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SUMMARY

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NUTRITION

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TECHNOLOGIES OF THE AGRO FOOD PRODUCTS PROCESSING

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GENETICS AND BREEDING

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IDENTIFICATION OF PIT-1 GEN USING PCR-RFLP AND GENETIC EVALUATION OF HACHING WEIGHT USING PATERNAL HALB SIB ON INDEGENOUS BREED SINGING COCKEREL PELUNG

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Abstract

This research was conducted at the Laboratory of Animal Breeding and Reproduction of Animal Husbandry Faculty, and Laboratory of Biochemistry of Mathematic and Natural Sciences Faculty Universitas Padjadjaran at June 2012. The objectives of this research were to know variation of Pit-1 gen of Pelung singing cockerel and evaluate genetic parameter of Hatching Weight (HW). The data comprised of 76 HW-records as progeny from 5 cocks and 15 hens of Pelung using Paternal Halb Sib and 29-blood samples. Variance component and heritability were estimated by Restricted Maximum Likelihood (REML) using Animal Model with the program of VCE 4.2. Fixed effect was sex and hatching period. Variation of chicken Pit-1 gen was analyzed using PCR-RFLP and used 5 primers (PR1, PR2, PR3, PR4 and PR5) and 4 restriction enzymes. The average hatch weight was 33.83 ±2.42 gr. The heritability value was 0.5 ±0.05 as high category. The genetic respond to selection was 2.55 gr. The accuracy of selection of was 0.707. While selection intensity was 1.92% for 3 hens and 1 cock (sex ratio 1 ♂3 ♀. Presence or absence of deletions in a PCR fragment of the result can be distinguished by differences in the electrophoresis migration of the fragment. The result showed that there was deletion of 57 bp of insertion fragment length of 387 bp. In this experiment the difference migration did not occur in all samples, that was implicated with reverse and forward primer (PR1).

Key words: *Artificial Insemination, Paternal Halb Sib, Pit-1 Gen, Singing Cockerel.*

INTRODUCTION

Pelung Chicken is one of the local chickens, which were developed by the community with the objective being to sound melodious crowing roosters, easy listening, big, long, playing and rhythmic. However, if it is not supported with good stamina, the sound quality may not appear as expected.

Although the criteria of performance and body weight are ignored, in the future the characteristics of Pelung Chicken (big body) will be lost.

On a semi-intensive maintenance Pelung adult, can achieve weight of 3.37 kg for cocks and 2.52 kg for hens, while the DOC body weight is 30.7 g for males and 31.6 g for females. The usefulness of Pelung Chicken as a source of animal protein, have higher productivity than other types of local chicken in Indonesia. The roosters have loud rhythmic and long floating crow. Pelung chicken do not have a specific pattern of feather color, many of them have a mixture color of red and black, yellow and white, and also shiny green color mix, but the most often found is the mixture of red and black.

Genetic diversity is important in breeding programs for the genetic optimization of the acquisition of certain properties can be achieved when there is enough opportunity for the selection of genes or the desired properties.

Beside that, genetic diversity plays an important role in the survival of the population. The loss of genetic diversity can reduce the chance of survival of the population.

Growth is weight gain until they reach adult size or per additional body mass per unit time. Specific growth occurs in young animals which is formed by the large bone, protein, and water networks.

Through a complicated on differentiation phase of the anterior pituitary and the regulation of prolactin gene (PRL), growth hormone (Growth hormone-GH) and thyroid-stimulating

hormone-ß (TSH-ß), then Gen Chicken PIT1 is recommended as a candidate gene to the nature of the production.

Somatic cells residing on fur or white blood cells can be used as a source for the DNA analysis. In the microsatellite study to obtain superior genes for the purification of Pelung chicken, so that the origin of the nation can be standardized based on Polymerase Chain Reaction (PCR) and Restriction Fragment Polymorphism (RFLP) (Bandiati, 2006).

Therefore, based on the availability of the genetic resources, it is essential to form seeds of Pelung chicken, which have criteria in accordance with the will of the breeder, for purposes of standardization and certification. The purpose of this study was to determine the variation of Pit-1 gene in blood samples and the response to selection in Pelung chickens base on hatching weight.

MATERIALS AND METHODS

1. Blood sampling as many as 29 samples, grouped into group A (A1, 2, 3, 4, 5); B (B1, 2, 3, 4, 5); C (C1, 2, 3, 4, 5); D (D1, 2, 3, 4, 5); E (E1, 2, 3, 4); F (F1, 2, 3, 4) and G1.
2. Pre-treatment sample storage (using va-cuumtainer containing EDTA anticoagu-lant).
3. Isolation of DNA samples using the Genomic DNA Purification Kit (Fermentas).
4. PCR analysis for each of the isolated DNA with four pairs of primers using Taq Dream Green PCR Master Mix.
5. Electrophoresis of PCR results to determine the quantity of DNA and determine the approximate size of the fragment amplification results using 1% agarose electrophoresis, specific to MR1 marker electrophoresis performed using 2% agarose electrophoresis to determine the insertion and deletion, another marker proceed to the next stage of analysis.
6. Cutting PCR results using four different restriction enzymes.
7. Electrophoresis of restriction enzyme cutting results using 1.5% agarose electrophoresis.

Table 1. Detailed Information of the Marker MR1-MR5 on PITI Gene in Chickens

Marker	Variation	location	Size	restrictions Enzymes	Primer	Statement analysis of variation
MR1	Insersions/ delesions 57 pb	Intron 2	387/ 330 pb	-	PR1 forw/ Rev	Analysis of insertions / deletions (display size of the ribbon electrophoresis)
MR2	C/T	Intron 5	599 pb	TaqI	PR 2 forw/ rev	RFLP
MR3	A/G	Intron 5	599 pb	MspI	PR 2 forw/ rev	RFLP
MR4	C/T	Intron 5	442 pb	EcoRI	PR 3 forw/ rev	RFLP
MR5	C/T	Ekson 6	483 pb	TasI	PR 4 forw/ rev	RFLP

Table 2. Order of Primer (forward/reverse 5′-3′)

Primer Name	Order of Primer		
PR1	Forw	:	gtcaaggcaaatattctgtacc
	Rev		tgcatgttaatttggctctg
PR 2	Forw	:	ggaccctctctaacagctctc
	Rev		gggaagaatacagggaaagg
PR 3	Forw	:	ggggattttgccactttaggg
	Rev		tgggtaaggctctggcactgt
PR 4	Forw	:	tgggaagaacagtttatggc
	Rev		tggctagcttgtaagggaatc

Isolation Method Using Genomic DNA Purification Kit (Fermentas) Component Kit:

- Lyses solution
- Precipitation solution (10x concentration)
- NaCl Solution (1.2 M)

Working Step:

1. Mix the 200 µL of blood samples of chicken (upper blood that has been stored on the vacumtainer with anticoagulants EDTA) with 400 µL lyses solution, pipette up and down, 10 minute incubation at 65°C (to be turned back several times during incubation).
2. Adding 600 µL of chloroform, inversion (alternating-turn tube) 3-5 times, 2 min centrifugation at 11,000 rpm.
3. Remove top aqueous phase, which contained DNA into new tubes had contained 800 µL 1x precipitation solution (80 µL precipitation 10x solution is added 720 µL sterile aqubidest), mix for 2 minutes (using a vortex), 2 min centrifugation at 11,000 rpm, discard supernatant.

4. Dissolve the pellet with 100 µL NaCl solution.
5. Add 300 µL of absolute ethanol cold, let the DNA precipitate for 10 minutes at-20°C, centrifuged for 4 min at 11,000 rpm, discard the supernatant. Can be added to the leaching method using 70% ethanol and then dried.
6. Dissolve the DNA in 100 mL sterile aquabidest Furthermore, the results of PCR was cut with restriction enzymes to determine the mutations found in fragments.

Marker	Variation	Restriction enzyme	Incubation Temp RFLP
MR1	Insertion/ delusion 57 bp	-	-
MR2	C/T	TaqI	65°C
MR3	A/G	MspI	37°C
MR4	C/T	EcoRI	37°C
MR5	C/T	TasI	65°C

Mixed cutting reaction by restriction enzyme (RFLP)

ddH_2O	9 µl
Buffer enzyme 10x	3 µl
Enzyme (10 u/ µl)	3 µl
DNAHasil PCR	15 µl
Final volume	30 µl

Electrophoresis of PCR Product:

Resulted DNA from electrophoresis purification used 1% polyacrylamide with silver staining colouring to see the pattern of band microsatellite, that was amplificated by primer.

Especially for MR1 marker electrophoresis performed using 2% agarose electrophoresis to determine the insertions and deletions, another marker proceed to the next stage of analysis.

PCR product was cut using four kinds of restriction enzymes, for this analysis used electrophoresis 1.5% agarose.

Genetic Evaluation:

Parameters were analyzed using REML method with repeated measurement pattern Animal Model (Grouneveld, 1998). Response selection by using the formula $R = i\, h^2\, s_P$

RESULTS AND DISCUSSIONS

Variations Polymorphism Pit-1 Gen:

At the holding cell isolation method optimazation with the Genomic DNA Purification Kit (Fermentas), used as a comparison sample of whole blood and uppers (serum). It was found that isolation by using whole blood (mixture) at stage 3 there will be no separate phase 3 well (the upper (aqueous phase), the middle (cell debris), and the lower (organic phase) as in isolation by using serum.

Isolation using whole blood at stage 3 there will be only two phases, namely solid phase (gel-like) on the top and the organic phase. Because it was later used as an isolated sample is part of the chicken blood serum. Furthermore, by using the PR1, all samples showed deduction Variations of PR1 primers in reverse and forward showed that the migration of the same relative to all samples and estimated (based on comparison with a marker) that is the size of the fragments between 250-500 bp, primer design based amplification size was 387 bp (when insertions) or 330 bp (when deletions). The results of further analysis to look for mutations that occur in fragment, that MR1 using PR1 showed 100% truncated insertions and deletions, MR2, that treated combination between PR2 with tag1 enzyme cut only 37% of total (11 samples out of 29 samples), MR3 that PR2 with MspI enzyme cut only 48.27% (14 samples from 29 samples), MR4 is PR3 with Eco RI enzymes cut 89.65% (26 samples out of 29 samples) and MR5 is a PR4 with Tas1 enzymes cut only 31.03% (9 samples out of 29 samples). According to Nie et al (2008) that the PIT1 haplotypes were associated with Hatching Weight Significantly ($P = 0.0252$), Body Weight at 28 days ($P = 0.0390$) and Shank Diameter at 56 days ($P = 0.0400$).

Structure of Research Data:

Structure of research data from 76 heads Pelung DOC consisting of 33heads females and 43 heads males, who are a offspring of the 5 sires (cocks) with 15 dams (hens). The structure of research data are listed in (Table 3). From (Table 3), shows the average of HW was 33.83 ± 2.42 gr with a coefficient of variation (CV) was 7% lower than the CV all ages, which means that the condition of the data into a uniform.

Table 3. Average Weekly Gain (AWG) Pelung Chicken. Absolute Growth Rate (AGR), Relative Growth Rate (RGR).

Age	N	Average	Sd	Min.	Max.	CV
Week		Gram				
0	80	33.83	2.42	27.06	38.50	7.00
1	80	57.88	5.42	46	70	9.37
2	80	96.17	9.44	75	115	9.82
3	80	146.57	19.88	110	202	13.56
4	80	215.78	25.61	158	267	11.87
5	80	299.15	34.03	206	366	11.38
6	80	396.89	45.62	259	491	11.49
7	79	502.35	57.53	332	631	11.45
8	79	622.54	73.94	413	782	11.88
9	79	751.78	94.87	501	962	12.62
10	77	891.70	112.75	578	1 157	12.64
11	76	1 029.34	133.32	697	1 337	12.95
12	76	1 157.77	153.46	759	1 463	13.25

In terms of average body weight gain is achieved per week, then it can be followed in (Table 4), in order to determine the point of inflection of postnatal growth in Pelung chickens:

Table 4. Average Weekly Gain (AWG) Pelung Chicken. Absolute Growth Rate (AGR), Relative Growth Rate (RGR).

N	Age	BW	AWG	LPR	RGR
		gram			
80	0	33.83			
80	1	57.88	24.05	3.44	0.055
80	2	96.17	38.29	5.47	0.052
80	3	146.57	50.40	7.20	0.042
80	4	215.78	69.21	9.89	0.039
80	5	299.15	83.37	11.91	0.033
80	6	396.89	97.74	13.96	0.028
79	7	502.35	105.46	15.07	0.023
79	8	622.54	120.19	17.17	0.021
79	9	751.78	129.24	18.46	0.018
77	10	891.70	139.92	19.99	0.017
76	11	1 029.34	137.64	19.66	0.014
76	12	1 157.77	128.43	18.35	0.011

In terms of body weight per week at make the diagram as the Y axis (ordinate) and age serve as its X axis, it will get the intersection between these two variables forming a growth curve, it is when in Generate growth curve using nonlinear regression exponential ($Y = ae^{bx}$), then when followed up to the age of adulthood (puberty) will look sigmoid shape, however in this study preferred only see the point of inflection on the growth of chickens Pelung by limiting the growth phase to the acceleration phase.

To be clear inflection point in sight, it can be showed to on a growth curve (Figure 1).

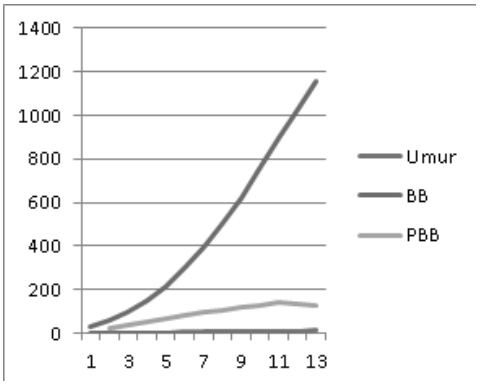

Figure 1. The Growth Curve since Hatched until 12 weeks old

The highest Average Weekly Gain (139.92 gr) was achieved at the age between 10 weeks old before the 11 weeks old. In the 12 weeks of absolute growth rate ranged (RGR) from 3.44 gr to 18.35 gr.

This value will continue to grow during puberty and feeding chickens, when it passed the fat accumulation.

In contrast to the relative growth rate obtained by dividing the absolute growth rate by half of the initial weight and final weight, which ranged from 0.055 gr to 0.011 gr will be changed to a negative value when animals are no growing any more, it is in accordance with the statement of Broody (1945).

Components and Heritability Body Weight range up to age 3 months.

The results of data analysis using REML with Animal Model with repeated measurement pattern (Table 5).

Table 5. Variance Component Ve, Va, VP and Heritability of HW, BW4, BW12 dan CW.

Hatching Period	Avg of BW of 0, 4, 12 and 1-12 weeks			
	0	4	12	1-12
V_e	2.95	328.25	11.775,15	4,218.681
V_a	2.95	328.25	11.775,15	699.752
V_P	5.80	656.49	23.550,31	4,918.433
Heritability	0.50	0.50	0.50	0.142

The highest selection intensity achieved on sex ratio of 1 male with 3 females, which is based on the highest rank in the genetic population,

the increasing HW on future generations is 2.55 gr above the population average.

The number of males used will also affect the fertility of the eggs, however, when the AI reproductive technologies was used, the problems will not be encountered. In this technology not only the proportion between males and females was considered, but also the quality of spermatozoa will make determination.

According to Iskandar (2005) Pelung chicken, Kedu chicken and Sentul chicken has an average spermatozoa number of 2.26 billion sperm per millimeter. Apart from that hen, the HW also affected by the weight of the hen. The too young or too old hen will produce eggs which did not have optimal weight for hatching eggs, because for HW occupies about 61-76% of the egg weight (Latour, et al, 1998).

CONCLUSIONS

Variation of reverse and forward primers PR1 showed that migration is relatively the same for all the samples and estimated (based on comparison with a marker) that is the size of the fragments between 250-500 bp, amplification size based on primer design was 387 bp (during insertions) or 330 bp (during deletions).

Variance components for HW trait consist of a variance of additive genetic (Vga) of 699.75. Variance of environment (Ve) was 4218.67; The Variance of phenotype (VP) is 4918.43. Increased HW on future generations is 2.55 gr above population average.

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PASTORALISM AND THE ROMANIAN HISTORY. SHEEP BREEDS-PEOPLE, LANGUAGES, GENES IN NORTHERN CARPATHIANS AND PANNONIA BASIN

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Abstract

In SE Europe there are 4 local Romanian groups of sheep breeds. *Old, useful genetic resources,* ***t****hey also are* ***historical and archaeological documents****. One of them, Valachian (erroneously named 'Zackel') reflects the Daco-Thracian descent of the Romanians; the other (Corkscrew horns Vallachian, erroneously named 'Racka", Tsigai; and Ruda) are of Roman descent. These breeds* ***could be useful to clarify some controversial history literature in SE Europe regarding the relationship between the peoples, their genes, and the languages they speak, especially since the genetic and linguistic trees of the human population in this region can be different.***

The North Carpathian countries *are believed to be populated by people of Slavic origin. The old Vallachian and Tsigai sheep presence in this area is connected to an old presence of immigrant or autochthon Valachs who may have lost forcefully or willingly their language, and being assimilated produced different deviations of Slaves from their genetic type to the Romanian one.*

Hungarians, inhabitants of the former Roman Pannonia, *are classified language-wise as Magyars, "which imposed its language on the local Romance-speaking population." (Cavalli-Sforza 2000). Palaeogeneticaly it is demonstrated that modern-day Hungarians* ***are genetically just only about 10% Magyars*** *and according to some estimation about 50% Slavic, 30% Romanian (an underestimation), and some 10% German and Gypsy. The Vallachian (Zackel) sheep, present in Pannonia ad least since the Middle Ages, Corkscrews horns Vallachian (erroneously named Racka), Tsigai and Ruda attest the former presence of Romanians in this region and attest that "Hungarians" are ethnically somehow also Romanians.*

The Slav populations of the South Pannonia Basin countries, *north of Jirecek lineare, according to some historical data (Noel Malcom 1994), some admixtures of Slavic people, Vlachs, and Valachs. Practically all the sheep breeds from the area are Vallachian ("Zackel", named Promenka), Tsigai, Corkscrew horns Valachian, and Ruda.*

In spite of their linguistic diversity, the "nations" which immigrated during the Middle Ages from this part of Europe are perhaps somehow genetically similar to the assimilated Valachs, perhaps themselves with a ***former large intertribal variation,*** *reflected by the intra and inter sheep group breed variation.*

Key words: *Archaeological document, assimilation, pastoralism, native sheep breeds, indigenous , immigrant, Pannonia.*

INTRODUCTION

Pastoralism, one of the oldest agricultural areas, had a great weight, not exclusively in the history of Romanians, but also in the miraculous conservation of their national and linguistic traits. Sheep milk and wool had a great role in the difficult survival of the Proto Romanians. Four local groups of sheep breeds registered in a vast SE Europe area suggest an actual or former Romanian presence. Thus these breeds, old, useful genetic resources, and even the sheep production system can also be regarded as historical and archaeological documents (Draganescu 1997). They could be useful for a clarification of some controversial historical literature in SE Europe regarding the relationship between the peoples, their genes, and the languages they speak.

We analyzed the situation of sheep breeds, peoples, languages, ethnicity in the areas located in the North-East of the Black See and in the Balkans. On these first findings we proposed a scheme (fig 1) of Romanian community organizations evolution in the large territory of their sheep breeds dispersion. We hope that the obtained data will help the paleogenetical, and historic research in clarifying the genetic relationships between people and even between the sheep breeds.

As the identification of language – genetic (ethnic) people relation, even the breed taxonomy in SE Europe seems to be more complex and has many unsolved complication and mysteries aspects, we continue the investigations in the Pannonia Basin. In this paper we will try to (1) remind and clarify some historical data on pastoralism and sheep breed extent in this area; (2) make a connection between the native sheep breeds and the former Roman and Romanized population; (3) reveal some aspects of people, language and genes in this area.

