţiuni generale de ihtiologie. Ed. Printech, Bucureşti, 9-15.

Rakocy J. E., Masser M.P., Losordo T.M., 2006. Recirculating Aquaculture Tank Production Systems: Aquaponics - Integrating Fish and Plant Culture. Southern Regional Aquaculture Center, Publication No. 454, 1-16.

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A BRIEF SURVEY OF LENGTH-WEIGHT RELATIONSHIP IN GIBEL CARP (*CARASSIUS GIBELIO* BLOCH, 1782) FROM CIŞMIGIU LAKE

Mala-Maria STAVRESCU-BEDIVAN[1], Petre Bogdan MATEI[2], Laura Daniela URDEŞ[3]

[1]University of Agronomic Sciences and Veterinary Medicine of Bucharest, Faculty of Agriculture, 59 Mărăşti Blvd, District 1, 011464, Bucharest, Romania, Phone: +4021.318.25.64, Fax: + 4021.318.25.67, Email: mala_stavrescu@yahoo.com
[2]"Grigore Antipa" National Museum of Natural History, Şos. Kiseleff No.1, 011341, Bucharest, Romania, Email: bogdan_matei@antipa.ro
[3]University of Agronomic Sciences and Veterinary Medicine of Bucharest, Faculty of Animal Sciences, 59 Mărăşti Blvd., Sector 1, 11464, Bucharest, Romania, Email: laurau_2005@yahoo.com

Corresponding author email: mala_stavrescu@yahoo.com

Abstract

A fishing survey conducted at the end of October 2014 in Cişmigiu Gardens from Bucharest, revealed that length - weight relationship (LWR) in the cyprinid Carassius gibelio was influenced both by environmental conditions and small length range of specimens caught.
The LWR for a total of 94 unsexed Gibel carp, examined from the very next day of sampling was calculated as: TW = 0.0055 TL $^{3.6303}$. Similar with other biometric case studies conducted on this species in the specialized literature, our report has limitations like narrow-sized specimens, but this is because in sample season, the fish had not yet reached full maturity. However, to our knowledge, this is the first contribution on length-weight relationship for Carassius gibelio belonging to an anthropic lake from Bucharest.

Key words*: Carassius gibelio, Cişmgiu Lake, length-weight relationship.*

INTRODUCTION

Length-weight relationships play an important role in fish biology (Froese et al., 2011; Zargar et al., 2012). LWR enables morphological comparisons between different fish species or populations from different habitats (Sangun et al., 2007).
Carassius gibelio Bloch, 1782 (Prussian carp or Gibel carp), a cyprind fish species spread in Europe, Siberia and Northeast Asia (De Giosa et al., 2014) was relatively few studied in Romania from biometric perspective, most studies focusing on the Danube populations (Gheorghe et al., 2012).
Here we aim to present such observations on gibel carp, a fish species introduced in the oldest public garden from Bucharest. From ecological point of view, Cişmigiu Lake is a young lacustrian ecosystem, artificially maintained through water emptying and the mud drainage in winter months (Zinevici et al., 2001), and this was the season when we easily collected the fish specimens.

MATERIALS AND METHODS

Overall, 94 specimens of Gibel carp were caught with the aid of a fishnet from Cişmigiu Lake on October 22, 2014. Sampling was carried out in three different points: near Monte Carlo Restaurant ($44^0$26'15" N, $26^0$05'22" E), Walnut Bridge ($44^0$26'16" N, $26^0$05'27" E) and Stone Bridge ($44^0$26'12" N, $26^0$05'30" E) (Figure 1). Fish specimens were preserved frozen and analyzed during the next three days in our laboratory from UASVM of Bucharest (Figure 2).
For each fish, the total weight in grams (TW ± 1 g) and the total length in cm (TL ± 1 mm) were measured. The length-weight relationship (LWR) was expressed as: $TW=aTL^b$, where *a*

(the intercept of the logarithmic form) describes the rate of change of weight with length and seasonal parameter *b* (the slope of the regression line in the logarithmic form) provides information about the type of growth (Froese, 2006; Sangun et al., 2007). The equation was log transformed (Log TW= Log a + b Log TL) to estimate the two coefficients of LWR. The b values should be within the expected range of 2.5-3.5 (Froese, 2006). When b > 3, positive allometric pattern of growth occurs, this means that the fish grows faster in weight than in length (Karachle and Stergiou, 2012). The slope and intercept were estimated with a nonlinear regression, by the least-square method, using PAST (Paleontological Statistics Software) version 3.04.