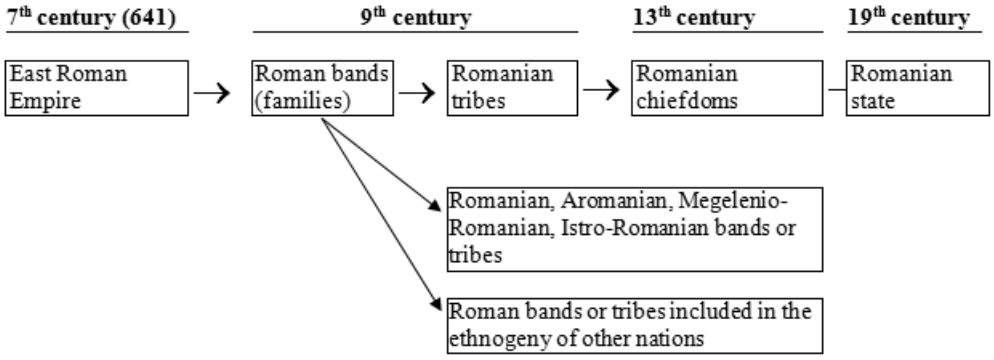

Figure 1. Society** evolution of the Proto Roman and Romanian population after the disappearance of the former East Roman Empire. (Draganescu 2007). One of their native sheep breeds started its formation before and three during the Roman Empire and evolved during the existence of Romanian bands, tribes and possible chiefdoms societies (Archiva Zootechnica 2007, 10:1-10)

RESULTS AND DISCUSSIONS

I. Some facts on pastoralism and sheep breeds in SE Europe.

"Please excuse me for persisting in my ideas that most sheep breeds of this part of Europe are connected to the former Vlack Valack existence."

Noel Malcolm. 1999

I.1. Proto Romanian pastoralism

The Romanized population from the former East Roman Empire, descends from the former Thraco-Geto-Dacian populations, and Roman immigrants, the ProtoRomanians, maliciously named by the new Byzantine power and by the new immigrants peoples Vlachs or Valachs, had to leave the history in the 7th century, when the official imperial language was changed in Byzance from Latin to Greek (641 year), and most of the peninsula has been occupied by Slavs. They retired for many centuries from the state, „imperial", social organization, to an ***obscured anonymous rural band and tribal life*** (fig 1). That can be an explanation of the absence of script material on them, fact which allowed many historical speculations.

The former Romans settlers and Romanized natives have managed to exist through the centuries, as Matley (1968) stated, ***"almost exclusively by herding of livestock on mountain pastures"***. In fact, so strong has herding become associated with the Vlachs that in part of Greece the term Vlahos is used to denote a shepherd and "Vlachostrata to denote transhumance routes," "with no ethnic connotations". Since the Middle Ages the term of Vlach has been used in Serbia and Bosnia as synonymous with mountain herder of any ethnic group". Perhaps this is what the Russian Tsar Nicolas II (1870) meant when he said that "Romania is not a nation, not a state, but a profession" witch is really a paraphrase of Bismark's words.

"The survival of the former Romans under these circumstances seems something of a miracle", noted Winnifrith (1985), "and perturbed the modern 'national' historians of this part of Europe. A large amount of 'historical' studies are dedicated to them". Seldom transhumance was mistaken by chance or deliberately to nomadism or to migration, their territorial location and even presence

contested. Winnifith (1985) however concluded***"It seem not to rash to guess that the Vlachs in the absence of any evidence to the contrary may have lived in the same way and in the same place for the previous fourteen hundred years"*** The transhumance, imposed by the mountains conditions (not possible to have forages for wintering in mountains), made possible the breeds dispersion and the tribal relations, the pasture disputes (the case of Vlahs and Saracatsans), but also the historical speculation by mistaking transhumance to nomadic and migration.

*People, language, genes palaeogenetics***.** Ages, as Matley (1968), Malcom (1996, 1999), Winnifrth (1985) noticed, referring to the south Pannonia Slavs area that *"most of the Romanized and Latin-speaking population had become spontaneously or deliberately assimilated by the surrounding newcomers others nationalities, but in spite of losing their language they still maintained the old way, pastoral of life"* and their breeds. As a result of forced assimilation appeared a difference between people name, language and their ethnicity (genes). ***Some people have a name, speech a language but really they have a different ethnicity,*** gene fund. ***The language designs a people and the genes another.*** A new genetic branch **– palaeogenetics** -appeared lately, allowing the clarification of the true relations (similarities, differences, evolution) between populations. The problem is scientifically interesting and sociological useful in establishing good inter-peoples relations. Sometimes it seems that genetic research (palaeogenetics) is more difficult (in intra breed people) and we supposed that some other cultural and economic indicators can help problem clarification. It is the case of local sheep breeds produced and utilized by different people. We note however that **there is no doubt that the conquering immigrants, from the Migration Period, didn't bring any sheep breeds to SE Europe.**

I.2. Phyletic native group of sheep breeds and the Proto Romanian ethnogeny

Sheep breeds. Generally each territorial community formed his breed. The name of breed was connected to the name of community the breeding region (Vlachico, Valachian) or to some breed characteristics (Corkscrew horns, Ruda from Aruda = fine soft wool, Tsigai = fine soft wool). With such nomenclature systems, which practically presented a short standardized description of the breed, it was easy to identify it, some history and the relationship with others breeds. These were the first signals for the identification of four breeds as "Valachian" and the former territory of them. However history complicates the problems.

Despite Valachs assimilation, **the name of breeds was seldom changed,** by chance**,** or by, an unjustified "national pride" (the language and the ethnicity are different things and must not be mistaken) **. It is symptomatic that on many sides of the borders of Balkan countries (Albania-Greece, Bulgaria-Serbia etc.) there are the same breeds seldom with different name, even though the borders are just from the 19th or 20th century and the breeds are there for centuries.** As a result the breeds name do not reflect always now, with some exception, their characteristic, phyliation, relationship, and that is not just a scientific damage but also a damage for a cooperation in their improvement, conservation, utilisation.

I.2a. Valachian ("Zakel") taxonomic heritage phyletic sheep group, attest the Thraco-Geto-Dacic descend and the territorial extension of Proto Romanians.

Valachian is the most important native heritage sheep phyletic group of Central and SE Europe, extended from Ural-Caucasus to Bohemia and Pindus mountains, adapted to low-input sustainable farming systems, still an integral part of most economy and ecology of this region, sustaining their natural landscape.

According to Bokonyi 1974 p. 181, 182, quoting Zeuner (1963 p. 187) "Zackelschaf" appeared in Mesopotamia in the 4th millennium BC and occurred in South East Europe as early as in the 2nd millennium BC" (Brendjes B, 1965 p.29). The explanation of actual name (Valachian) and extension area can be explain perhaps by the fact that in was introduced in Europe by or to the Thraco-Geto-Dacians, and suggest possible language similarities of their language to the Latin of Vlachs.

Some errors persist in their identification, denomination, classification:

-Their phyletic old name Zackel, not used for any breed, is scientifically incorrect, being the translation into German of word "strepsiceros" from the Latin name of a breed (O. a. strepsiceros = Corksrew horns Valachian) from the old Egyptian phyletic group.
-The breeds' identification, denomination and classification was made initially isolated in different country, not always on those criteria, and in some countries frequently changed by unclear reasons. As a result some errors persist creating some chaotic situation. In the phyletic group are included breeds from others phyletic group (Ruda group, Old Egyptian group) and not clear included his breeds.
-Genetic diversity of Valachian breeds is very large, produced by very many generation of evolution in divers ecological niches, divers pastoral systems (sedentary, transterminance, transhumance), and divers community breeding in small regions. Most breeds have an island structure and are possible that sometimes the very different "island of a breed" is denominated just "variety", a not correct taxonomical category, and sometimes-different breeds. More or less empirical approach of the problem mislead to a "taxonomical" complication.

I.2.b **The Corkscrew horns Valachian, Tsigai and Ruda breeds attest the Roman descend of the Proto Romanians.**

Corkscrew horns Valachian It is a breed from the Egyptian-Mesopotamian phyletic sheep group. It seem that is the sheep of a former Roman colonizers brought to the Serbian, Montenegro, Pannonia area from the Middle east-Egyptian area, who survived there as Valach band or tribe until the 17-18th century, being gradually Serbicized, or Hungarcized**.** It is very well conserved in Hungary, where it was considered as national breed, in Romania and Serbia.
With his name is a whole ***comedy of errors***. Buffon (1768) after a description of Colinson presented it under the name of ***Valachian*** sheep (Brebis valachienne); Darwin (1865) presented the breed under the same name. The Serbs, knowing their owners, use to call the breed ***Valaska Vitoroga*** (Corkscrewhorns Valachian) or in Montenegro "Baluska". The Romanian peasants from Romania call this sheep "Serbian", and the Hungarians and the Germans from S-W Romania ***Racka***, but most of them forgot that Rakz means in their language "Serb", and thought that is a Hungarian national breed, brought by them. Maior (1899) spell the breed name Raczka and explain that it mean "Serbian", but the Romanian scientist didn't notice the translation. Cornevin (1890) presented the breed as being "from Montenegro". Nathusius (1890) wrote its history and, translating the Linnaean breed name- (***O. a. strepticeros***) into German, named it ***Zackel*** (strepsiceros=zackel), and created the basis of ***confusions to Valachian***, because for the Germans living in Romania Zackel was synonymous to Valach (mountain peasant) and Corkscrew sheep had the wool a little similar to Valach sheep". As a result the Valachian breeds have been named Zackel, and the Corkscrew horns Valachian sheep, the descendant from the old Egyptian-Mesopotamian sheep, have been erroneously introduced in some phyletic group with the descendant of Thraco-Geto-Dacic, clear morpho-ecological different taxonomical group.
The breed is still conserved by Romanian peasants on the Romanian-border, in Serbia and very well in Hungary, sometimes as a "Hungarian historical breeds", (Draganescu 1997).

Tsigai. It is a breed from the Merino phyletic sheep group. Kulesov (1894) noted "The (Tsigai) skull was so similar to Merino that was possible to differentiate them just on the label", and "If we consider Merino as it was 100-150 years ago, the differences to Tsigai are negligible". The same findings were noted also by others scientist (Adametz, Teodoreanu etc).
It seems that this is the sheep of a former Roman colonists introduced during the Roman time (105-275 A.D.) to Carpathian Bend from where it was dispersed in SE Romania and by transhumance in North Carpathians, North Bulgarian, NE Pontic areas, possible in East Pannonia and sold to Turkey, Albania, Bulgaria. Other hypothetical origin is not valid because its name used in all countries is strictly a Romanian word, meaning soft, fine wool, and all dispersion routes are known. They have a semi fine wool, white dominant to black (as in Merino), brown, reddish or white face and legs.
Ruda is a sedentary, phyletic group of breeds, with a white uniform but coarser wool than

Tsigai, generally with black face and legs. A different conformation (lopped ears, Roman nose) and a larger weight is related to Italian Bergamasca. Introduced perhaps by Romans in more plain area of Balkan Peninsula and seldom confused to Tsigai (Serbia, Croatia, even Hungary) or to Valachian, was noticed just by Mason (1986) as independent phyletic group. We note that the confusion to Tsigai was facilitated by the fact that owners from Serb-Croat-Hungarian border were perhaps Valach and they don′t have in their vocabulary the word Ruda, a Vlach synonym of Tsigai.

II. **People, languages, genes in Northern Carpathians and Pannonia Basin**

"*They imposed their language on* the province, a frequent outcome of conquest."

Cavalli Sforza 2000 p. 154

The historical document registered in prehistorically time on Pannonia Basin as well as in all Central and SE Europe a great diversity of tribes, seldom considered people even it is not clear if they had different languages not speaking of genes.

The acquaintance of the scientific truth, important in our era, imposes a clarification of real sociological, cultural and genetic structure of populations.

Old, useful genetic resources, **t**he sheep breeds are also **historical and archaeological documents**. One of them-Valachian (erroneously named 'Zackel') reflect the Daco-Thracic descent of the Romanians; the other (Corkscrew horns Vallachian-erroneously named 'Racka", Tsigai; and Ruda) reflect their Roman descent.

These breeds **could be useful for a clarification of some controversial history literature in SE Europe regarding the relationship between the peoples, their genes, and the languages they speak, especially since the genetic and linguistic trees of the human population in this region can be different.**

II.1. Sheep breeds, people, languages genes in North Carpathian countries

The main local sheep breeds in North Carpathians, from the Romanian border to their end at Austrian border are Valachian and Tsigai.

Their name is conserved in Czechia and Slovakia. The Valachian name was changed in Poland, were in denominated now Polish mountain breed, even in the years 1930 they imported for improvement rams from Romania (Draganescu 1995). In Ukraine, in the former Czechoslovak and Romanian area, the Valachian is named Ukraine mountain sheep breed. Actually, these breeds can be found not just in mountains but also in some lowlands.

The presence of these breeds can be connected to the mountain existence of a group of old diverse highland communities, "people", more or less assimilated to the country where5 they live, but with specific ethnographic and even linguistic characteristics.

It is possible that the mountains were for them a refuge. **Survival of a language, of a people is more likely to happen in refuges (isolated place-like mountainous regions − resistant to invaders (Braudel 1985, Cavalli Sforza 2000). In** a word they are named Gorals there is highlanders. The main highlander communities of such indigenous people are the following.5

Moravian Wallachia. Located in the easternmost part of Moravia, Czech Republic, near the Slovakian border.

The name Wallachia was formerly applied to all the highlands of Moravia and neighbouring Silesia, although in the nineteenth century a smaller area came to be defined as **ethno-cultural** Moravian Wallachia.

I*t is the single community where is* maintained his real communities name Vlachs. Are accepted as former Vallachians some more communities named **Gorals** along southern Poland (Podhale, Zywiec), northern Slovakia in 4 separate groups: in northern Spiš (34 villages subdivided in two groups), Orava and Kysuce (2 villages) and smaller groups in 7 other enclave villages and Czech Republic (Silesian Beskids, Zywiec Beskids) in northern.

They are considered, it seem with some subjectivity, as partially descended from Romance-speaking Vlachs who migrated into the region from the 14th to 17th centuries and were absorbed into the local population.

We accepted (1995) that in this centuries there were perhaps some transhumance shepherd from Meridional Carpathians, especially Tsigay owners who visited Nard Carpathians, but it is difficult to accept that there were no sheep, no autochthonous Vallach, even

lowlanders before the Slav immigration in this area.

In the remaining Carpathians area there are now 3 communities, **Hutsuls** (Ukraine, Romania) considerate by some students as been original Romanians, Lemkos (in Poland, Slovakia, Ukraine**),** Boykos (in Ukraine, Poland, Slovakia) with a sort of Proto Slavonic languages but with some Vallachian ethnographic and even professional characteristics.

Language, Genes, People in North Carpathian area. The identification of genetic people differences in this area is somehow difficult because practically all of them belong to the Indo-European genetic family. For these reasons we insist for some clarification starting from others information, in special sheep production. **The** sheep breeds from the North Carpathian area suggest a great language similitude between the peoples of the area, but also suggest some genetic similitude with the Romanians, produced by the assimilated Vallachian. The last hypothesis seems to be by the small numbers of palaegenetic studies published until now who didn't note significant differences between peoples. It is an interesting problem for genetic researches to clarify the problem, and also the small people's communities, possible descents from the old tribe who use to live in this area.

II.2. Sheep breeds, people, languages genes in Pannonia

The history of sheep breeds in the ancient Roman province of Pannonia, which correspond roughly to modern Hungary, are well presented by Bokonyi (1974) in an interesting study on the History of Domestic Mammals in Central and Eastern Europe. The indigenous breeds have been breed especially up to the middle of 18th century. They became almost extinct because of introduction and spreading of the merinos. We retain that from local breeds the **Vallachian (Zackel**, it seems name the Medieval breed of Hungarian sheep), were, present in Pannonia at least since the Middle Ages. In his place were introduced now from Romania so called "Gyiimes Racka" a variety of Romanian Valakian (Tsurcana). The most important local sheep breed seem to be now the **Corkscrew horns Vallachian** (it seem named **Hungarian Zackelschaft** or erroneous **Racka (=Serbian), named by Buffon-1768- and by Darwin −1865-"Valachian"and by Serbs Valaska Vitoroga**). It is considered as national breed introduced by conquering Hungarians or other people and tribes − Avars, Pechenegs, Jazygians, Cumans"- (Boconyi 1962 quoted by Dunka, 1984). However its presence has been identified archeologically by bones only from 16th century (Bokonyi). The 3rd local breed, introduced perhaps from Romania (some students write erroneously that they came from Balkan and Asia Minor, were the breed doesn't exist), and now imported from Slovakia. The 4th local breed is **Ruda,** erroneously named Dairy Tsigai even on the Serb-Croat border from which it was imported.

The presence of these breeds put the problem whether the "Hungarians" are somehow ethnically also Romanians**.** The paleogenetics studies of Cavally Sforza (1994, 2000) attest the supposition asserting that" ***Latin was the administrative language in the ancient Roman province of Pannonia, which corresponds roughly to the modern Hungary, but Pannonia was invaded by the Uralic speaking Magyars at the end of ninth century A.D. " (p.114) "The conquest resulted in a Magyar monarchy, which imposed its language on the local Romance speaking population," (p.151).*** Some others recharges attest that "the number of conquerors was large but did not constitute the majority of population-perhaps less than 30 percent of the total" (p.151). According to some estimation about 50% Hungarian's genes fond are genetically Slave, 30% Romanian (an underestimation), and some 10% German and Gypsy. The identification of genetic people differences in this area is somehow difficult because practically all of them belong to the Indo-European genetic family. For these reasons we insist for some clarification starting from other information, particularly, the sheep production

II.2. Sheep breeds, people, languages genes in the South Pannonia Basin.

The "nations" from this part of Europe are more or less genetically related, their genetic and linguistic trees being different (As Cavalli Sforza noticed for Hungarians) ".
Noel Malcolm. 1999

The Slav populations of the South Pannonia Basin countries, north of the Jirecek line are (Malcom 1994, 1999) some admixtures of Slavic people with Vlachs, and Valachs. At the Slav immigration the former Romanized population used to live in this area (1999 p 2328) and and now "after Greece, Yugoslavia has the second large number of Vlachs within its borders (45)

Practically **the sheep breeds** from the this area are: Vallachian, Tsigai, Corkscrew horns Valachian, and Ruda (2003 Sr. Stoianovic-Serbia and Montenegro, D. Koman-Slovenia, M.Posavi, Croatia-FAO reports). The sheep production system is Valach and many Vlach words connected with pastoral life were absorbed into Serbo-Croat dialects, as well as in all SE Europe. As some data can produce taxonomic confusion, we will present just some opinions on them.

In the **Vallakian phyletic group**, ("Zackel", named in Slavic languages "Promenka=long lock wool") are included many breeds from other phyletic groups; (Ruda, Corkscrew Vallahian etc.) and the breed name is often changed. The changed name hides the group and the breed's type, their origin, and their relationship. Mason (1988) presents the breed Piva from North Montenegro, as named also Durmitor (Romanian word). The Bosnian Vlasic, is clear, as Matley (1968) noted, the breed of some transhumance Vlachs, „completely Slavicized, many converted to Islam during the Turkish occupation"; more even in Malcom opinions the Valachia („Zackel") breeds from Bosnia are originally from Romania and have changed name.

For **Corkscrw horns Vallahian** breed, it is honourably for the Serbs the use the correct name, also accepted by Buffon and Darwin. The acceptation of Tsigai as immigrated from Romanian has however some scientific complications. The "Tsigai" have some complications. The **"Somborski" Tsiga**, named by Hungarian Milk Tsigai, from the Serbo-Croatian border is not Tsigai, but **Ruda** breed. It can't be imported from Romania, because in Romania it wasn't (it was introduced from Bulgaria where perhaps it came from Sombor). The second "Tsigai" "Cokanski", from the the Serbo-Romanian border is also Ruda perhaps, and can also be the true Tigai introduced by the Romanian transhumant shepherds.

The Slavonic people from the area have more or **less different languages**, certainly different from Vlach Valach, and Istrian dialects of the Romanian language, assimilated people. Even the genic structure of each language was more or less identified and the conclusion seems to be that practically all of them belong to the **Indo-European genetic family**. (table 1) Intnsive *c*ollaboration between experts of genetics, historians and archaeologists in the research of the ethnogenesis of populations").Until a further research will clarify the problem, it is interesting to elucidate the magnitude of Vlach and Valach assimilation noted by some studies in the last decades excepting the presently not assimilated known **Romanian tribe (Istria, Timok Valley, Banat etc),.**

Malcom noted that, "this enables us to see that in the late fifteenth century there were at least **35,000 Vlachs in Hercegovina**, and in the sixteenth century as many as **82,692** mainly Vlach households (including some non-Vlach martolosi, with similar privileges) **in theSmederovo region to the south of Belgrade** For a time, as A.I. Popovici (1939) noted also, **Belgrade was almost a Vlach city.** Malcom noted also "So important was the Vlach element in the creation of this Bosnian Orthodox population that, three centuries later, the term 'Vlach' was still being used in Bosnia to mean 'member of the Orthodox Church**.'**

For Vlachs or Morlacks on the north and north-western frontier of Bosnians, Ferdinand II established in 1630 a document known as the **'Statuta Valachorum''Law of the Vlachs'.**

In Zagreb, there is, from 13th, century a **Vlaška Ulica** (*Vicus Latinorum*). Some of these pastoral Vlachs also penetrated as far as the central Bosnia, where medieval place-names in the regions of Sarajevo and Travnik indicate their presence: Vlahinja, Vlaskovo, Vlasic.

Most of these early Dalmatian and Bosnian Vlachs seem to have led quiet, secluded lives in the mountains.

But in Hercegovina itself, where there was a large concentration of Vlachs, a more military and aggressive tradition developed. There are many complaints in Ragusan records of raids by these neighbouring Vlachs during the

fourteenth and fifteenth centuries. The Vlachs of Hercegovina were horse-breeders and caravan-leaders

Giovanni Lovrich noted in the 1770s that the Croatian Morlachs all had flocks of 200, 300 or 600 sheep, and when he asked why they were so reluctant to till the soil, they replied: '

Our ancestors didn't do it, so neither shall we.'

Some writers, especially Serbian ones, have argued that the term 'Vlach' was used just to mean 'shepherd' and did not imply any ethnic or linguistic difference--so that most of these people were really just Serbs with sheep.

This view is rejected by the leading modern expert on Vlachs in the early Ottoman Balkans, who insists that they were regarded as distinct population

The great South Danubian Vlachs presence is attested also by the Turk Imperial low from 9/22 May 1905 by which they are recognized civil rights (schools, churches in their language etc.), right which was not observed by the new national states.

One explication and method of Vlachs assimilation in all Balkan area is that they have always been bilingual (as today), since they were never the administrators, they haven′t the right to have schools and churches in their language.

As a result of Vlachs assimilation many Slavs have, as Hungarians, Romanian ancestry, but, one cannot possibly calculate precisely the percentages for the 'Vlach' ancestry of them. Some (mainly in Croatia) became Catholics, and quite a few were Islamicized in Bosnia.

To call someone a Serb today is to use a concept constructed in the nineteenth and twentieth centuries out of a combination of religion, language, history and the person's own sense of identification.

Table 1. Y_DNA haplogroup among SE Europe Region (A.Imreh synthesis)

on/Haplogroup	I1	I2a	I2b	R1a	R1b	G2a	J2	J1	E1b1b	T (+ L)	Q	N1c1
Bosnia-Herzegovina	2.5	50	0.5	13.5	4	2	6	1	14.5	2.5	0	0
Bulgaria	3	20	1	18	18	1	20	0	16	1	1	0
Croatia	8	42	1	29	8	1	3.5	0	6	1.5	0	0
Czech Republic	11	9	4	34	22	5	6	0	6	1	1.5	0.5
Greece	4	10	1.5	12	12	3.5	25	2	27	3	0	0
Hungary	8	15	2.5	32.5	17	5	7	0	9.5	1	1	1
Poland	6	9	1	56.5	16.5	2	1	1	3.5	0	0	0
Romania	1.5	17.5	2	22	22	1	24	0	6	2	2	0
Serbia	2	29	4	15	7	1	10	1	24	7	0	0
Slovakia	6	10	1	40	23	1	4	0	11	1	2.5	0.5
Ukraine	2	12	1	50	4	4	10	0	8	2	5	2

CONCLUSIONS

The 19th, 20th century **formation of national states** in SE Europe, a historical, human and ethical development necessity, also had some unpleasant effects, *tendency to denature the history* for vainly reasons or territorial vindication, some tendency to emphasise again the forced assimilation of foreign people. The modern international opinions and even legislation break such actions. Perhaps our findings tend to give a scientific basis for a break.

The peoples from this part of Europe are perhaps somehow relatives, genetically similar (table 1) by assimilating part of the Romanized population from the former East Roman Empire. In seems that the inter people genetic variation is much lower than the intra peoples variation, cultural and economic development deserving more attention.

The ***Proto Romanians*** probably also had **a large intertribal variation** reflected by the 4 inter sheep group variation and by the intra group breed variation. The linguists studied this aspect, which is also reflected by the sheep breeds, aspect who deserve perhaps more scientific attention; the Moldavian Black and Grey Valachian (Tsurcana) sheep attest that the Moldavian ethnogenesis took place in

Moldavia; they didn't come from Transylvania, as an old legend asserts.

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HISTORY OF GENETIC EVALUATION METHODS IN DAIRY CATTLE I. DAUGHTER-DAM COMPARISONS

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Abstract

The procedures used on genetic evaluation in dairy cattle are presented, during the last century. These procedures have evolved greatly over the years, from the simple dam-daughter comparison to animal model, from single trait to multiple trait analysis, and from lactation to test-day model. Nowadays, more emphasis is put on the incorporation marker genetic information, in order to get so named GEVB-genomic breeding value.
From historical point of view, there are four category of methods: 1) Methods based on averages (1902-1952); 2) methods based on selection index procedure (B.L.P;1952-1970.); 3) Methods based on mixed model equations (B.L.U.P.; 1971-2000) and 4) Methods based on BLUP and Genomics (2001-present).
The aim of this paper is to give an overview of the genetic evaluation methods in dairy cattle, starting with first category of methods: "the Methods based on averages" or Daughter-Dam Comparisons.
This group of methods cover the period 1906-1950, and take in account the following 12 indexes. For each index the formula is given and also the main advantages and disadvantages are presented.

Key words: *Selection index, daughter-dam comparison, heritability, regression.*

INTRODUCTION

The idea to use the best animals for reproduction is rather old, being mentioned by VARRO, 2000 B.C. The same idea was resumed under different forms in the 18th and 19th centuries. Thus, H. BRANTH (cit. by BONNIER, G., 1936), a Danish farmer, said (1891) that *"the ability of a cow to produce more or less milk fat, from the feed it eats, depends on heredity"*.
BRANTH's ideas have been further developed by SEDELHOLM (cit. by BONNIER, G., 1936), who verified them in his own farm (1900). Practically, Sedelholm compared the daughters with the dams in terms of milk fat, proving that the bulls have a variable influence on daughter records. Historically, this was *the first real attempt to apply selection by progeny in cattle*.
After 1920, the research to identify the best animals in a dairy cattle population entered a new stage with the focus on the genetic evaluation of the bulls.

Several indexes were developed during this period for the genetic evaluation of dairy bulls, most of them being variations of the basic method (dam-daughter comparison). Most indexes rely on the average record of the daughters and dams and on a linear regression which can take values from 0 (Daughter - mean index) to 0.5 (Intermediate index).
A general approach of the selection indexes that have as variables both dam records ($\overline{Y}$) and daughter records ($\overline{X}$) was proposed by LUSH (1933;1944). Within this context he presented a new formula and showed how an index can be obtained starting from the general formula:

$$\hat{I} = a + c \cdot \left(\overline{X} - b \cdot \overline{Y}\right)$$

where a , b and c are constants; $\overline{X}$ = average record of bull's daughters; $\overline{Y}$ = average record of the dams.
The main objective of all indexes proposed by the different authors was to eliminate dam influence and to highlight the genetic potential of the bulls. No index is perfect because the

sources of error cannot be removed completely, just minimized. Therefore, the genetic potential of a bull can only be estimated, within predictable limits. The bulls can be classified on the basis of their genetic potential and retained for breeding depending on the present intensity of selection.

In order to obtain an acceptable precision of the bull index one of the first recommendations was that the bull has to be evaluated on the basis of several dam-daughter couples. As an overview, in table 1 are enumerated the most important indexes, frequently cited in the literature.

The Högström′s index was formulated in base of GALTON′s idea, namely that each progeny inherits ¼ from each parent, the rest coming from other ancestors.

Hansson (1913) continued the investigations in the same farm which Högström had analyzed and proposed a new formulation to estimate the genetic value of bulls for milk fat.

The same Index has been proposed later by YAPP (1925) and mentioned in the literature as YAPP′s index. Because Hansson was the first to propose this index, it was referred in literature as HANSSON-YAPP′s index, as the "index of parental equality", as the "intermediary index" or as the "American index". This index has been used for the genetic evaluation of the Ayrshire and Holstein breeds and by the American Club for Dairy Cattle.

From the early stages of genetic evaluation of bulls, farmers noticed that the average record of a sufficiently large number of daughters can be used to measure the genetic potential of bulls. At least six daughters must be used to obtain an acceptable accuracy (Davidson, 1925; Lush, 1931). R. R. Graves (1925) seems to be the first to present how the index is calculated and to use it under USA conditions.

Based on the study of Gowen (1930) regarding the crosses in cattle, Goodale (1927) proposed the Mount Hope index. In his study, Gowen reached the conclusion that in the first generation of crosses between a breed with higher milk yield and a breed with lower milk yield, the average daughter production is not half way between the parental breeds but closer to the level of the higher parental breed, while the fat percentage is closer to the level of the lower parental breed.

The results of these experiments lead to the conclusion that when animals with different production levels are mated, the average production of milk is about seven tenths of the difference between the parental levels above the level of the higher parent; the fat percentage is about four tenths of the difference above the lower parent.

Gifford (1930) showed that the average record of the progeny is a sufficiently accurate indicator of the bull′s genetic transmission ability (1/2 of the breeding value). The main attribute of GIFFORD′s method is that it allows using the records of all the daughters of a bull, even if their dams have not been tested.

All indexes presented so far relied on the phenotypic difference of the dam and daughter average records without explicitly taking into account the number of dam-daughter pairs (n). In order to eliminate this deficiency, Wright (1932) proposed a new index which takes this aspect into consideration and incorporated the number of daughter-dam pairs (n) per bull.

Bonnier (1936) showed that by using this index, Wright intended to give a higher weight to the bulls with a higher number of daughters. Thus, two bulls with the same phenotypic differences of the daughters, but with a different number of daughters, will have different genetic values, the bull with more daughters having a higher value.

First index proposed by Bonnier (1936) was the *''regression index''* with variable coefficients. The regression coefficient (b) was estimated with the least squares method, by minimizing the difference of the potential yield of a cow and its actual yield.

When the regression has an intermediate value (b = 0.5), the regression index coincides with the Hansson-Yapp index. When b is variable, therefore different from 0.5, the values of the two indices are no longer similar. This shows that the Hansson-Yapp index is a particular case of the regression index, the latter having a wider scope.

HÖGSTRÖM's INDEX (1906)	Finnaly, the formula proposed for the evaluation of bulls was: $\hat{I} = 4 \cdot \overline{X} - \overline{Y} - 2 \cdot A$, where A is the breed average.
PEARL 's INDEX (1919)	When a=0; b=c=1, the dam-daughter comparison index is obtained: $\hat{I} = \overline{X} - \overline{Y}$
HANSSON-YAPP INDEX (1913; 1925)	$\hat{I} = 2 \cdot \overline{X} - \overline{Y}$ which is equivalent with the fact that the value of an average progeny will always be half way between its parents. According to Lush (1944) the same formula is obtained when a=0; b=0.5 and c=2.
MOUNT HOPE's Index (1927)	a) When daughter average is above dam average: $\hat{I}_{Milk} = \overline{X} + 0.429 \cdot (\overline{X} - \overline{Y})$; $\hat{I}\_\%\_Fat = \{\overline{X} + 1.5 \cdot (\overline{X} - \overline{Y})$ b) When daughter average is below dam average: $\hat{I}_{Milk} = \overline{X} - 2.333 \cdot (\overline{Y} - \overline{X})$; $\hat{I}\_\%\_Fat = \{\overline{X} - 0.667 \cdot (\overline{Y} - \overline{X})$ The total Index: $\hat{I}_{Total} = \hat{I}_{Milk} \times \hat{I}_{Fat}$
GIFFORD's INDEX (1930)	When constants a and b are null, and constant c is 1, an index relying only on daughters' average is obtained: $\hat{I} = \overline{X}$
WRIGHT's Index (1932)	$\hat{I} = A + \frac{n}{n+2}\left(2 \cdot \overline{X} - \overline{Y} - A\right)$
NORTON' Index (1933)	$\hat{I} = \overline{X} + (\overline{X} - e) \Leftrightarrow I = 2 \cdot \overline{X} - e$
BONNIER's Indexex (1936)	a) ''regression index'' : $\hat{I} = \frac{\overline{X} - b \cdot \overline{Y}}{1 - b}$ b) '' Index of minimal variance'': $\hat{I} = a \cdot \overline{Y} + (1 - a) \cdot \overline{X}$
LUSH's Index (1941)	$\hat{I} = (2 \cdot \overline{X} - A) - Average\left(\frac{n \cdot h^2}{1 + (n-1) \cdot R}\right) \cdot (\overline{Y} - A)$ $\hat{I} = (2 \cdot \overline{X} - A) - Average\left(\frac{\overline{Y} - A}{2}\right)$
RICE's Index (1944)	$\hat{I} = A + (\overline{X} - e)$
ALLEN's Index (1944)	$\hat{I} = A + 2 \cdot (\overline{X} - e)$

Figure 1. Proposed Daughter-Dam Indexes (Table 1)

The second index proposed by Bonnier (1936) was *'' Index of minimal variance" to* minimize the difference between the true genetic value of a bull (I) and the estimated genetic value of that bull.

Finally, in order to obtain an index with the lowest variance, constant a is incorporated in

the general equation established by BONNIER (1996).

Historically, the index proposed by J. L. Lush is the first one to take into consideration the heritability and repeatability as genetic parameters to calculate the breeding value.

Another improvement of the index is the term of comparison used for the daughters. Thus, Lush proposed to replace the dam average by the farm average, which seems to be an advantage because the daughters whose dams had not been tested could not be included in the calculations for the candidate bulls.

Given the average productive life of the dams, the number of dam records (n) usually varies between 2 and 4. In this case, the value of the heritability of the average, varies between 0.39 (n=2) and 0.49 (n=4), considering a heritability of 0.28 and a repeatability of 0.43 (Lush, 1941). In base of these values, Lush rewrites initial formula. Dam selection was an important source of errors which affects the breeding value of the candidates for selection. This happens when the dams are not a representative sample of the population. In other words, some bulls are mated to better (selected) dams, which will shift the breeding values of these bulls.

When the dams are not selected, and each one has one record, the daughters will exceed their mothers by $(\bar{Y} - A) \cdot (1 - 0.5h^2)$. At the same time, the average daughter record is shifted in an opposite direction, by an amount of $(\bar{Y} - A) \cdot (0.5h^2)$, which will also favor the bulls mated with superior dams (Lush, 1941).

If the dams have several records, the heritability of a single record is replaced by the heritability of multiple records (h_m^2). The amount of shift generated by dam selection is also affected by the level of heritability and by the number of records; the shift will increase with the decrease of n and of the heritability. Therefore, one way to dampen dam selection effect, which shifts the breeding values of candidate bulls, is to take into consideration several records (lactations), while heritability should tend as much as possible towards the level of the repeatability (Lush, 1941).

H. W. Norton Jr. (1933, cit. by LUSH, 1933) suggested an index based on daughter records regressed towards dam records (Allen, 1944; Rice, 1944). In his study, Norton relied on the records extracted from the genetic registry of the Holstein breed. He analyzed daughters whose dams were recorded in the registry. After grouping the dams by classes of production, Norton calculated the average yield of the daughters, which he called "the expected average daughter record" (e). He also proposed a modification of the Hanson-Yapp index, replacing the average dam records with the expected average daughter record.

V. A. RICE (1944), suggested a new method to evaluate bulls based on the expected daughter average record and the breed average record. The method compares the average daughter record with their expected average record and the difference is added to the breed average record. His index was officially adopted in the United States in 1945, and allowed comparing bulls within the breed, not just within the farm(s) where the daughters were; this enabled a better classification of the candidates for selection.

Allen (1944) proposed a modification of Norton's index, by which twice the deviation between the average progeny record and the expected record (e) is added to the population (breed) average.

Allen's index with Rice's index does not double the deviation between the average progeny record and the expected record. However, by definition, the genetic value of a bull is double the deviation of its progeny from the population average, doubling the deviation proposed by Allen's index seems appropriate. Allen's index is identical with the Hansson-Yapp index, but the similarity is valid only when the regression has the value of 0.5. Therefore, the additional accuracy of Allen's index appears only when the value of the regression is different from 0.5.

Over the years, this index have been subjected to rigorous comparative analysis in order to highlight the merits or shortcomings.

Gowen (1930) studied the agreement between the breeding value of the bulls calculated with the selection indices available at his time (Gifford, Pearl, Wright, Mount Hope) and the daughter records, used the correlation method to measure the agreement between the breeding value of the bulls and the average records of their future daughters.

The results showed that the highest agreement was obtained with the Gifford index, while the

lowest concordance was noticed for the Pearl index. On the basis of these results, Gowen proposed a new index to calculate the breeding value of the bulls on the basis of two sources of information: average daughter records corrected for the age at calving, combined with mother records.

Edwards (1932) based on the idea that an ideal index should not change bull classification irrespective of the dams, made a comparison of five indices (Pearl, Hansson-Yapp, Wright, Mount Hope, Gifford), in terms of accuracy of breeding value estimation. First, he calculated the value of each type of index using the three types of averages (general, low production, high production) average, and after determines the differences between indices calculated using the low and high averages and the index inferred from the general average. Thus, Edwards noticed that Gifford′s index, in based of the lowest average difference was considered to be the best. Edwards justified this by Galton′s theory according to which the average daughter records are expected to vary less around the population (breed average) than the dam average records. Edwards reached two important conclusions for the genetic evaluation of the bulls:

a) if the purpose is to achieve stability in bull ranking as new data are added to the evaluation process, the best method was the index with minimal variance;

b) if the purpose is to predict with the highest accuracy the records of the future daughters, the Hanson-Yapp index is the best Rice (1933) conducted an analysis of three indices, (Hansson-Yapp, Mount Hope, Gifford), conclude that any index must meet at least three criteria in order to be useful:

a) it must be readily understandable by the user. If an index is too complex and the users have problems understanding, its utility will be hampered;

b) the index must include both dam and daughter records;

c) by its numerical value the index must state clearly how much it can improve the production level of the evaluated trait

These results show that no index is universally valid. Therefore, adequate indexes must be used for different objectives of selection.

The main disadvantage of the Daughter-Dam Indexes is the fact that the daughters are not contemporary with their dams when records are produced. For instance, there is a gap in excess of 2.5 years between the first lactation of the dams and of their daughters. Changes in the environmental factors can appear in this interval, even within the same farm, which may alter the expression of the genetic potential of the animals. Even more drastic changes may occur if the dams and daughters perform under different environments (farms/production units).

Environmental differences between farms also have an influence on breeding values of candidate bulls. The selection methods most affected by the environmental differences are the Gifford index (average records of the daughter), and the Pearl index (dam-daughter comparison). The other indices are less affected.

In order to overcome the problem of the adverse environmental influences, the USDA decided in the early 60s to replace the dam-daughter comparison by the herdmate comparison.

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GENETIC DIVERSITY OF KARYA AND ÇINE ÇAPARI SHEEP

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Abstract

Genetic diversity of Karya (n=117) and Çine Çapari sheep (n=123), which is indigenous breed of Turkey, were investigated by 10 ovine microsatellite markers proposed by the Food and Agriculture Organization (FAO). A total of 105 and 115 were observed in Karya and Çine Çapari sheep breed respectively in this study. A wide range of genetic variability was observed as allele number from 7 (OarCP34) to 14 (DYMS1) for Çine Çapari and 6 (OarCP34) to 14 (OARJMP58) in Karya sheep breed. The estimated observed heterozygosities (Ho) were between 0.450 and 0.950 in Çine Çapari and 0.541 and 0.841 in Karya sheep breed. The highest genetic identity (0.8131) was observed between Karya and Çine Çapari sheep breed. The results obtained in the present study will help to interpret the genetic structure of indigenous Karya and Çine Çapari sheep.

Key words: *Çine Çapari sheep, genetic diversity, Karya sheep, microsatellite, indigenous sheep.*

INTRODUCTION

Native breeds are the primary elements of animal breeding and they have complied with ecological, social and economical conditions of different geographies. These elements have taken shape within the process of thousands of year's agricultural society throughout history of humanity. Together with industrialization, sociological and economical conditions rapidly changing have introduced the necessity of benefiting more from native breeds. Statistical methods in breeding studies done especially in livestock over the past century have found a common field of application (Beuzen and et al. 2000). Thanks to rapid improvement in molecular biology today, base sequence of DNA can be defined, the place of the genes can be determined, the relationships between the genes can be examined and some genes can be transferred from living creature to living creature. As it's in the other species, molecular genetics methods for identification of genetic structure in livestock have developed rapidly in recent years and the usage of the methods has become widespread (Arranz et al. 1998, Baumung et al. 2004, Pariset et al. 2003, Montaldo, 1998). Specific microsatellite genetic markers to DNA areas are commonly used to identify genetic variety in animals (Bruford et al., 1996).