Figure 1. Sample stations from Cişmigiu Lake, Bucharest

Figure 2. Gibel carp specimens from Cişmigiu Lake

RESULTS AND DISCUSSIONS

In our fish sample, the TL ranged from 3.7 to 10.8 cm, with a mean of 5.283 cm. The TW ranged from 1.00 to 23.00 grams, with a mean of 3.394 grams. All specimens proved to be juveniles and were not sexed in this analysis.

The linear regression of the log-transformed values was calculated as: Log (TW) = - 2.2581 + 3.6303L Log (TL), with a value of 0.913 for the determination coefficient (r^2), at 95% confidence limits of the parameters *a* and *b* (Figure 3).

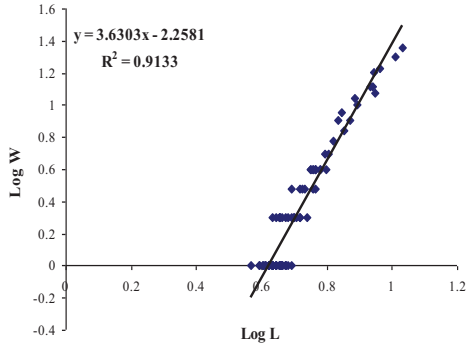

Figure 3. Least square regression of Log W x Log L for gibel carp sampled from Cişmigiu Lake, October 2014

The corresponding nonlinear equation, showing the weight-length relationship was represented by: $TW = 0.0055 \times TL^{3.6303}$ (Figure 4). Growth type for gibel carp juveniles was determined as positive allometric, since parameter *b* has a value greater than 3.

The slope value recorded for *Carassius gibelio* from Cişmigiu Lake exceeded the maximum value reported by FishBase for this species in the Romanian waters (www.fishbase.org).

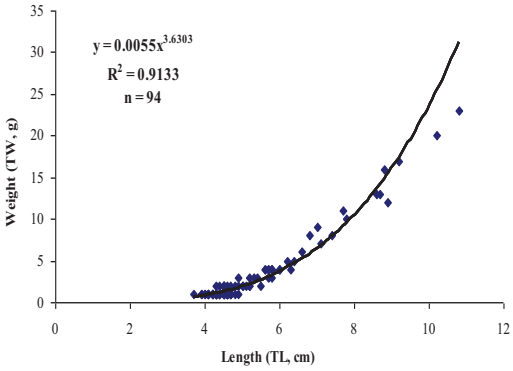

Figure 4. Relationship between length and weight for gibel carp sampled from Cişmigiu Lake, October 2014

The high value of *b* is often related to small length range of specimens caught (Froese, 2006; Treer et al., 2011). Actually, in our case, the overestimation of the slope may be due to the fact that fish sample did not coverd, by chance, the full size ranged, as recommended (Froese et al., 2011), although the lake was randomly sampled. Thus, with few exceptions, most gibel carp from Cişmigiu Lake seemed to be immature when they were caught.

Also, the exponent *b* may vary with trophic state of the lake, with low values in eutrophic rather in oligotrophic lakes (Tsoumani et al., 2006). Hence, in the absence of chemical analysis of the water, our high value of parameter *b* in the LWR might suggest the presence of an oligotrophic lake. Yet again, having fish with an average length slightly above 5 cm, we can not rush to any conclusions in this regard.

The anthropic lake from Cişmigiu Garden is filled every year by omnivorous fish like *Carassius gibelio*. For one- and two- year old gibel carp, green and blue-green algae are dominant in food spectrum, while older specimens preferr the zooplankton, like copepods (Rogozin et al., 2011).

In eutrophic lakes, the zooplankton is reduced, which correlates to fish growth (Treer et al., 2010). Studies regarding the seasonal dynamics of Cişmigiu Lake zooplankton (Zinevici et al., 2001), showed that the minimum value of diversity was riched in October, meaning the exactly same month of the year when we sampled the gibel carp. This last aspect might explain why fish were represented by almost only young specimens in all three stations from the studied lake.

On the other hand, sample season might influence the weight-length relationship (Bobori et al., 2010; De Giosa et al., 2014). Although the *b*-value seems to be in general significantly lower than 3, indicating hypoallometric growth for *Carassius gibelio* recorded in cold season (De Giosa et al., 2014), it should not be forgotten that our sample was formed by very small fish specimens. Small range of the gibel carp sample could be linked with the environmental conditions, in that small amounts of food are available in cold month of the year.