Çine Çapari sheep breed are raised in highlands of Aydin province. It has been localized in the borders of Aydin province. With so many scientific study done by Adnan Menderes University, the properties of the breed, its current condition are introduced and it's suggested that it's at risk as genetic resource and the breed is taken under conservation (Karaca et al., 1999a,b,c; Karaca et al., 2004; Karaca and Cemal, 2005; Binbas and Cemal, 2007). Karya sheep has developed in consequence of systemless back-crossing of Sakiz, Kivircik or Sakiz x Kivircik cross-bred rams with native breeds (Ödemis, Çine Çapari, Dagliç etc.) in Western Anatolia in the last 20 years period by animal breeders. Since Karya which is a genotype having high reproductive performance and milk yield is preferred by animal breeders, it has become widespread in Western Anatolia in recent years (Karaca et al. 2009). The purpose of this study is to determine the genetic diversity in DNA level of Karya sheep that they are commonly raised in Western Anatolia day by day and Çine Çapari which is under the threat of extinction by microsatellite DNA (STR, SSR) markers.

MATERIALS AND METHODS

Obtaining DNA sample from two hundred and forty animals from Karya (N=117) and Çine Çapari sheep (N=123) (Figure 1) were genotyped with 10 microsatellite markers that selected from the list recommended by FAO (2004). Three multiplex groups were formed with 8 out of the 10 microsatellites. Annealing temperatures of MAF65 and DYMS1 were not appropriate for the other 3 multiplex groups. Therefore, these two microsatellites were amplified by Polymerase Chain Reaction (PCR) separately. Table 1 shows details for the considered microsatellites.

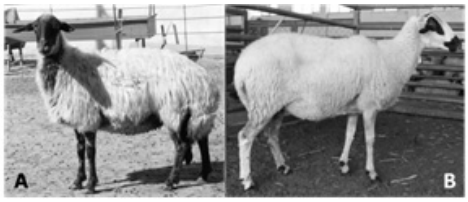

Figure 1. Çine Çapari (A) and Karya sheep (B)

Table 1. Details of considered microsatellites

Locus Name	Primers	Base Pair (bp)
OarCP34	**F:** GCTGAACAATGTGATATGTTCAGG **R:** GGGACAATACTGTCTTAGATGCTGC	112-130
OarFCB304	**F:** CCCTAGGAGCTTTCAATAAAGAATCGG **R:** CGCTGCTGTCAACTGGGTCAGGG	150-188
OarFCB193	**F:** TTCATCTCAGACTGGGATTCAGAAAGGC **R:** GCTTGGAAATAACCCTCCTGCATCCC	96-136
OarJMP29	**F:**GTATACACGTGGACACCGCTTTGTAC **R:** GAAGTGGCAAGATTCAGAGGGGAAG	96-150
OarFCB128	**F:** ATTAAAGCATCTTCTCTTTATTTCCTCGC **R:** CAGCTGAGCAACTAAGACATACATGCG	96-130
BM8125	**F:** CTCTATCTGTGGAAAAGGTGGG **R:** GGGGGTTAGACTTCAACATACG	116-122
OarJMP58	**F:** GAAGTCATTGAGGGGTCGCTAACC **R:** CTTCATGTTCACAGGACTTTCTCTG	145-169
OarVH72	**F:** GGCCTCTCAAGGGGCAAGAGCAGG **R:** CTCTAGAGGATCTGGAATGCAAAGCTC	121-135
DYMS1	**F:** AACAACATCAAACAGTAAGAG **R:** CATAGTAACAGATCTTCCTACA	159-211
MAF65	**F:** AAAGGCCAGAGTATGCAATTAGGAG **R:** CCACTCCTCCTGAGAATATAACATG	123-135

DNA was isolated from blood samples using a DNA extraction kit. Specific genomic regions were amplified by Polymerase Chain Reaction (PCR) in accordance with the touchdown PCR technique. The thermal cycling conditions are given in the Table 2.

For every microsatellite locus, the amplification reaction took place in a total volume of 25 µl and contained the following constituents in the final concentrations indicated in brackets; dNTP's (0·2 mM for each one), MgCl2 (2.0 mM), primers (0.25 mM for each one), and Taq DNA polymerase (1 unit reaction–1). Approximately 100 ng of genomic DNA was used as template for each of PCR amplification. Fragmentanalysis was achieved using the Beckman Coulter CEQ 8000 Genetic Analysis System. Obtained data was analyzed by the Beckman Coulter CEQ Fragment Analysis Software.

Table 2. Thermal cycling conditions according to Touchdown PCR

Loci	Denaturation (°C)	Annealing (°C)	Extension (°C)
OarCP34	95 *45 sec*	60-58 *45 sec*	72 *45 sec*
OarFCB193			
OarFCB304			
OarJMP29	95 *45 sec*	61-57 *45 sec*	72 *45 sec*
OarFCB128			
BM8125			
OarJMP58	95 *45sec*	60-56 *45 sec*	72 *45 sec*
OarVH72			
MAF65	95 *45sec*	59-57 *45 sec*	72 *45 sec*
DYMS1	95 *45sec*	52-50 *45 sec*	72 *45 sec*

The data were analyzed using GenAlEx (Peakall and Smouse, 2006), PowerStatsV12 (Brenner and Morris, 1990), MEGA 4 (Tamura et al., 2007), Arlequin 3.5 (Excoffier and Lischer, 2010) and POPGENE (Yeh et al., 1997) softwares. A dendrogram based on Nei′s (1978) genetic distances was obtained using UPGMA (Unweighted Pair Group Method with Arithmetic Mean) method.

RESULTS AND DISCUSSIONS

In the study, 105 allele in Karya sheep and 115 in Çine Çapari sheep from 10 microsatellite locus has been observed. Average allele number in a locus has been respectively 11.5 and 10.5 in Karya and Çine Çapari sheep. The results obtained are in compliance with the literature (Cemal et al., 2013; Yilmaz and Karaca, 2012). The locus which has the highest allele has respectively appeared as OarFCB193 (17) and OarJMP58 (14) in Karya and Çine Çapari sheep. The locus which shows the lowest polymorphism in Karya and Çine Çapari sheep has been OarCP34. The significant differences in allele numbers in populations indicates to high genetic diversity (Table 3).

Table 3. ASR (bp), nA, nE, Ho, He and PIC values in considered microsatellites

Loci	ÇINE ÇAPARI							KARYA						
	N	ASR	na	ne	Ho	He	PIC	N	ASR	na	ne	Ho	He	PIC
OarCP34	121	112-124	7	4.48	0.843	0.777	0.74	113	112-122	6	4.25	0.841	0.765	0.74
OarFCB193	121	96-136	17	8.67	0.950	0.885	0.87	115	96-140	13	3.32	0.774	0.699	0.69
OarFCB304	121	160-190	11	3.80	0.727	0.737	0.70	115	148-188	13	5.44	0.800	0.816	0.77
OarJMP29	122	116-156	13	4.29	0.820	0.767	0.74	113	110-158	13	4.88	0.770	0.795	0.78
OarFCB128	117	100-128	10	4.86	0.692	0.794	0.77	111	100-134	11	3.90	0.541	0.744	0.74
BM8125	119	108-138	13	4.78	0.782	0.791	0.76	113	108-130	8	3.35	0.726	0.702	0.68
OarJMP58	122	141-171	13	4.32	0.713	0.769	0.74	115	143-169	14	5.92	0.748	0.831	0.82
OarVH72	122	123-143	8	3.88	0.730	0.742	0.71	114	123-139	9	5.89	0.798	0.830	0.81
MAF65	111	125-141	9	4.38	0.568	0.772	0.74	113	121-139	7	3.44	0.823	0.709	0.66
DYMS1	120	169-201	14	4.31	0.450	0.768	0.74	114	181-203	11	5.13	0.763	0.805	0.78
Mean			**11.50**	**4.78**	**0.73**	**0.78**	**0.75**			**10.50**	**4.55**	**0.76**	**0.77**	**0.75**
St.dev.			**3.064**	**1.407**	**0.141**	**0.041**	**0.048**			**2.838**	**1.033**	**0.084**	**0.053**	**0.055**

Observed heterozygosity values obtained from Karya sheep are in the given range (Yilmaz and Karaca, 2012; Grigaliunaite et al., 2003, Tascon et al., 2000). Expected heterozygosis values obtained from Çine Çaparı sheeps has been higher than the studies made with the other sheep breeds (Tapio et al., 2005; Handley et al., 2007; Pramod et al., 2009; Tascon et al., 2000; Grigaliunaite et al., 2003). This situation can be explained with the high level of polymorphic information content of locus studied. Genetic similarity and genetic distance between Çine Çaparı and Karya sheep have been given in Table 4. Dendrogram belonging to genetic distance between populations studied has also been given in Figure 2.

Table 4. Genetic similarity (above diagonal) and genetic distance (below diagonal) between Çine Çaparı and Karya sheep

	Çine Çaparı	Karya
Çine Çaparı	******	0.8131
Karya	0.2069	******

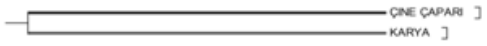

Figure 2. Dendrogram based on Nei's genetic distances between Çine Çaparı and Karya sheep

Above diagonal values present in Table 4 gives the genetic similarities of the breeds. As it's understood from the table, there has been a genetic similarity in high level between the populations. It has been suggested that Çine Çaparı sheep can provide contribution to Karya sheep (Karaca et al., 1999a; Karaca et al., 2004; Karaca and Cemal, 2005; Karaca et al., 2009). When this situation is taken into consideration, obtainment of these genetic similarity values is seen as a normal result. Factorial Correspondence Analysis (FCA) graphic has been drawn to show how the individuals in population are separated. The results obtained are given in the following multidimensional platform (Figure 3).

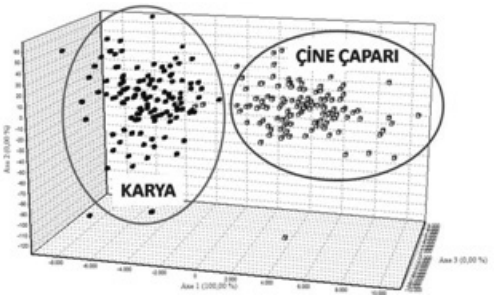

Figure 3. Factorial correspondence analysis (FCA) of Cine Capari and Karya sheep

When FCA graphic (Figure 2) has been examined, it's mentioned that the individuals in Çine Çaparı and Karya sheep populations constitute a group between each other. Dense clustering in Çine Çaparı and Karya sheep populations can be observed or there can be animals which remain between two clusters or enter into the other cluster. This result obtained shows the necessity of maximum microsatellite locus usage in characterization.

If the population performs specific assumptions, simple mathematical engagement which is known as Hardy- Weinberg law is in question to calculate genotype frequencies from allele frequencies. The information obtained from 10 microsatellite locus has been determined by using χ^2 test in terms of suitability for Hardy-Weinberg equilibrium and it's given in Table 5.

Table 5. Chi-Square test values belong to 10 microsatellites in all population

Loci	Çine Çapari Sheep				Karya Sheep			
	DF	X^2	Prob	Sign	DF	X^2	Prob	Sign
OARCP34	21	20,96	0,461	NS	15	12,326	0,654	NS
OARFCB193	136	180,51	0,006	**	78	37,164	1,000	NS
OARFCB304	55	141,41	0,000	***	78	115,917	0,003	**
OARJMP29	78	308,40	0,000	***	78	81,850	0,361	NS
OARFCB128	45	138,66	0,000	***	55	152,422	0,000	***
BM8125	78	216,77	0,000	***	28	15,923	0,967	NS
OARJMP58	78	254,78	0,000	***	91	238,865	0,000	***
OARVH72	28	26,87	0,525	NS	36	88,909	0,000	***
MAF65	36	183,45	0,000	***	21	16,580	0,736	NS
DYMS1	91	365,78	0,000	***	55	44,779	0,836	NS

*** P< 0.001; ** P< 0.05

When the results of ki-square (X^2) test made in terms of suitability for Hardy-Weinberg equilibrium on the basis of population are evaluated, it's seen that 8 locus in Çine Çapari population and 4 locus in Karya population aren't in Hardy-Weinberg equilibrium. When it's considered that these locus given in Table 5 aren't in Hardy-Weinberg equilibrium and there are selection studies carried out in Karya population and protection program applied in Çine Çapari sheep, it's seen as a normal situation. These findings are in compliance with the findings informed by Yilmaz and Karaca (2012) and Cemal et al. (2013).

CONCLUSIONS

In this study, genetic diversity of Karya sheep, which have high reproductive performance and milk yield has been preferred by breeders in the western Anatolia region, and Çine Çapari sheep, which is under conservation indigenous breed of Turkey, have been determined by using 10 ovine microsatellite markers proposed by the Food and Agriculture Organization (FAO) in DNA level.

The results obtained have shown that there is high level of genetic similarity between Karya and Çine Çapari sheep populations. In addition to this, in this study it's suggested that genetic diversity in current gene pool belonging to these two breeds is significantly high. In Western Anatolia, in sheep genotypes over the last 20-30 years, there is a change with the effect of consumer demands. This situation has enabled fat tail breeds such as Ödemis, Çine Çapari and Dagliç, especially present in Western Anatolia to turn into a form which has thin tail by cross-breeding with Sakiz, Kivircik or Sakiz x Kivircik cross-breed rams (Karaca and Cemal, 1998, Karaca and Cemal, 2005, Karaca et al., 2009). The information obtained in the meaning of genetic similarity supports scientific information suggested by previous phenotypic methods.

Consequently, the findings obtained from this study have provided significant contribution to the literature as being the first study regarding identification of two breeds in molecular level.

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BIRTH WEIGHTS AND GROWTH PERFORMANCES OF HAIR GOAT KIDS RAISED IN DENIZLI PROVINCE OF TURKEY

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Abstract

The aim of this study was to determine birth weights and growth performances of hair goat kids till 5 months (150 days) of age at the extensive breeding conditions in Denizli province of Turkey. The analyzed data from hair goats in 54 breeders' flock (4 multiplier and 50 base flocks) was collected in years 2011 and 2012. The least square means for kids' birth weight were found as 3.29 kg.The differences created by flock type, birth type and sex, except years, on birth weight of kids were found statistically significant (P< 0.01).The least square means for live weight of kids at an average age of 150 days were 27.15 kg. This traits was significantly affected by sex (P< 0.05) and age of kids (P< 0.01) only. The average daily gain of kids for first five months of life was 156.32 g. Among the investigated factors, only the age of kids have a significant effect (P< 0.01) on average daily gains (ADG) of kids. The numbers of researches conducted on hairy goats are very limited. Therefore, these results will be a guide for other researches on this goat breed.

Key words: *Hairy goat, kid, birth weight, growth rate, average daily gain.*

INTRODUCTION

Hair goats constitute the majority of the existence of the goats in Turkey. 7.126.862 heads of the goats from total 7.277.953 heads of the goats raised in 2011 in Turkey are hair goats (TUIK, 2013). The goat breeders make this production at almost zero expense excepting of their efforts by using natural resources. Hair goat, which is a natural part of the ecosystem and especially mild temperature zone, is one of the important gene pool for our country as it is an important means of living of the interior part of the forest and forest side villages (Anonymous, 2008a; Kaymakçi et al., 2008; Dellal et al. 2010; Kaymakçi et al. 2010a).

Hair goat raised in each part of Turkey is commonly raised in Aegean region. Within this region, Denizli province is one of the provinces that the goat is most commonly raised. It attracts attention that hair goat constitutes the majority of the existence of the goats in Denizli province.

The goats are extensively raised in high regions, in forest-side maquis shrubland. Five hundred fifty six breeders are registered to Denizli Province Association of Denizli Province Sheep and Goat Breeders established in the year of 2006 in Denizli Province with 84830 heads of goats.

In the warmer months of the summer season, the breeders immigrate to higher areas called as upland from the places they are found and they come back to their villages in the autumn. There is not the habit of common flock management. Each family provides the management of their own flock. As hair goat is generally raised in the region for meat production, it′s quite important to define the growing properties in the goats. When growing properties are called in goats, birth weight, average daily weight gain and marketing weight come to the forefront. These characteristics are affected by some factors such as gender, birth type, birth time, and breeding type (Goodwin, 1971, Hassan, 1987; Osinowo et al., 1990; 1992; 1993).

The characteristics of hair goat in our country have not completely been defined. The definition of some of the characteristics of hair goats within the scope of "National Genetic Improvement Project for Small Ruminants at Breeders' Conditions" put into operation in the

year of 2005 by General Directorate of Agricultural Research and Policy within the body of Republic of Turkey Ministry of Food Agriculture and Livestock and also sub-projects regarding breeding of hair goats are put in to operation in 2011 throughout the country. "Denizli Province Hair Goat Breeding Project" started in Denizli Province take part in these projects. In this project, which is based on breeding program regarding open nucleus breeding system and structured directly in breeder conditions, it has been targeted to determine the characteristics of production of hair goats and breed them. In this research article, it has been aimed to define some growth properties of the goats born in 54 hair goat enterprises (4 elite, 50 basis) in Denizli Province within the scope of this project.

MATERIALS AND METHODS

Hair goats born between the years of 2011 and 2012 in 54 enterprises that raise hair goats in Denizli province have constituted the animal material of the research. The distribution of births months have been given in Figure 1. Birth weights of the goats have been determined by sensitive digital hand scales within 24 hours following the birth.

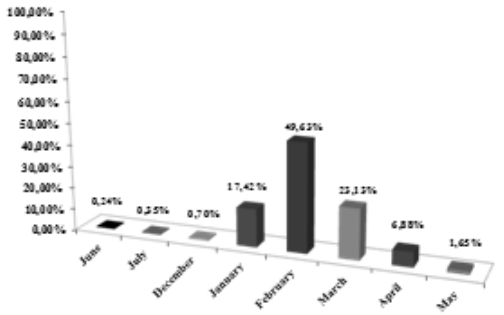

Figure 1. The distribution of births by months

When the goats reach to the age of 5 months, their living weights have been determined by electronic scale having 50 gr sensitivity. As there is a wide variation between the enterprises in terms of birth times live weight control made in certain times in goats has been analyzed by classifying as to be 150 days. The average daily weight gain of the goats has been calculated for the process between the birth and 150th day.

Figure 2. Photographs of Hair goats raised in Denizli province of Turkey

GLM procedure has been used in SAS (SAS, 1999) package statistic program in order to obtain smallest squares averages and make variance analysis of properties discussed.

RESULTS AND DISCUSSIONS

Birth weights, 150th day live weights and average daily weight gain at the 150th day have been determined in order to determine growth properties of the goats. The findings regarding birth weights of the goats in all the flocks have been given in Table 1. As a result of analysis of general average birth weight of the kids has been found as 3.29 kg.

It has been informed that birth weight in Saanen goats is 3.22 kg (Bolacali and Küçük, 2011), birth weight in Saanen x Hair goats cross-breeds (G1) is 2.82 kg (Simsek et al. 2007), birth weight in Damascus goats is 4.17 kg (Taskin et al., 2000), and birth weight in colored Angora goat and Ankara goat x colored Angora goat is respectively 2.17 kg and 2.13 kg. When the related literature is evaluated, it's seen that the value obtained for birth weight in hair goat in this study is lower than Damascus goats and higher than the others. This situation can arise from the difference of the breeding conditions in addition to difference of the breeds. Excepting the years, the difference in sexuality, birth type and stratums is statically found significant ($P< 0.01$) in the differences arisen between the layers, the maintenance and management differences in the farms come to the forefront as determinant. There has been a very small difference between the years. There is superiority in terms of birth type and males in terms of gender. It is an expected situation in addition that it is in accordance with the literature (Taskin et al., 2000; Bolacali ve Küçük, 2011, Simsek et al., 2007; Odabasioglu et al., 2007).

Table 1. Least square means for birth weight, 150th day weight and average daily gain of Hair goat kids

Factors	N	Birth Weight (kg)	Weight at 150 days of age (kg)	Average Daily Gain (g)
Years		**P=0.219**	**P=0.331**	**P=0.408**
2011	1976	3.31±0.025	27.24±0.219	156.83±1.391
2012	2643	3.28±0.024	27.05±0.208	155.81±1.321
Flock Type		**P=0.000**	**P=0.691**	**P=0.579**
Multiplier	476	3.16±0.037	27.21±0.324	156.89±2.061
Base	4143	3.43±0.016	27.08±0.139	155.75±0.883
Birth Type		**P=0.000**	**P=0.946**	**P=0.716**
1,00	3952	3.42±0.019	27.15±0.166	156.63±1.054
2,00	667	3.17±0.033	27.14±0.286	156.01±1.816
Sex		**P=0.000**	**P=0.011**	**P=0.041**
Male	2484	3.46±0.024	26.90±0.207	155.05±1.314
Female	2135	3.13±0.025	27.39±0.220	157.59±1.402
Reg (Linear)				
Age of kids	-	-	0.112±0.008***	-0.284±0.053***
Birth Weight	-	-	0.239 ± 0.132^{NS}	$1..373\pm0.839^{NS}$
General	**4619**	**3.29±0.022**	**27.15±0.190**	**156.32±1.209**

**P<0.001, NS: NonSignificant

On the contrary to the other regions, weaning in the hair goats is made in later period (when the goats is 6-8 months). Weaning age is also evaluated as marketing period in the region. Live weight average of 5 months belonging to 4619 heads of kids evaluated in the study has been found as 27.15 kg. Live weights in 5 months obtained in the study and when average daily weight gain increases in this period is evaluated, the effect of gender on live weight is statistically significant (P< 0.05).

The effect of other factors evaluated on 150^{th} day live weight and average daily weight gain increase is not significant. The coefficient of linear regression on 150^{th} day live weight and average daily weight gain is statically significant (P< 0.01). The linear regression of birth weight on live weight and average daily weight gain is not statically significant.

In the research made in Ankara goats by Öztürk and Goncagül (1994), live weight of 6^{th} month has been informed as 21.7 kg. In another study made in Ankara goats, live weight of 6-months has been informed as 20.85 (Yurtseven et al., 1998). Average live weight at 150^{th} day of Sanen goats has been informed as 17.37 kg by Bolacali and Küçük (2011). When the findings regarding 150^{th} day live weights have been evaluated, it attracts attention that general live weight average is relatively higher than some breeds that are raised in our country.

In addition to this, female goats has shown higher performance (P< 0.05) than males. This finding is not in accord with the literature (Yurtseven et al., 1998; Taskin et al., 2000; Bolacali and Küçük, 2011, Simsek et al., 2007; Odabasioglu et al., 2007; Öztürk and Goncagül, 1994).

The effect of the year that is evaluated, flock type (base, multiplier), birth type and gender factors on average daily weight gain increases isn't statistically significant. The superiority shown in females in terms of 150^{th} day live weight has shown a similar situation for this property. Average daily weight gain increases obtained between 0-150 days has found higher than the values informed in the studies made in Saanen and Saanen crossbred goats Bolacali and Küçük, 2011; Karadag and Köycü, 2011). This difference can be attributed to breed difference and breeding system.

CONCLUSIONS

When it is thought that hair goats constitute the majority of the goat population of our country, it is mentioned that the studies regarding the definition of this breed is in limited number. When the researches made related to the subject are examined, it is seen that these are in

experimental level and the population is not in the formation to be able to define. Another respect is that the studies made have more focused on crossbreeding studies than definition of the breed. Reduction policy has been followed regarding hair goat population in our country within the last 15-20 years period in the context of goat forest relationship and intensive goat raising has been supported. While these practices are being done, the breeders who make a living from this breed have been ignored. Positive policies regarding hair goat population has been put in place with the attempts of non-governmental organizations and Ministry of Food, Agriculture and Livestock.

The most important of them is "National Genetic Improvement Project for Small Ruminants at Breeders' Conditions", which was put in practice in 2011 by General Directorate of Agricultural Research and Policy (TAGEM) with these sub-projects put into practice in so many parts of the country, descriptive information of hair goats, which are our gene resource, started to be suggested in a wide population. The sub-project, which is called "Denizli Province Hair Goat Breeding Project" put into practice in 2011 in Denizli Province within the scope of these projects, is quite important in terms of defining the properties and specificities of hair goats at breeders' conditions.

The data evaluated in this article include the performance qualifications belonging to the goats obtained in the first 2 years (2011 and 2012) of this project that its first step will continue 5 years. The superiority suggested on behalf of females in terms of live weight and average daily weight gain come to the forefront as an interesting finding to be required to put emphasis on. Consequently, when it is compared with the other genotypes that the breeding is made in our country in terms of the properties taken into consideration, it is seen that hair goats have a potential not to be underestimated.

When this situation is taken into consideration, making similar researches and increasing the data regarding the subject will provide significant contribution to the literature.

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STUDY OF GENETIC DIVERSITY OF THREE PORTUGUESE CATTLE BREEDS BY 93 MICRO SATELLITE MARKERS

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Abstract

The objectives of this work were to assess the genetic diversity within and between three Portuguese cattle breeds using 93 microsatellites markers. Blood samples were collected from 50 individuals of each breed, and ninety-three microsatellites were analysed to get thorough information about genetic diversity and interrelationships among three Portuguese cattle breeds: Mirandesa (MIR), Maronesa (MAR), and Barrosã (BAR). Estimates of genetic variability, observed (Ho) and expected heterozygosity (He), allelic richness for each locus were determined. The alleles were classified in three classes according to their frequency: common alleles (observed in the three sub-populations), private alleles (alleles observed in one sub-population) and rare alleles (non-private alleles with a frequency < 0.01 over the whole population). The number of rare alleles found was 52 in MAR, 33 in MIR, and 30 in BAR. The number of private alleles found was 5 in MIR and BAR, and 2 in MAR. The MIR showed the lowest genetic diversity, and the highest genetic distance to the other two breeds. The three breeds could be considered as genetically distinct populations. This study shows that measures should be taken in order to preserve the genetic diversity of MIR, MAR, and BAR cattle breeds.

Key words: *Cattle Conservation Genetic diversity Microsatellites.*

INTRODUCTION

Considering the animal and plant species used, extensive livestock production systems are highly heterogeneous and contributes to maintain the ecological balance. The European Union (EU), in general, and Portugal, in particular, has interest in the preservation of these production systems since they contributes to reduce environmental pollution, to maintain or increase the biodiversity and to preserve the typical landscape across EU regions. In Portugal, several autochthonous cattle breeds are classified as endangered by the MADRP (2008). In general, they present a good adaptation to adverse environmental conditions, making these breeds particularly suited for the extensive productions systems.

EU consumers are, also, sensitive to the management practices that improves the welfare of livestock animals, and are willing to pay more for these certified animal products. Thus, these high quality products may contribute to the preservation of the rural world and its diversity as well as to increase the profitability of the extensive production systems, which can contribute to the conservation of autochthonous breeds endangered.

Traits, genotypes and alleles with possible economic interest are at risk of being lost (Mateus et al., 2004b). But, genetic diversity is the basis for the sustained ability of a breed to respond to selection programs, for adaptation to environmental changes, like: climate, diseases, management and husbandry practices (Boettcher et al., 2010). Livestock breeds with small population size are prone to a rapid increase of the inbreeding coefficient, and to losses of genetic diversity, which at long term is the primary key to the survival of animal

populations. The reduction of fitness of the populations due to the inbreeding depression effects is well known, and a severe reduction of the populations size (genetic bottleneck) increases the risk genes loses. Thus, the conservation of endangered livestock breeds relies on the conservation of their genetic diversity, and at the initial stage of conservation plan the rate of inbreeding should be minimised (Baumung and Sölkner, 2003) to avoid the losses of genetic variability gained during the breeds differentiation process (Cañón et al., 2011). According to FAO (2007) those livestock breeds classified as endangered should be included in conservation programs, in order to preserve their adaptation characteristics, value for food and agriculture, and because of their cultural and historical value (Ramljak et al., 2011).

Several studies (Jordana et al., 2003; Mateus et al., 2004c; 2004a) have been conducted to study the genetic diversity of Portuguese cattle breeds, however those studies were based in 16 to 30 microsatellite markers.

Thus, the objectives of this work were to assess the genetic diversity of within and between three autochthonous Portuguese cattle breeds using 93 microsatellites markers.

MATERIALS AND METHODS

Samples and microsatellite markers

Blood samples from 131 adult animals were collected, and the animals were selected using the pedigree information in order to ensure that animals were not closely related.

This study was conducted with three Portuguese cattle breeds: Mirandesa (MIR, http://www.mirandesa.pt/caracteristicas.htm), Barrosã (BAR, http://www.carnebarrosa.com /index.asp?p=r) and Maronesa (MAR, http://www.marones.pt/conteudo.php? idm=9); bred at north of Portugal. These breeds where selected because of their geographical proximity, and because of their importance for high quality meat production which is protected by Protection Designation of Origin (PDO).

A total of 93 microsatellite markers previously described by Ramljak et al. (2011) (http://onlinelibrary.wiley.com/doi/10.1111/j.1439-0388.2010.00905.x/suppinfo) were analyzed to estimate several parameters of genetic diversity. These loci were recommended by the International Society of Animal Genetics (ISAG) /FAO for the analysis of genetic diversity in cattle breeds (FAO/ ISAG 2004).

Samples and microsatellite markers

The genomic DNA was extracted using the QIAamp Blood-Kits (Qiagen) protocols. The summary information concerning the 93 microsatellites markers can be checked at http://onlinelibrary.wiley.com/doi/10.1111/j.1439-0388.2010.00905.x/suppinfo. The PCR products were analysed on ABI377 and ABI310 DNA Sequencers (Applied Biosystems) at the Animal Genetics and Husbandry laboratory of the Ludwig-Maximilians-University Munich. Genotypes were assigned using GENESCAN ANALYSIS 3.7 NT (Applied Biosystems) and GENOTYPER 3.7 (Applied Biosystems). To ensure the accuracy of genotyping, all animals, including international control samples (as declared by the European Cattle Genetic Diversity Consortium), were genotyped twice in two independent courses.

Statistical analysis

The adegenet package (Jombart and Ahmed, 2012) from the R software (R Development Core Team, 2011) was used to calculate the allele frequencies, the mean number of alleles per locus and breed, the Nei′s genetic distance (DA, Nei, 1987), the observed (Ho) and expected (He) heterozygosities. The Fisher′s exact test, with standard Bonferroni corrections, was used check for the deviation from Hardy-Weinberg equilibrium (HWE).

The Wright F-statistics (FS_{IT}, FS_{ST}, and FS_{IS}; Weir and Cockerham, 1984), were calculated for each locus and across breeds using hierfstat package (Goudet, 2005) and the population pairwise FS_{ST} was computed using adegenet package (Jombart and Ahmed, 2012) from the the R software (R Development Core Team, 2011).

The alleles were classified in three categories according to their frequency: common alleles (cA), observed in all 3 sub-populations; private alleles (pA) alleles observed in one sub-population; and rare alleles (rA) non-private alleles with a frequency < 0.01 over the whole population.

RESULTS AND DISCUSSIONS

Overall genetic variability

Across the 93 microsatellite loci, a total of 554 alleles where detect for BAR, 465 for MIR, and 578 for MAR breed. The number of alleles at the 93 microsatellite loci are shown in Figure 1., and ranged from 3 (L01) to 18 (L75). The mean number of alleles per locus was 6.22 for MAR, 5.96 for BAR, 5.00 for MIR, and the number of private alleles occurred at very low frequencies (< 0.011) for the three breeds. These results are in accordance with those presented by Medurogac et al. (2009) and Ramljak et al. (2011) in studies with Central European cattle breeds and by Costa et al. (2012) in a study with Cuban cattle breeds.

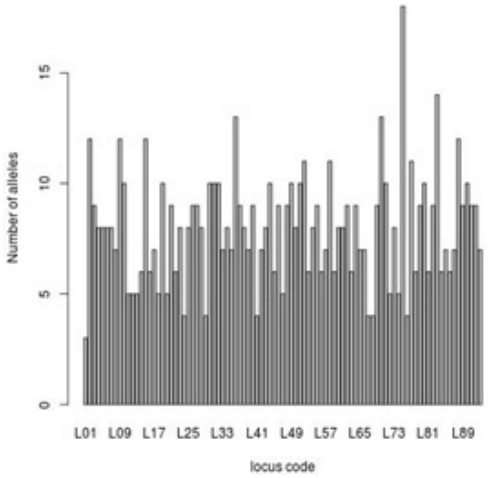

Figure 1. Alleles number per locus at the 93 microsatellite loci

Across breeds, the expected heterozygosity varied from 0.122 (L01) to 0.882 (L75), and the observed heterozygosity ranged from 0.113 (L01) to 0.781 (L57). The mean observed heterozigosity was lower (P< 0.001) than the mean expected heterozigosity as can be observed from the Figure 2.

The overall loci estimates of inbreeding, evaluated by the F_{IS} statistic, showed that the three cattle breeds presents a reduced heterozygosity due to within population inbreeding (FF_{IS} = 0.0724). The breed differentiation, evaluated by the F_{ST} statistic (0.0988), indicates that only 9.88% of the total genetic variation can be attributed to differences among the cattle populations. Thus, 90,12% of the genetic variability can be attributed to the individuals within the populations.

Genetic diversity within breeds

The within-breed genetic variability measures are presented in Table 1. The mean number of alleles per locus was 6.22 for MIR, 5.96 for BAR, and 5.00 for MIR, which is lower than the mean of the three breeds. The MAR breed presented the higher number of total (578) and rare (52) alleles, and the number of private alleles were low for all three breeds (5 for MAR and MIR, and 2 for MAR). These allele richness indicators are lower than those reported by Ginja et al. (2010) in a study with 13 Portuguese cattle breeds with 39 microsatellite markers.

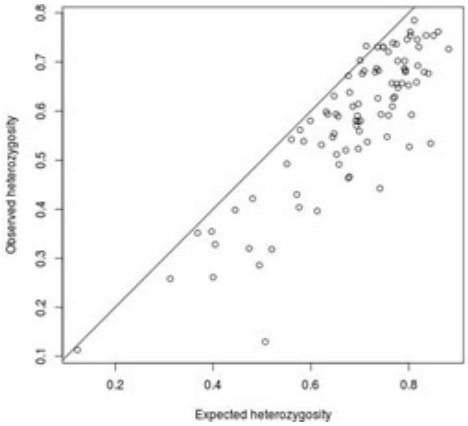

Figure 2. Observed versus expected heterozigosity for the 93 microsatellite loci

However, our results are in line with those presented by Medurogac et al. (2009) and Ramljak et al. (2011) for Central Europe cattle breeds and by Costa et al. (2012) for Cuban cattle breeds.

Table 1. Genetic variability at the 93 microsatellites loci for the three breeds studied

Breed	mA	tA	rA	pA	He	Ho
BAR	5.96	554	30	5	0.64	0.60
MAR	6.22	578	52	2	0.64	0.54
MIR	5.00	465	33	5	0.56	0.49
Mean	5.72	532	38.3	4	0.62	0.54

mA = mean number of alleles; tA = total number of alleles; rA = number of rare alleles; pA = number of private alleles; He = mean

expected heterozygosity (unbiased estimate Nei, 1987); Ho = mean observed heterozygosity.

The MAR presented the highest (Ho = 0.60 and He = 0.64) genetic diversity, and the MIR breed presented the lowest (Ho = 0.49 and He = 0.56) genetic diversity. These results corroborates those attained by Mateus et al. (2004) and Ginja et al. (2010), where the MIR also presented the lowest heterozigosity among all Portuguese cattle breeds. The three breeds presented Ho lower ($P < 0.001$) than the He (Figure 3), and the exact test for HWE within breed showed a deviation ($P < 0.001$) from the equilibrium.

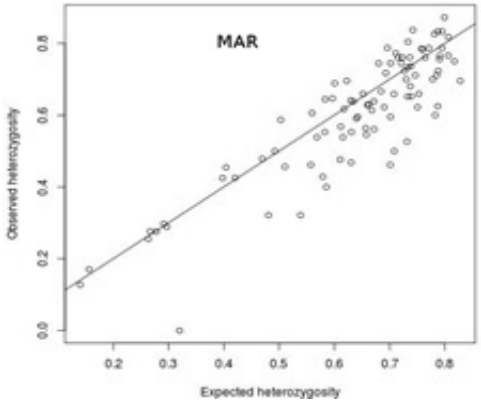

Figure 3. Expected versus observed heterozigosity for the three breeds studied

This observation is common in domestic animal populations (Costa et al., 2012), and the reduction in the heterozigosity can have several causes: selection against heterozygous animals (Wahlund effect) and inbreeding effects (Maudet et al., 2002).

Table 2 shows the frequency distributions of the inbreeding coefficient for three cattle breeds. The mean and median individual inbreeding coefficient for the analysed samples was 12.2 and 10.4% for MIR, 10.1 and 8.1% for MAR, and 10.7 and 6.96 for BAR. These results are in line with the results for heterozigosity. The inbreeding, produced by mating between relatives, is one of the causes for the losses of heterozygosity (Nei, 1987).

Populations under random mating, the genes are equally related within and between individuals, and the $F_{ST} = F_{IT} = 0$. Estimates of F_{ST} and F_{IT} that differ significantly indicate departures from random mating. In our study, both, F_{ST} and F_{IT} are positive ($F_{ST} = 0.131$ and $F_{IT} = 0.219$), thus we can assume that differences in the allele frequencies may be attributed to the effects of random genetic drift. Thus, the genetic differentiation (9.88%) can be attributed to an increase in the mean inbreeding coefficient.

Breeds interrelationships

Pairwise estimates of genetic differentiation (F_{ST}) and Nei's genetic distance (DA) among the three cattle breeds are shown in Table 2. The estimates of pairwise F_{ST} were all significant ($p < 0.01$), thus indicates that the three breeds can be considered genetically independent (Figure 5).

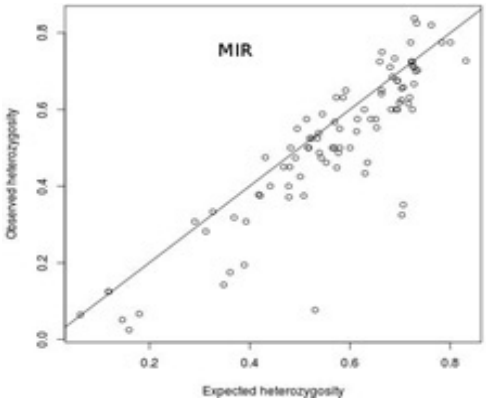

Figure 4. MIR

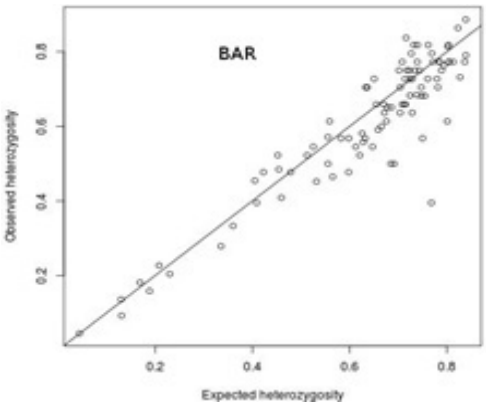

Figure 5. BAR

The Nei's genetic distance presented the highest values among MIR and BAR (0.477) and MIR and MAR (0.466).

Table 2. Pairwise estimates of FST below the diagonal, and Nei

Breed	BAR	MIR	MAR
BAR	-	0.477	0.345
MAR	0.174	-	0.466
MIR	0.09	0.157	-

A principal components analysis, based on Nei's genetic distances, corroborates these results, showing that all three breeds are genetically independent (Figure 5). Thus, both MAR and BAR are genetically well differentiated from MIR (F_{ST} = 0.157 and 0.175, respectively), and this clear genetic differentiation of MIR can be attributed to the occurrence of a strong genetic bottleneck. This evidence of a strong genetic subdivision (see F_{ST} values) between MIR and both MAR and BAR corroborates the results attained by Ginja et al. (2010), that showed that MIR presented the higher genetic differentiation among all Portuguese cattle breeds.