Interpretation of present results it should also take into account that most of the LWR studies on *Carassius gibelio* have dealt before with natural reservoirs, not anthropic areas.

Only one study reported, so far, the presence of *Carassius gibelio* in Cişmigiu Garden (Gavriloaie, 2008). Aside from gibel carp, brown bullhead is another naturalized species in the fauna of Cişmigiu Lake, a few specimens of *Ictalurus nebulosus* being caught during this brief survey.

As a result of water emptying of the lake in this part of the year, if it had not been collected, the fish would have come anyway on garbage, food for gulls or simply would be frozen due to the low temperatures recorded in the sampling season.

CONCLUSIONS

The LWR of *Carassius gibelio* sampled from Cişmigiu Lake, determined from regression of Log weight on Log length, indicated a positive allometric type of growth.

However, our results should first be interpreted within the context of a very small-sized of fish caught, which implies that majority of gibel carp sampled in October did not reach the maturity age. Compared with previously case studies on gibel carp biometry, the *b*-value reported in this survey should be considered an example of how narrow length range of fish may influence the LWR results.

For further analysis, we intend to observe the evolution of LWR and the condition factor of gibel carp over a full year, covering all seasons. Also, biometric parameters of the fish will be correlated with sex specimens and physico-chemical features of the water that will reveal the real trophic state of the lake.

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REFERENCES

Bobori D.C., Moutopoulos D.K., Bekri M., Salvarina I., Munoz A.I., 2010. Length-weight relationships of freshwater fish species caught in three Greek lakes. Journal of Biological Research-Thessaloniki, 14: 219-224.

De Giosa M., Czerniejewski P., Rybczyk A., 2014. Seasonal changes in condition factor and weight-length relationship of invasive *Carassius gibelio* (Bloch, 1782) from Leszczynskie Lakeland, Poland. Advances in Zoology, 1-7, http://dx.doi.org/10.1155/2014/678763.

Froese R., 2006. Cube law, condition factor and weight-length relationships: history, meta-analysis and recommendations. Journal of Applied Ichthyology, 22 (4): 241-253.

Froese R., Tsikliras A.C., Stergiou K.I., 2011. Editorial note on weight-length relations of fishes. Acta Ichthyologica et Piscatoria, 41 (4): 261-263.

Gavriloaie I.C., 2008. Contributions to the knowledge of Bucharest city. AACL Bioflux, 1: 21-26.

Gheorghe D.C., Nica A., Cristea V., Răzlog G.P., 2012. Growth and mortality estamation parameters for the Prusian carp (*Carassius gibelio*, Bloch, 1782) population from Danube River (km 170 - 196). UASVM Iasi, Lucrari Stiintifice Journal, Seria Zootehnie 57 (17): 164-169.

Karachle P.K, Stergiou K.I., 2012. Morphometrics and allometry in fish. In: Wahl C.M., 2012. Morphometrics. New York: Cornell University, 65-68. (Agricultural and biological science). DOI: 10.5772/34529.

Rogozin D.Y., Pulyayevskaya M.V., Zuev I.V., Makhutova O.N., Degermendzhi A.G., 2011. Growth, diet and fatty acid composition of Gibel Carp *Carassius gibelio* in Lake Shira, a Brackish Water Body in Southern Siberia. Journal of Siberian Federal University. Biology 4 (1): 86-103.

Sangun L., Akamca1 E., Akar M., 2007. Weight-length relationships for 39 fish species from the North-Eastern Mediterranean Coast of Turkey. Turkish Journal of Fisheries and Aquatic Sciences 7: 37-40.

Treer T, Matulic D., Bogdanovic G., Anicic I., Safner, R., Piria M., Sprem N., Tomljanovic T., 2010. The condition of allochtonous fishes in the Mediterranean Vransko Lake. J. Appl. Ichthyol. (2010), 1–3, https://bib.irb.hr/datoteka/512660.Vransko-onlineLibraryTPS.pdf .

Zargar U.R., Yousuf A.R., Mushtaq B., Jan D., 2012. Length–weight relationship of the crucian carp, *Carassius carassius* in relation to water quality, sex and season in some lentic water bodies of Kashmir Himalayas. Turkish Journal of Fisheries and Aquatic Sciences 12: 683-689.

Zinevici V., Parpală L., Macovei F., 2001. Zooplankton structure in Cişmigiu Lake. Revue Roumaine de Biologie Serie de Biologie Animale, 46 (1-2): 39-51.

***http://www.fishbase.org