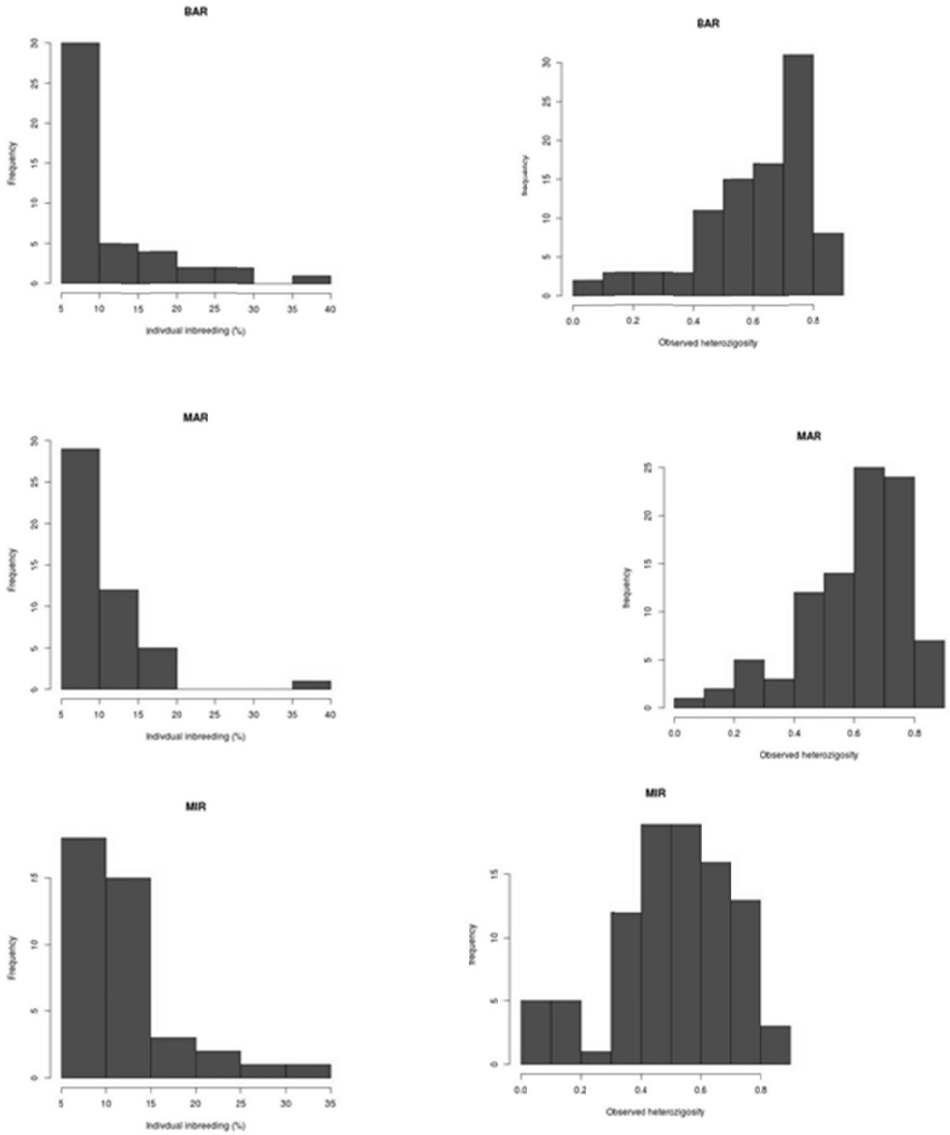

Figure 6. Frequency distributions of the individual inbreeding coefficient and heterozigosity for three cattle breeds

This results for MIR can be attributed to the increase of the inbreeding coefficient, in a short period of time, as stated by Laval et al. (2000). It is well known that populations subjected to genetic bottleneck lead to an increase of the genetic distance, distorting the topology of the evolution trees (Nei et al., 1983; Nei, 1987).

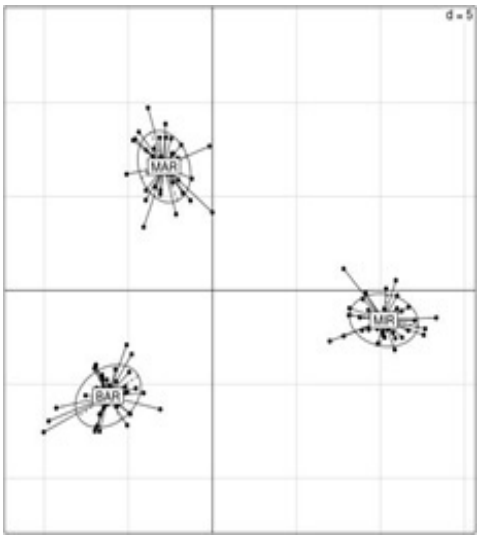

Figure 7. Principal components analysis of the Nei

CONCLUSIONS

The present study showed that a significant amount of genetic variation is maintained in the three cattle populations.

The three breeds could be considered as distinct genetic populations, however the MIR is the more genetically distance from both MAR and BAR. The MIR maintains an important genetic isolation from MAR and BAR. Populations with small effective size, needs breeding programs properly managed to avoid the losses of genetic diversity.

Thus, accurate pedigree records are essential to define matings among individuals in order to minimize the increase of the inbreeding coefficient.

Finally, it is clear that conservation measures should be developed to minimize the inbreeding in these three cattle breeds.

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CALPASTATIN GENE POLYMORPHISM IN ÇINE ÇAPARI AND KARYA SHEEP

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Abstract

This study was carried out to determine Calpastatin gene polymorphism in native Çine Çapari and synthetic Karya sheep in Turkey. Calpastatin is an endogenous inhibitor of calpain. This gene has a key role on meat tenderness after slaughter, and also has been known as candidate gene in muscle growth efficiency. Calpastatin gene was located on 5th chromosome of sheep. Randomly taken blood samples were collected from 97 Çine Çapari and 90 Karya sheep raised in Western Anatolia. Intron I from L domain of the ovine calpastatin gene was amplified by PCR to produce a 565 bp fragment. Then, PCR products were digested with restriction endonuclease enzyme MspI. Digested products were separated by electrophoresis on agarose gel and visualized with gel documentation system. The digestion of the PCR products by MspI enzyme produced fragments of 306 and 259 bp. Data analysis was done using PopGen32 software. In Karya sheep population MM, MN and NN genotypes were identified with 0.296, 0.496 and 0.208 frequencies, M and N allele frequencies were identified with 0.544 and 0.456, respectively. In Çine Çapari sheep population MM, MN and NN genotypes were identified with 0.543, 0,388 and 0.069 frequencies, M and N allele frequencies were identified with 0.737 and 0.26, respectively.

Key words: *Sheep, Cine Capari, Karya, Calpastatin, PCR-RFLP.*

INTRODUCTION

Calpastatin (CAST) is an endogenous calpain-inhibitor protein (Takano et al., 1999). Page et al. (2002) demonstrated that calpain plays a leading role in the tenderness of meat by degrading myofibrillar proteins during the process of rigor mortis after slaughter. Many researchers working on the genetics of livestock raised for meat production investigated the calpastatin gene and its physiological role on meat tenderness (Boehm et al., 1998; Huff-Lonergan et al., 1996; Killefer and Koohmaraie, 1994; Lonergan et al., 1995). The differences between the levels of calpastatin was studied among various species (Koohmaraie et al., 1991), breeds (Shackelford et al., 1994; Shackelford, 1995), and muscles (Geesink and Koohmaraie, 1999).

Several studies have been conducted to identify the calpastatin gene polymorphisms in different animal species including mice (Hitomi et al., 2000), goats (Javanmard et al., 2010), swine (Choi et al., 2006), sheep (Palmer et al., 1998), and cattle (Juszczuk-Kubiak et al., 2004). The calpastatin gene, which plays a key role in regulating meat tenderness following slaughter, is also regarded as one of the potential gene affecting muscle development (Byun et al., 2008). The calpastatin gene is located on chromosome 5 in the sheep genome (Khederzadeh 2011; Palmer et al., 1998).

The first study to identify calpastatin gene in sheep genomes was conducted by Palmer et al. (1998). As a result of this PCR-RFLP based study, two distinct alleles of the calpastatin gene (M and N) were identified in Dorset sheep. Later on, the presence of these alleles was also confirmed in studies on different sheep breeds (Gábor et al., 2009; Gharahveysi et al., 2012; Khan et al., 2012; Khederzadeh, 2011; Mohammadi et al., 2008; Nanekarani et al., 2011a; Nanekarani et al., 2011b; Shahroudi et al., 2006; Suleman et al., 2012; Szkudlarek-Kowalczyk et al., 2011); moreover, certain single nucleotide polymorphisms (SNP) were identified as a result of DNA sequencing studies on calpastatin gene regions (Gregula-Kania, 2011). However, there are no reports of an attempt to identify this gene in Turkey′s native sheep breeds.

This study was performed to identify calpastatin gene polymorphism in the native Cine Capari sheep breed, which is conserved as a genetic resource, and in Karya sheep, a synthetic genotype for which the breeding practices have become prevalent in Western Anatolia, at the DNA level by using PCR-RFLP.

MATERIALS AND METHODS

A total of 187 animals (97 Cine Capari sheep and 90 Karya sheep) were analyzed in the study. Blood samples of 4.5 ml were collected from the *vena jugularis* into anticoagulant K3 EDTA-containing vacuum tubes and DNA isolation was performed using a commercially available isolation kit (Invitrogen, Medsantek, Izmir). A PCR mixture containing PCR buffer (1X), $MgCl_2$ (2 mM), dNTP mixture (0.2 mM), forward and reverse primers (0.25 µM), *Taq* DNA polymerase (1U), genomic DNA (~100 ng), and sterile ddH_2O was prepared in a final volume of 25 µl. To amplify the suitable calpastatin gene region for a polymorphism analysis, the primer pair that was also used by Khederzadeh (2011) was synthesized. Forward and reverse primer sequences were;
CAST-F:CCTTGTCATCAGACTTCACC
CAST-R:ACTGAGCTTTTAAAGCCTCT,
respectively. The PCR conditions consisted of the following steps: 2 minutes of pre-denaturation at 95°C, followed by 35 cycles 1 minute of denaturation at 95°C, 1 minute of annealing at 65°C, 2 minute of extension at 72°C, and the last step that final extension was incubated for 10 minutes at 72°C. Amplified DNA regions were digested with *Msp*I restriction enzyme (Fermentas) for genotyping. For restriction digestion, 3 µl of 10X Buffer Tango, 1 µl of ddH_2O and 1 µl of *Msp*I (Fermantas) enzyme were added to the PCR products and this mix were incubated at 37°C for at least 6 hours. Digested PCR products were stained with SafeView Nucleic Acid Stain (NBS Biologicals), run in 2% agarose gel at 65 V for 2 hours, and visualized in a gel visualization system to determine the genotypes. Genotype and allele frequency analysis and Hardy-Weinberg equilibrium test were carried out using PopGene 32 software (Yeh et al., 1997).

RESULTS AND DISCUSSIONS

Resulting bands from PCR-RFLP were stained with SafeView, run in agarose gel electrophoresis, and visualized in a gel visualization system (Figure1). Following the digestion of the 565 bp PCR product, which was amplified using the primer pairs to discriminate between M and N alleles of the calpastatin gene, two bands with lengths of 306 bp and 259 bp were observed for the MM genotype, a single 565 bp band was observed for the NN genotype, and three bands with lengths of 259, 306 and 565 bp were observed for the MN genotype. Bands with different lengths were produced as a result of a single point mutation (CCGG à CCAG) in the calpastatin gene, which removes the MspI restriction cut site (...CCGG...) (Gregula-Kania and Monika, 2011).

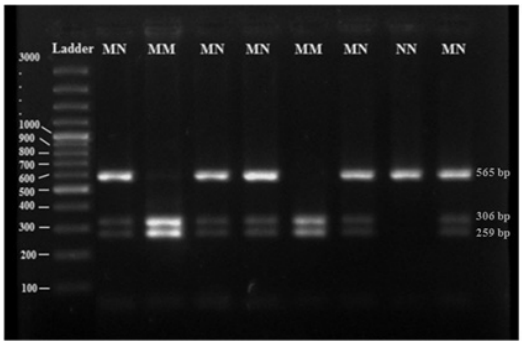

Figure 1. Gel picture of M and N alleles of the Calpastatin gene.

The allele and genotype frequencies are presented in Table 1. In the Karya samples, the allele frequencies were not significantly different, whereas the frequency of the M allele was significantly higher in Cine Capari sheep. This finding is in line with other studies (Gábor et al., 2009; Gharahveysi et al., 2012; Khan et al., 2012; Khederzadeh, 2011; Mohammadi et al., 2008; Nanekarani et al., 2011a; Nanekarani et al., 2011b; Nassiry et al., 2006; Suleman et al., 2012; Szkudlarek-Kowalczyk et al., 2011). When genotype frequencies are considered, the frequency of the NN genotype is significantly low in both sheep genotypes. In Karya sheep, the highest frequency was observed for the MM genotype, whereas the highest frequency was observed for the MN genotype in Cine Capari breed.

Table 1. The allele and genotype frequencies of the CAST gene in Karya and Cine Capari sheep.

		Allele Frequency		Genotype Frequency		
Breed/Genotype	**n**	***CAST* M**	***CAST* N**	**MM**	**MN**	**NN**
Karya	90	0.544	0.456	0.543	0.388	0.069
Cine Capari	97	0.737	0.263	0.296	0.496	0.208
Total	**187**	**0.644**	**0.356**	**0.415**	**0.458**	**0.127**

The observed and expected number of calpastatin genotypes in Karya and Cine Capari sheep and the result of the Hardy-Weinberg equilibrium tests with chi-square were showed in Table 2. Both genotypes were found to be in Hardy-Weinberg equilibrium.

Table 2. The observed and expected number of genotypes in Karya and Cine Capari sheep and the result of the Hardy-Weinberg equilibrium test

		Observed			Expected				
Breed	**n**	**MM**	**MN**	**NN**	**MM**	**MN**	**NN**	**2sd=1**	**P**
Karya	90	23	52	15	26.673	44.645	18.681	2.281	0.131
Cine Capari	97	51	41	5	52.702	37.594	6.704	0.713	0.399
Total	**187**	**74**	**93**	**20**	**77.661**	**85.702**	**23.656**	**1.273**	**0.259**

CONCLUSIONS

There are no previous studies to identify calpastatin gene polymorphism in Turkish sheep breeds. The aim of this study was to identify calpastatin gene polymorphisms in Cine Capari sheep, a native genetic resource facing danger of extinction, and in synthetic Karya sheep, which was obtained as a result of rotational crossbreeding practices of breeders in the Western Anatolian region, using the PCR-RFLP method. In conclusion, calpastatin gene polymorphims were identified in both of sheep studied in the present study. Two alleles, *CAST* M and *CAST* N, were determined in the calpastatin locus and the most frequent was the M allele. This study can be regarded as a reference study and it will be possible to perform future studies on other native sheep breeds of the country. Future studies to identify calpastatin polymorphisms in other native breeds and to determine the changes in development and meat quality with respect to calpastatin genotypes would be useful. Furthermore, this study detects a single nucleotide change only in the investigated genomic region. DNA sequence analysis of the calpastatin locus will prove if genomic differences exist in our breeds.

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GENETIC CHARACTERISATION OF POPULATIONS OF CATTLE OF HOLSTEIN BREED, CULTIVATED IN THE REPUBLIC OF MOLDOVA

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Abstract

Are present the results of research and analysis of the antigenic spectrum of blood groups of animals in the Holstein breed SRL 'DOCSANCOM' and STE 'Maximovca'. In both certified herds in EAB locus there is a high frequency of antigen G_2, O_2, Y_2, E'_2, O ', Q', G ', which is characteristic for the Holstein breed, many breeds black-and-white root as well as Moldavian type black-and-white cattle. By EAC-locus10 of the studied antigens R_1 antigen is not revealed among animals STE 'Maximovca'. High frequency of antigen E, R_2, W and X_2 characterizes the analyzed populations of animals. For single-factor locuses EAJ, EAL, EAM and EAZ observed almost the same concentration of the corresponding antigen in compared animal populations. The average frequency of antigen, or saturation of the studied populations of antigenic factors in the population of animals herd SRL 'DOCSANCOM' is 23.7%, in the animal herds STE 'Maximovca'-24, 5%. The genetic distance between populations of both analyzed populations is of little importance - 0.0687, so they sufficiently close to each other. Concentration of main allele in the herd SRL 'DOCSANKOM' is 0,6213, the herd STE 'Maximovca'-0.6667, of rare 0.2129 and 0.2727 respectively. The degree of homozygosity in the analyzed population is low, at 5.0% (SRL 'DOCSANKOM') and 6.0% (STE 'Maximovca').

Key words: *Holstein breed, groups of blood, alleles, the index of antigenic similarities.*

INTRODUCTION

The breeding work more goes to the level of genetic analysis of selection processes in cattle breeds. Without knowledge of the genotype of the animal can not fully judge his individuality, heredity and variation, focusing only on the phenotypic traits.

At modern stage of development the selection process in dairy cattle breeding highly relevant is the use of allelic forms of the genes responsible for blood group. Codominant inheritance according to the rules of Mendel allelic genes controlling blood group and their wide variety, to differentiate specific features of the animal directly characterize the genotype (Popov, 1994, 1998). This helps to widen the forms controlling the state specific breeds, types herds from the position of the gene level heredity (Sozinov, 1992; Prokhorenko et al., 1996; Serdyuk et al., 2000).

An essential complement of immunogenetic evaluation of the gene pool of breeds make up research materials in a separate step, which can be regarded as micropopulation.

Therefore, the aim of our study was to immunogenetic characteristic of a population of Holstein breed herds of Dutch selection, imported to the Republic of Moldova.

MATERIALS AND METHODS

Material for investigation served the blood sampled from animals of Holstein breed of Dutch breeding herds of cattle SRL 'DOCSANCOM' (n = 202) and STE 'Maximovca' (n = 33).

Blood samples from the animals, the assay of hemolysis of erythrocytes, and the study of blood groups was performed by the standard technique, 1983. Blood group determined hemolytic test using 49 reagents of cattle, unified in international comparative tests, which detect antigens controlled by allelic genes 9 genetic systems. Frequency of antigens and alleles EAB locus (q) determined the standard method.

Immunogenetic indices of similarity (r) and distance (d) between populations were determined by the formula Serebrovsky (1970), the use of genetic similarity (r)-by subtracting d of unity. Identification of alleles EAB locus and subsequent analysis of allele-fund carried out on the following genetic parameters: total number of alleles EAB locus, the total frequency of alleles: basic, rare, the degree of homozygosity (Merkuryeva et al., 1983). The materials obtained were treated on a personal computer.

RESULTS AND DISCUSSIONS

As a result of research and analysis of the spectrum of blood group antigen of animals Holstein breed found that in SRL 'DOCSANCOM' was found 4 (Q, T_1, B ', U') and STE 'Maximovca'-13 (Z', G_1, P_1, P_2, Q, T_1, T_2, P', Y', B″, R_1, M, U″) antigen of the 49 studied.

By EAA-locus frequency of antigen A2 was 0,3713 (SRL 'DOCSANCOM') and 0,4848 (STE 'Maximovca').

By EAB-locus of the 25 studied antigens in analyzed herds haven't been identified 3 (Q, T_1, B ') and 9 (G_1, P_1, P_2, Q, T_1, T_2, P', Y', B',) antigens respectively. Revealed low incidence or absence of antigens I_1, P_1, B ', K', P ', Y'.

It should be noted that in both herds is observed high frequency of antigen G_2, O_2, Y_2, E'_2,, O ', Q', G ', which is characteristic for the Holstein breed, many breeds black-and-white root and Moldovan type black and white cattle (Focsa et al., 2001), Figure 1. As can be seen, there is almost the same (with minor fluctuations antigen O') the concentration of the above antigens in both animal populations.

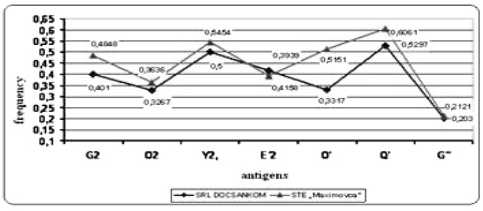

Figure 1. The frequency of some antigens EAB locus

By EAC-10 locus of the studied antigens R_1 antigen was not detected in animals STE 'Maximovca'. High frequency of antigen E, R_2, W and X_2 characterized a population of animals (Figure 2).

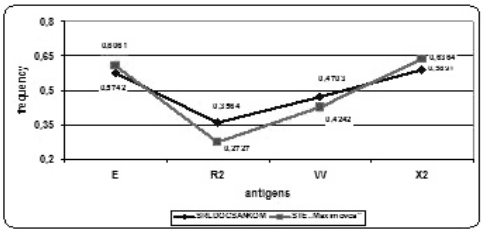

Figure 2. The frequency of some antigens EAC locus

By EAF-locus frequency of F antigen varies from 0,9356 (SRL 'DOCSANCOM') to 1,0 (STE 'Maximovca'). The frequency of V antigen varies from 0,0024 (STE 'Maximovca') to 0,1831 (SRL 'DOCSANCOM').

For single-factor locus EAJ, EAL, EAM and EAZ observed almost the same concentration of the corresponding antigen in the comparable populations of animals (Figure 3).

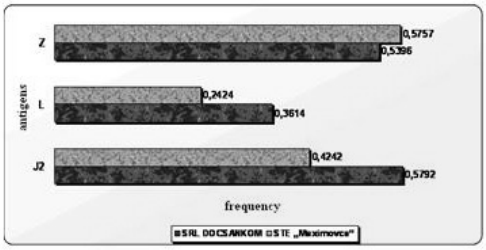

Figure 3. The frequency of antigen J2, L and Z

Antigen M is not detected at animals STE 'Maximovca', but in the herd SRL 'DOCSANCOM' its frequency is 0.0099.

By AES-locus of six studied antigens at the animals of both populations was not detected antigen "U", a significant difference in the frequency of other antigens haven't been identified.

Evaluation saturation of studied population antigenic factors showed (Figure 4) that the animals population of herd SRL "DOCSANCOM" it is 23.7%, at animal herd STE 'Maximovca' a little more – 24.5%.

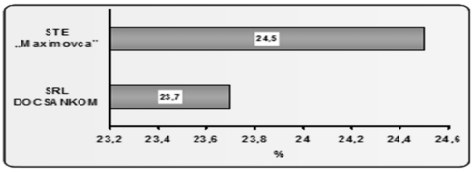

Figure 4. The saturation of cattle populations with antigenic factors

About similarities and some differences between the analyzed populations of animals can be seen Holsteins comparing their genetic structure to EAB-locus alleles.

Genetic structure of the Holstein breed animals studied population presented in Table 1 and Table 2.

Table 1. Genetic structure of the Holstein breed (SRL "DOKSANCOM") respect to alleles EAB locus

No.	allele	n	p	No.	allele	n	p
1.	B_1P'	1	0.0025	41.	$Y_2B'D'Q'$	1	0.0025
2.	$B_2G_1I_1P_1$	1	0.0025	42.	$Y_2B'G'$	1	0.0025
3.	B_2G_2	3	0.0074	43.	$Y_2D'I'Q'$	1	0.0025
4.	$B_2G_2O_2$	1	0.0025	44.	$Y_2D'G'O'$	2	0.0050
5.	$B_2G_2Y_2I'O'Y'$	1	0.0025	45.	$Y_2D'G'O'G?$	1	0.0025
6.	$B_2G_2E'_2I'J'_2O'$	1	0.0025	46.	$Y_2E'_2$	3	0.0074
7.	B_2I_1	1	0.0025	47.	$Y_2E'_2I'J'_2O'$	1	0.0025
8.	B_2O_1	13	0.0322	48.	$Y_2E'_2J'_2O'$	1	0.0025
9.	$B_2O_1Y_2D'$	6	0.0148	49.	Y_2G'	1	0.0025
10.	$B_2O_1Y_2E'_3G'P'Q'G?$	2	0.0050	50.	$Y_2G'I'P'$	1	0.0025
11.	$B_2O_1Y_2G'P'Q'G?$	1	0.0025	51.	$Y_2G'J'_2K'O'P'Q'G?$	1	0.0025
12.	B_2O_1B'	5	0.0124	52.	$Y_2G'O'G?$	2	0.0050
13.	$B_2O_2Y_2$	2	0.0050	53.	$Y_2G'G?$	3	0.0074
14.	$B_2Y_2G'I'O'P'Q'G?$	1	0.0025	54.	Y_2I'	1	0.0025
15.	$B_2G'O'Q'$	1	0.0025	55.	$Y_2I'O'Y'$	1	0.0025
16.	$B_2J'_2O'$	1	0.0025	56.	Y_2O'	1	0.0025
17.	G_2O_2	5	0.0124	57.	$B'G'O'$	1	0.0025
18.	$G_2O_2T_2G'O'$	1	0.0025	58.	$D'E'_1G'Q'$	1	0.0025
19.	$G_2O_2Y_2$	1	0.0025	59.	$D'G'I'Q'$	1	0.0025
20.	$G_2Y_2B'I'$	1	0.0025	60.	$D'G'J'_2K'O'$	2	0.0050
21.	$G_2Y_2E'_2Q'$	61	0.1510	61.	$D'G'O'$	23	0.0569
22.	$G_2E'_2$	1	0.0025	62.	E'_2	4	0.0100
23.	$G_2I'O'G?$	1	0.0025	63.	E'_2G'	1	0.0025
24.	I_1O_2	3	0.0074	64.	E'_2I'	2	0.0050
25.	I_1B'	2	0.0050	65.	$E'_2J'_2O'$	2	0.0050
26.	I_2	47	0.1163	66.	E'_2Q'	2	0.0050
27.	I_2Q'	1	0.0025	67.	$E'_2G?$	1	0.0025
28.	O_2	16	0.0396	68.	$G'J'_2K'O'G?$	1	0.0025
29.	O_2T_2	1	0.0025	69.	$G'O'Q'$	1	0.0025
30.	O_2Y_2	1	0.0025	70.	$G'O'G?$	1	0.0025
31.	O_2Y_2D'	1	0.0025	71.	$G'G?$	1	0.0025
32.	O_2B'	1	0.0025	72.	I'	5	0.0124
33.	$O_2D'G'Q'$	1	0.0025	73.	$I'Q'$	2	0.0050
34.	$O_2E'_2$	1	0.0025	74.	$J'_2K'O'$	8	0.0198
35.	O_2G'	1	0.0025	75.	$J'_2K'O'Q'$	1	0.0025
36.	O_2I'	3	0.0074	76.	O'	9	0.0223
37.	$O_2J'_2K'O'$	1	0.0025	77.	Q'	22	0.0544
38.	P_1I'	2	0.0050	78.	$G?$	17	0.0421
39.	Y_2	2	0.0050	79.	„b"	14	0.0346
40.	Y_2B'	1	0.0025				

Table 2. Genetic structure of alleles EAB locus of Holstein cows (STE " Maximovca")

No.	allele	n	p	No.	allele	n	p
1.	$B_2G_2I_2$	1	0.0151	16.	Y_2G'O'Q'G?	1	0.0151
2.	B_2O_1	2	0.0303	17.	Y_2I'O'	1	0.0151
3.	$B_2O_1Y_2$D'	1	0.0151	18.	Y_2O'	1	0.0151
4.	B_2O_1B'	1	0.0151	19.	B'Q'	1	0.0151
5.	G_2O_2	2	0.0303	20.	D'Q'	2	0.0303
6.	$G_2Y_2E'_2$Q'	13	0.1970	21.	E'_1	1	0.0151
7.	G_2D'	1	0.0151	22.	G'O'	1	0.0151
8.	I_2	7	0.1061	23.	G'O'G?	1	0.0151
9.	O_1	1	0.0151	24.	I'	2	0.0303
10.	O_1D'	1	0.0151	25.	I'Q'	2	0.0303
11.	O_1D'G'Q'	1	0.0151	26.	O'	1	0.0151
12.	$O_1J'_2$K'O'	4	0.0606	27.	Q'	1	0.0151
13.	O_2B'	1	0.0151	28.	G?	4	0.0606
14.	Y_2D'G'I'O'	1	0.0151	29.	„b"	4	0.0606
15.	Y_2D'G'O'	2	0.0303				

As can be seen, in the herd SRL 'DOKSANCOM' are revealed 79 alleles, in the herd STE 'Maximovca-29 alleles.

In both analyzed herds greatest distribution are alleles B_2O_1, $G_2Y_2E'_2$Q', I_2, I', G" and the negative allele „b". Results of studies found 19 similar alleles in the analyzed populations of animals, Figure 5.

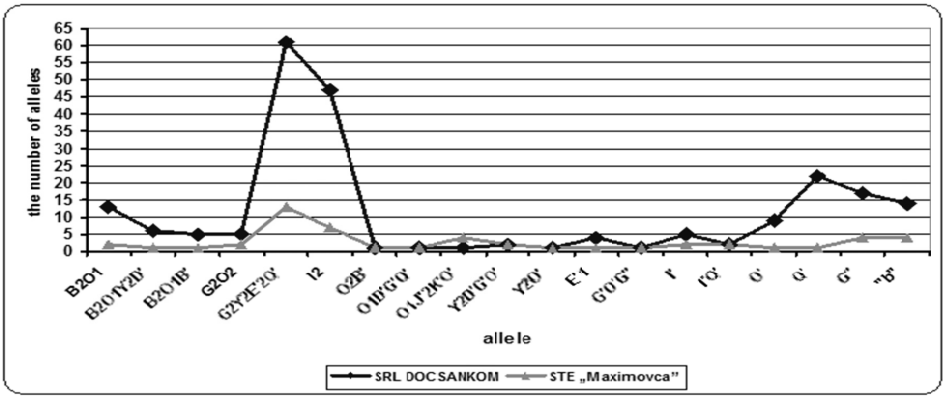

Figure 5. The concentration of the same allele in the analyzed populations of animals of Holstein breed

In the research results (Popov, NA, Eskin, GV, 2000) allelofonde Dutch, Holstein, the German Black and White breed is present most of the alleles detected in the analyzed populations of animals Holsteins.

Intensive use of the gene pool of black-and-White, Dutch and of Holstein breed as improving in many countries of the world leads to a general genetic convergence. In this regard, a high frequency occurs in the case of allele D'G'O' (0.0569) in the herd SRL 'DOCSANKOM'.

In our earlier study was identified allele D'G'O 'herds in agricultural firm 'Friendship', STE 'Maximovca' and bull-producing cows of the republic, the incidence of which was 0.0087, 0.0395 and 0.0041, respectively (Smirnov et al., 2007). This allele has been introduced by bull Diamond producer in 1287 of Holstein breed (line Soiling Troyon Rokita 252803).

The genetic distance between populations of both analyzed herds of Holstein cattle has little meaning – 0.0687, so they are close enough to each other-the index of genetic similarity is 0.9313, which indicates that the homogeneity of breed. Similar results were obtained in studies (Svyazhenina, 2012)-the value of genetic similarity indices in Holstein cows of different origin was 0.815 – 0.873, from which comes that in the Tyumen region Highly productive herd of Holstein cattle of breeding, which is almost no different from the representatives of the European selection in genetic plan.

Objectively genetic characteristics of the analyzed populations of animals of Holstein breed reflect such factors as the rate of homozygosity (Ca), the number of effective alleles (Na), the degree of genetic variability (factor V), Table 3.

Table 3. The genetic variability of populations of Holstein breeds cattle

No.	Indices	Name of farms	
		SRL „Docsankom"	STE „Maximovca"
1.	We investigated, goals	202	33
2.	The number of installed alleles: - total - the major - rare	 79 15 64	 29 11 18
3.	The total frequency of alleles: - the major - rare	 0.6213 0.2129	 0.6667 0.2727
4.	Coefficient of homozygosity, Ca	0.0501	0.0602
5.	Number of effective alleles, Na	19.9	16.6
6.	The degree of genetic variability, V	95.5	96.9

As can be seen, the concentration of the principal allele in the herd SRL 'DOCSANKOM' was 0.6213, in the herd STE 'Maximovca'-0.6667, 0.2129 and 0.2727 rare respectively. The degree of homozygosity in the analyzed populations is low, at 5.0% (SRL 'DOCSANKOM') and 6.0% (STE 'Maximovca'). A low coefficient of homozygosity (Ca) indicates of significantly higher genetic diversity of studied population of Holsteins breed cattle.

As is known, the state of breed allelofond the level of homozygosity reflects a number of effective alleles. It is found that the animal population of the herd SRL ('DOCSANKOM') was more homozygous, the number of effective alleles equal 19.9.

In general allelofonde of studied herds dominated marker alleles typical Holstein cattle, and revealed differences, their specificity, give the right to assert the valuable in breeding for both populations of cattle.

CONCLUSIONS

Was established a high frequency of antigen G_2, O_2, Y_2, E'_2, O', Q', G' in studied populations of SRL' DOCSANKOM' and STE 'Maximovca' that are characteristic of Holstein breed, breed of Black and White stalk including the Moldavian type of Black and White breed.

Was established 19 alleles identical in both studied populations, the most widespread alleles were B_2O_1, $G_2Y_2E'_2Q'$, I_2, I', G' and recessive allele 'b'.

Level of homozygous in the studied populations was low and amounted to 5.0% (SRL 'DOCSANKOM') and 6.0% (STE 'Maximovca').

It was established that the Holstein breed population of SRL 'DOCSANKOM' is homozygote and the number of effective alleles was 19.9.

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CHEMICAL COMPOSITION AND NUTRITIONAL VALUE OF THE FODDER GROWN IN THE CONDITIONS OF THE REPUBLIC OF MOLDOVA

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Abstract

The chemical composition and nutritive value of fodder depends on many factors, the most important of which are the conditions for plant growth (climate, soil, fertilizers, agricultural machinery), species, stage of plant development, the way of harvesting, storage conditions. The aim of the research was to study the differences in the chemical composition of the fodder grown in different zones of the Republic of Moldova, the identification and comparative analysis of their actual nutritional value, analysis and comparison with the data given in specialty literature. The laboratory studies of the local fodder in Moldova, selected at the farms of the State Enterprise 'Moldsuinhibrid' (Orhei) and E.T.S. (Maximovka) revealed differences in chemical composition and general nutritive value that depends on the area of cultivation, as well as in comparison with the data used in calculating the recipes of fodder mixtures and combined fodder for animals and poultry. Differences in chemical composition and general nutritive value were observed in almost all fodder in the Republic of Moldova, selected from different places, as well as in comparison with the data used in the country to balance the diets of cattle and poultry.

Key words: *chemical composition, fodder, nutrition, breeding zone.*

INTRODUCTION

Livestock production largely depends on the high-grade feeding of animals. According to the World Health Organization (WHO), 52-55% of the nation's health is determined by the food quality. One of the causes of people′s health deterioration is the catastrophic shortage of micronutrients: vitamins, minerals and other biologically active substances. In parallel to this problem, in the conditions of increased human impact on the biosphere, the production of ecologically pure crop and livestock production is particularly acute.

Numerous studies have established that in many regions, depending on the ecological state of the animal habitats and the exposure of the animals to toxicants, the concentration of heavy metals in animals and animal products, is usually several times higher than their content in the soil and fodder, and that it exceeds the maximum allowable rate (Talanov and Chmielewski, 1991; Kashtanov et al., 1999; Bokova et al., 2000; Ermacov and Tyutikov, 2008).

Due to the deficiency of protein, vitamins and minerals in fodder, the metabolism in animals is disturbed, the animals often suffer from various diseases, the productivity is reduced, and the nutritional value of the livestock products is also low.

The rational use of the forage reserve involves carrying out an analysis of fodder by pedo climatic zones and obtaining of large amounts of low-cost fodder rich in nutrients. Nutrient content in fodder depends on fodder composition, stage of vegetation, soil fertility, etc., and the concentration of minerals varies in accordance with the phase of vegetation of cereals and the species of animals, including technological age groups (Voynar, 1960; Babenco et al., 1980).

In this context, the study of the chemical composition and nutritive value of fodder produced in Moldova and the determination of their quality become essential.

The level of crude protein, the essential amino acids and metabolisable energy in the recipes for livestock and poultry has a significant impact on productivity and depends on the breed, genetic potential, nutrition and maintenance technology (Pop et al., 2006; Stepurin and Vrancean, 2008; Caisin, 2010).

MATERIALS AND METHODS

In order to conduct the research, in the period of March-July 2011, samples of fodder were collected at the mixed fodder factory of the Stat Enterprise for pigs breeding 'Moldsuinhibrid' (Orhei town) and at the Technical and Experimental Station (village of Maximovka, the Republic of Moldova), which afterwards were tested on the content of moisture, crude and digestible protein, crude cellulose, fat, ash and nitrogen-free extractives. Conventional methods of zootechnical analysis were used (Petukhova et al., 1989).

The analysis of the fodder was carried out in the laboratory of the Department of General Animal Husbandry of the State Agrarian University of Moldova and the Laboratory of Nutrition and Fodder Technology of the Research and Practical Institute of Biotechnology in Animal Breeding and Veterinary Medicine. The determination of amino acids was conducted by the Institute of Physiology and Sanocreatology, and the determination of macro-and micro minerals was carried out by the Institute of Chemistry, Academy of Sciences of Moldova.

The estimation of the actual general nutrient value of the fodder was performed by calculation according to the data of the chemical composition of fodder, the digestibility of its nutrients using the energy equivalent of the conversion of digestible nutrients into energy.

RESULTS AND DISCUSSIONS

According to the results of the chemical analysis of the fodder and recycling wastes it was established that the dry matter content varied considerably in the fodder grown in different zones of Moldova, as well as in comparison with literature data (Kalashnikov, 2003) (Table 1, 2).

Table 1. The content of dry matter and water in the natural fodder of Moldova,%

Fodder	SE 'Moldsuinhibrid'		E.T.S. Maximovka		Variations in dry matter content in the fodder of different zones in comparison with literature data
	total moisture	dry matter	total moisture	dry matter	
Corn grain	13.65	86.35	18.64	81.36	-3.64 - +1.35
Barley grain	13.17	86.83	13.42	86.58	+1.58 - +1.83
Wheat grain	14.19	85.81	15.06	84.94	-0.06 - +0.81
Oat grain	12.07	87.93	14.65	85.35	+0.35 - +2.93
Soybean grain	11.38	88.62	15.06	84.94	-0.06 - +3.62
Pea grain	12.97	87.03	12.60	87.40	+2.03 -+ 2.40

According to the results of the analysis, in the tested fodder the level of crude protein (Table 3) differed from the data in reference literature. Significant differences in the content of this substance were found depending on the zones of cultivation of forage crops; thus, according to the chemical analysis the quantity of crude protein in the corn grain was of 68.08-70.80 g, in reference literature this amount is equal to 92.0 g, so that the difference in the content of crude protein is 21.20-23.92 g, or 23.04-26.00%.

A lower quantity of crude protein in the local fodder in comparison with literature data was observed in the wheat grain-16.10 g, in oat grain-12.91 g, in pea grain-6.01, and in sunflower seed cake and meal-25.70 g; a bigger amount of crude protein, g/kg protein in comparison with literature was observed only in the barley grain (by 11.09 and 23.80 g), in wheat bran and alfalfa grass meal.

Table 2. The content of dry matter and water in the plant based raw material,%

Fodder	SE 'Moldsuinhibrid'		E.T.S. Maximovka		Variations in dry matter content in the fodder of different zones in comparison with literature data
	total moisture	dry matter	total moisture	dry matter	
Extruded corn	8.43	91.57	-	-	-
Extruded peas	8.35	91.65	-	-	-
Extruded barley	7.66	92.34	-	-	-
Extruded wheat	7.90	92.10	-	-	-
Extruded soybean	10.31	89.69	-	-	-
Sunflower oilcake	5.70	94.30	10.98	89.02	+4.02-+9.30
Sunflower seed meal	10.22	89.78	-	-	-0.22
Soybean meal	10.31	89.69	11.94	88.06	+3.06-+4.69
Wheat bran	14.51	85.49	-	-	+0.49
Alfalfa grass meal	8.30	91.70	13.67	86.33	+1.33

Table 3. Content of crude protein in the fodder in the Republic of Moldova, g/kg

Fodder	SE 'Moldsuinhibrid'	E.T.S. Maximovka	Variation in the content of crude protein in fodder in comparison with literature data
Corn grain	68.08	70.80	-23.92 -21.20
Barley grain	96.09	108.80	+11.09 +23.80
Wheat grain	133.62	116.90	+0.62 -16.10
Oat grain	95.09	116.90	-12.91 +8.90
Soybean grain	323.83	-	+4.83 -
Pea grain	211.99	192.30	-6.01 -25.70

Actually significant differences in the content of major nutrients were observed in all kinds of fodder and waste products from the processing of plant based raw materials, depending on the location of fodder sampling, as well as in comparison with literature data (Table 4, 5, 6).

These data indicate that the balancing of fodder rations for cattle and poultry on the basis of literature data, versus the basis of an actual fodder analysis results in significant differences with respect to the required level of their needs for nutrients.

The importance of mineral substances in animal feeding is extremely high, even though they have no energy value (Table 7, 8, 9). Depending on the zone of the country the macro-and micronutrients varies.

Table 4. The content of crude fat in fodder in Moldova, g/kg

Fodder	SE 'Moldsuinhibrid'	E.T.S. Maximovka	Content of crude fat in comparison with literature data
Corn grain	42.39	40.10	-2.90 -0.61
Barley grain	19.95	23.10	-2.05 +1.10
Wheat grain	11.19	13.50	-8.81 -16.50
Oat grain	44.03	38.60	+4.03 -1.40
Soybean grain	184.12	-	+38.12 -
Pea grain	13.06	17.90	-5.94 -1.10

Table 5. Content of crude cellulose in the fodder in Moldova, g/kg

Fodder	SE 'Moldsuinhibrid'	E.T.S. Maximovka	Content of crude cellulose in comparison with literature data
Corn grain	20.88	11.70	-22.12 -31.30
Barley grain	51.98	66.70	+2.98 +17.70
Wheat grain	25.71	9.90	+8.71 -7.10
Oat grain	129.07	53.40	+32.07 -43.60
Soybean grain	74.81	-	+4.81 -
Pea grain	70.81	95.40	+16.81 +41.40

Table 6. The content of major nutrients in the raw waste products from the industrial processing of plant based raw material, g/kg

Fodder	SE 'Moldsuinhibrid'			E.T.S. Maximovka			By A. Kalashnikov, 2003		
	Crude protein	Crude fat	Crude cellulose	Crude protein	Crude fat	Crude cellulose	Crude protein	Crude fat	Crude cellulose
Extruded corn	75.13	27.03	27.32	-	-	-	-	-	-
Extruded peas	204.51	6.22	107.31	-	-	-	-	-	-
Extruded barley	103.90	18.27	52.77	-	-	-	-	-	-
Extruded wheat	142.89	19.80	46.48	-	-	-	-	-	-
Extruded soybean	358.62	125.59	85.27	368.60	9.50	130.8	-	-	-
Sunflower oilcake	260.44	231.39	233.94	231.20	196.9	130.8	405.0	77.0	129.0
Sunflower seed meal	331.65	12.17	201.76	-	-	-	429.0	37.0	144.0
Soybean meal	126.64	45.13	87.91	-	-	-	151.0	41.0	88.0
Wheat bran	613.77	127.70	23.96	-	-	-	535.0	108.0	-
Alfalfa grass meal	-	-	-	199.90	12.10	142.0	189.0	29.0	211.0

Table 7. The content of macronutrients in the fodder in Moldova, g/kg

Fodder	Ca		P		K		Na	
	*	**	*	**	*	**	*	**
Corn grain	0.067	0.5	3.10	5.2	3.81	5.2	0.021	1.3
Barley corn	0.712	2.0	4.39	3.9	6.68	5.0	0.054	0.8
Wheat grain	0.561	0.8	2.87	3.6	3.79	3.4	0.033	0.1
Oat grain	0.799	1.5	3.08	3.4	4.35	5.4	0.059	1.8
Soybean grain	1.601	4.8	4.98	7.1	17.21	21.7	0.040	3.4
Pea grain	0.476	2.0	3.28	4.3	8.75	10.7	0.016	0.3

Table 8. Content of macronutrients in the waste products from the industrial processing of plant based raw material, g/kg

Fodder	Ca		P		K		Na	
	*	**	*	**	*	**	*	**
Extruded corn	0.137	-	2.51	-	3.60	-	0.014	-
Extruded peas	0.857	-	4.56	-	8.48	-	0.038	-
Extruded barley	0.397	-	3.59	-	5.01		0.045	-
Extruded wheat	0.612	-	4.08	-	5.24	-	0.025	-
Extruded soybean	2.113	-	6.27	-	17.15	-	0.029	-
Sunflower oilcake	2.699	5.9	9.39	12.9	14.87	9.5	0.046	1.3
Sunflower seed meal	4.038	3.6	14.36	12.2	16.00	8.0	0.040	0.9
Soybean meal	0.617	2.0	6.50	9.6	8.34	10.9	0.026	0.9
Wheat bran	24.623	27.0	8.30	18.0	4.01	6.9	2.875	12.2

Plants that grow in soils containing an insufficient amount of nutrients are responsible for fodder which does not possess full value or quality.

The analysis of local fodder on the content of amino acids showed that both the content of lysine and methionine is significantly lower than the average data referred to in literature (Table 10).

Table 9. Content of micronutrients in the fodder and waste products from the industrial processing of plant based raw material, g/kg

Fodder	Fe		Cu		Zn		Mn		Co	
	*	**	*	**	*	**	*	**	*	**
Corn grain	42.93	303.0	3.07	2.9	22.69	29.6	22.69	3.9	0.91	0.06
Barley grain	860.19	50.0	6.86	4.2	30.11	35.1	30.11	13.5	0.92	0.26
Wheat grain	46.73	40.0	4.63	6.6	32.12	23.0	32.12	46.4	0.90	0.07
Oat grain	98.56	41	2.68	4.9	22.62	22.5	22.62	56.5	0.94	0.07
Soybean grain	156.63	125	9.12	14.2	36.84	33.0	36.84	27.3	0.94	0.09
Pea grain	61.75	60.0	7.71	7.70	38.35	26.7	38.35	20.2	0.91	0.18
Extruded corn	186.53	-	1.96	-	20.20	-	20.20	-	0.96	-
Extruded peas	98.37	-	5.56	-	37.48	-	37.48	-	0.97	-
Extruded barley	267.04	-	5.20	-	21.75	-	21.75	-	0.97	-
Extruded wheat	78.71	-	6.46	-	28.12	-	28.12	-	0.96	-
Extruded soybean	212.07	-	12.61	-	40.78	-	40.78	-	0.93	-
Sunflower seed meal	176.72	215	25.23	17.2	73.74	40.0	73.74	37.9	0.98	0.19
Soybean meal	208.96	332	36.59	24.1	85.25	40.8	85.25	48.5	0.94	0.416
Wheat bran	84.67	170	11.08	11.3	60.35	81.0	60.35	117.0	0.90	0.10

Table 10. The content of amino acids in grain, g/kg

Amino acids	Corn grain		Pea grain		Barley grain		Oat grain		Soybean grain	
	*	**	*	**	*	**	*	**	*	**
Lysine	3.00	2.1	20.48	14.2	5.06	4.1	5.31	3.6	18.71	21.1
Methionine	0.80	3.3	0.51	1.8	0.83	3.6	0.39	4,6	2.07	1.8
Cysteine	1.37	-	2.30	-	1.69	-	2.11	-	3.24	-
Threonine	2.06	-	5.27	-	2.46	-	2.08	-	5.69	-
Serine	3.18	-	9.68	-	4.45	-	4.19	-	11.40	-
Glutamic acid	13.64	-	54.83	-	35.08	-	26.75	-	63.37	-
Proline	5.05	-	10.21	-	11.93	-	5.17	-	13.44	-
Glycine	3.11	-	10.43	-	4.99	-	5.24	-	11.88	-
Alanine	4.39	-	10.53	-	4.90	-	5.26	-	11.97	-
Valine	3.09	-	10.37	-	5.34	-	4.52	-	9.85	-

*fodder selected at the SE „Moldsuinhibrid",Orhei; **literature data by A. Kalashnikov, 2003*

The calculations to determine the actual overall nutritional value of fodder in Moldova revealed that, in the analyzed fodder the content of the exchange energy for pigs and poultry (Table 11) differed depending on the location of sampling, as well as on the indicators listed in the normative literature; significant differences were observed in the data both on nutrition value of cereal crops and products of their processing.

Table 11. The nutritional value of fodder and waste products from the processing of plant based raw material in exchange energy, Mj

Fodder	SE 'Moldsuinhibrid'		E.T.S. Maximovka		by Kalashnikov (2003) for:	
		poultry	pigs	poultry	pigs	poultry
Corn grain		13.94	11.48	12.43	12.80	13.81
Barley grain		12.21	14.83	19.63	12.43	11.20
Wheat grain		13.22	11.25	11.02	13.60	12.34
Oat grain		13.85	10.40	10.76	10.80	10.75
Soybean grain		16.04	-	-	15.00	12.97
Pea grain		13.77	15.11	15.19	13.10	10.46
Extruded corn		14.49	-	-	-	
Extruded peas	14.20		-	-	-	-
Extruded barley	12.88		-	-	-	-
Extruded wheat	13.80		-	-	-	-
Extruded soybean	14.93		-	-	-	-
Sunflower oilcake	11.76		11.53	11.62	12.30	9.62
Sunflower seed meal		8.26	11.53	10.01	13.70	9.83
Soybean meal	-		12.81	12.33	14.50	11.09
Wheat bran	10.52		-	-	9.30	7.20
Alfalfa grass meal		-	8.55	8.35	7.20	5.86

CONCLUSIONS

The results of the chemical analysis of local fodder showed considerable differences on nutrient content in comparison with literature data. Large deviations were found in the content of crude fat: in oat grain by 4.03 g/kg and soya by 38.12 g/kg; the content of crude protein in corn grain was lower by 32.2-34.92 g/kg, in barley grain by 4.2-16.91 g/kg, in wheat grain by 16.1 g/kg, and in oat grain by 12.91 g/kg.

Overall nutritional value in metabolic energy of local fodder in Moldova varied depending on the location of sampling and compared with the data in specialty literature.

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THE METABOLIC UTILIZATION OF IRON AND COPPER IN THE YOUNG SWINE ORGANISM

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Abstract

The aim of this study was to evaluate the influence of different iron and copper levels from the compound feed over the bioproductive performances of young swine, also the evolution of the contained iron and copper in blood, liver, fat and muscles, in the conditions in which during 2 weeks iron and copper marked with radioactive isotope ^{56}Fe and ^{64}Cu have been used. The researches have been made on the number of 75 pigs from the LS-345 Peris Synthetical Line, being divided in 3 batches uniformly by body weight (11.5 kg). The swine nutrition from all the 3 batches has been realized with the same compound feed, the differences being in the different proportion of the two microelements (iron and copper) from vitamino-mineral premix (batch no.1 received 95 mg iron/kg compound feed and 6 mg copper/kg, batch no. 2-85 mg iron/kg compound feed and 5 mg copper/kg, batch no. 3-75 mg iron/kg compound feed and 4 mg copper/kg). The best bioproductive performances (average daily gain, average daily consumption, specific consumption) have been obtained by the young swine in which's compound feed level has been of 85 mg iron/kg and 5 mg copper/kg. After giving the radioactive isotopes the highest values of iron have been observed in liver and muscles, moreover in case of copper, they can be found in liver. For the young swine copper is a growing biostimulating factor, influencing positively the iron metabolism.

Key words: *bioproductive performances, copper, iron, young swine.*

INTRODUCTION

Swine meat represents an important source of energy, protein, minerals and vitamins. The synthesis of the muscle and fat tissues, as well as other components of the body depends on the adequate nutrient intake through the food which the swine receive and which can be used efficiently for growth (Savu and Petcu, 2002) . Under these conditions, it is necessary to pay a special attention to food since the youth time.

Microelements are present in very small quantities in the tissues of swine, but they perform essential functions for the functioning of the organism, possessing the role of enzymes activators.

Iron is an essential element, being involved in the active transport of oxygen and in the tissue breathing (Deng et al., 2010) . The iron needs are increased during the youth period, deficiency being caused by an inadequate alimentary intake or due to the deficiency in copper, because of which iron cannot be embedded in the biologically active substances (Knutson, 2007).

Iron is an essential microelement in raising swine, playing an important part in haematopoiesis and in the synthesis of some enzymes which participate in different phases of the metabolism (Tapaloaga, 2008). The copper metabolism has interrelationships with that of iron, meaning that the first one is required in the formation of haemoglobin, in increasing the intestinal absorption of iron and in its mobilization from the deposits of ferritin (Pyatskowit J.W., Prohaska J.R., 2008).

Although the researches from the domain are numerous, many of the effects of the trace elements have remained still unclear. In this respect, the paper will present the influence of different iron and copper levels from the compound feed over the bioproductive performances of young swine, also the evolution of the contained iron and copper in blood, liver, fat and muscles, in the conditions

in which iron and copper marked with radioactive isotope ^{56}Fe and ^{64}Cu have been used.

MATERIALS AND METHODS

The researches have been made on the number of 80 pigs from the LS-345 Peris Synthetical Line, being divided in 3 batches uniformly by body weight (11.5 kg) and the ratio between sexes (Table 1).
The swine nutrition from all the 3 batches has been realised with the same compound feed, with the starter recipe (Tabel 2).

Table 1. The experimental scheme

Specification	Batch		
	E1	E2	E3
Swine number (heads)	25	25	25
Initial weight (kg)	11.5	11.2	11.7
Iron (mg/kg feed)	95	85	75
Copper (mg/kg feed)	6.0	5.0	4.0

Table 2. The structure and the parameters of the compound feeds used in experiment

Specification	Phase I
Maize	67.00
Soya meal	22.41
Sunflower meal	4.00
Fish meal I	2.50
L-lysine	0.35
DL-methionine	0.09
Threonine	0.06
Calcium carbonate	1.19
Dicalcium phosphate	0.90
Salt	0.50
Vitamino-mineral premix	1.00
TOTAL	100.00
The recipes parameters	
ME (kcal/kg)	3155
PB (%)	18.01
Lysine (%)	1.21
Methionine+cystine (%)	0.63
Methionine (%)	0.40
Trypthofan (%)	0.58
Treonine (%)	0.67
Calcium (%)	0.89
Phosphorus (%)	0.45
Brute cellulose (%)	3.09

The differences are in the different proportion of microelements (iron and copper) from vitamino-mineral premix utilised. The experimental batch no. 1 received the vitamino-mineral premix which assured a content of compound feed of 95 mg iron/kg and 6.0 mg copper/kg. The experimental batch no. 2 received the vitamino-mineral premix which assured a content of compound feed of 85 mg iron/kg and 5.0 mg copper/kg. The experimental batch no. 3 received the vitamino-mineral premix which assured a content of compound feed of 75 mg iron/kg and 4.0 mg copper/kg.
The source of iron has been represented by the iron sulphate and the source of copper by copper sulphate. During the last 24 days of the experiment in the feed of ten young swine from each batch has been introduced iron sulphate and copper sulphate, containing the radioactive isotopes ^{56}Fe and ^{64}Cu.
The main observed targets in the experiment have been the evolution of bioproductive parameters (average daily gain, average daily consumption, specific consumption). The evolution of the content of iron and copper in blood, liver, fat, muscles, has been determined using iron and copper marked with radioactive isotopes ^{56}Fe and ^{64}Cu which have been used during the last 20 days of the experimental period. From the each batch were sacrificed three animals at 8, 16 and 24 days from the beginning of feeding to establish the way of repartition of marked iron and copper in the pigs′ organism. The radioactivity of iron and copper marked with the ^{56}Fe and ^{64}Cu isotopes from blood, liver, intestine, muscles samples have been measured with the spectrometer with liquid scintillation Beckman LS-6500.
In order to analyse from the point of view of the metabolic iron and copper usage in the body of young swine, the amount of haemoglobin and ceruloplasmin has been determined from the blood collected from five animals per batch at the beginning of the experimental period, as well as at two and three weeks from the start of the experience

RESULTS AND DISCUSSIONS

The influence of the microelements levels administrated to the young swine over the bioproductive performances unregistered by them during the whole experimental period is presented in table 3.
From the analyses of the obtained results it can be observed that the experimental batch no. 3 in which′s feeding the recipe with the lowest values in iron and copper content has been

used, registered average daily gains less than the other two experimental batches, between which the differences have not been unsignificant. It can be noticed under these conditions that copper is a growth biostimulator for swine, the levels of about 6.0 and 5.0 mg copper/kg, achieving growth gains which are relatively (395 g/head/day for the first experimental batch, respectively 400 g/head/day for the second experimental batch).

Table 3. The bioproductive performances of swine registered in the experimental period

Batch	Average daily gain (g/head/day)	Average daily consumption (kg/head/day)	Specific consumption (kg compound feed/kg gain)
E1	$395^{a}\pm7.15$	$1.28^{a}\pm0.22$	$3.24^{a}\pm0.78$
E2	$400^{a}\pm9.11$	$1.26^{a}\pm0.20$	$3.15^{ab}\pm1.01$
E3	$387^{ab}\pm7.16$	$1.25^{a}\pm0.18$	$3.23^{a}\pm1.00$

a – there are no significant differences between the batches ($P > 0.05$)

* ab – there are significant differences between the batches ($P < 0.05$)

The daily consumption of the compound feed varied between 1.25-1.28 kg/head/day at the experimental batches.

The specific consumption achieved a lower value (3.15 kg compound feed/kg gain) in the case of the second experimental batch, in which′s feed have been administrated different average levels of iron and copper, the differences towards the other two batches, being significant.

For looking after the way of repartition of iron and copper in the pigs′ organism after the ingest of the marked microelements has been measured the evolution of the iron and copper radioactivity from blood, liver, fat and muscles (Tables 4 and 5).

Table 4. The evolution of the total radioactivity of iron in pigs′ organism (DPM/g) (x 103)

Batch	Sacrifice at:	Iron in:			
		blood	liver	small intestine	muscle
E1	8 days	211.45	93.45	180.14	88.39
	16 days	289.75	137.15	269.41	107.16
	24 days	358.16	210.28	301.84	170.64
E3	8 days	197.12	100.12	170.26	103.69
	16 days	255.87	167.34	253.32	131.65
	24 days	330.45	231.74	290.64	196.81
E3	8 days	179.56	84.17	159.28	75.37
	16 days	245.93	122.19	243.82	95.31
	24 days	304.12	183.62	274.36	149.18

Table 5. The evolution of the total radioactivity of copper in pigs′ organism (DPM/g) (x 103)

Batch	Sacrifice at:	Copper in:			
		blood	liver	small intestine	muscle
E1	8 days	99.04	60.27	77.12	83.45
	16 days	135.15	85.14	98.52	104.85
	24 days	164.19	100.26	118.26	135.72
E2	8 days	85.42	54.26	69.27	77.27
	16 days	120.65	75.94	89.16	96.27
	24 days	149.51	92.63	103.26	119.36
E3	8 days	79.31	50.25	61.16	70.25
	16 days	107.14	70.25	81.23	89.74
	24 days	128.49	90.53	97.17	108.24

All in all, by analysing the evolution of the total radioactivity of two microelements in pigs′ organism, at the first experimental batch, which has been administrated the biggest quantity of iron and copper, it has been established the biggest amount of microelements from blood, liver, small intestine and muscle. A progressive increase of iron and copper radioactivity in the experimental period has been observed during the 24 days of feeding.

In what matters the evolution of the total radioactivity of iron in pigs′ organism it is noticed that the highest values are in blood, followed by the intestine, liver and muscle. This situation explains the fact that iron is found in the haemoglobin component from the red cells that of mioglobine from muscles. At the level of the small intestine, the absorption of iron takes place, after which a part is deposited in the intestinal epithelium, in liver, most of the quantity being used in the haemoglobin synthesis.

Higher values of the total radioactivity of iron have been registered at the end of the experimental period at the first batch with 358.16 DPM/g in blood and with 301.84 DPM/g in the small intestine, comparative to the samples from liver and muscle (231.74 DPM/g and 196.81 DPM/ml).

In the organism of swine from the second experimental batch have been registered medium values, the lowest being found at the swine from the third experimental batch, in which′s food have been administrated the lowest quantities in iron and copper.

Similar results obtained by Gipp et al., 2013; Collins et al., 2010, are observed, such as the absorption of iron is negatively influenced by

the presence of big concentrations of some microelements, such as copper.

The iron radioactivity values determined from the samples taken from the liver and muscles of swine from the first experimental batch have been lower compared to the ones from the second experimental batch, even if batch E1 received a bigger quantity of iron and copper in food. As a consequence, the copper in excess influences negatively the deposit of iron in liver and muscle.

Higher values of the radioactivity of copper have been determined at the pigs from batch E1 in blood (164.19 DPM/ml), muscle (135.72 DPM/ml) and in the small intestine (118.26 DPM/ml). The lower value was observed in liver (100.26 DPM/ml). Progressive developments of radioactivity of copper have been observed at all the batches.

At the second experimental batch, at which the copper and iron have been in intermediate limits, lower values of radioactivity have been observed compared to batch E1, but bigger than batch E3. Copper is absorbed in the small intestine, from where it reaches blood, being present in all the tissues, but mostly in muscle.

The established values obtained experimentally demonstrate an accumulation of radioactive iron and copper bigger in blood, where the two microelements reach after the absorption from the small intestine. The iron is deposited in liver, which copper can be found in a higher proportion in muscles.

The deposit of iron in liver and muscle is due to the medium values of copper which make the recommended levels of iron and copper to be of 85 mg iron/kg and 5.0 mg copper/kg feed for the young swine.

In order to analyse from the metabolic point of view the usage of iron and copper in the young swine organism the quantity of haemoglobin and ceruloplasmine from blood have been determined, the results being presented in Table 6.

It can be noticed the fact that in the case of the administrated iron quantities the values of haemoglobin have been close, being observed a small decade in parallel by diminishing the administrated iron quantity by the compound feed.

Ceruloplasmine, the way under which copper can be found in plasma, is a protein with a role in the iron metabolism which transforms it into bivalent iron and trivalent iron which is easy to be used by the body.

Table 6. Blood parameters determined during the experimental period

Batch	Period	Haemoglobin (g/100 ml)	Ceruloplasmine (mg/dl)
E1	Initial	11.3	46.1
	2 weeks	12.3	45.4
	3 weeks	12.7	48.5
E2	Initial	10.9	46.5
	2 week	12.2	48.6
	3 weeks	12.5	51.3
E3	Initial	11.2	46.2
	2 weeks	11.9	45.1
	3 weeks	12.3	47.8

The experimental determined values have shown that at medium values of copper administrated by the use of (5 mg/kg feed), ceruplasmine has registered a slight increase in the taken blood samples, therefore iron has been better used in the young swine organism. In this way, the values of the bioproductive performances can be explained, obtained by the second experimental batch.

In order to appreciate the influences of iron and copper fluctuations over the bioproductive performances of the young swine, an informatics program has been developed which allows the simulation program of the metabolic usage of iron and copper in the organism (Figure 1). The program has been written in the programming language Java, in NetBeans.

By the use of the primary experimental data, the program allows a simulation of the bioproductive performances and blood parameters which can be obtained in the case of use of different levels of iron and copper.

The program allows the graphical representation of the influence of iron and copper over the accumulation of the two mineral microelements in blood, liver, small intestine and muscle.

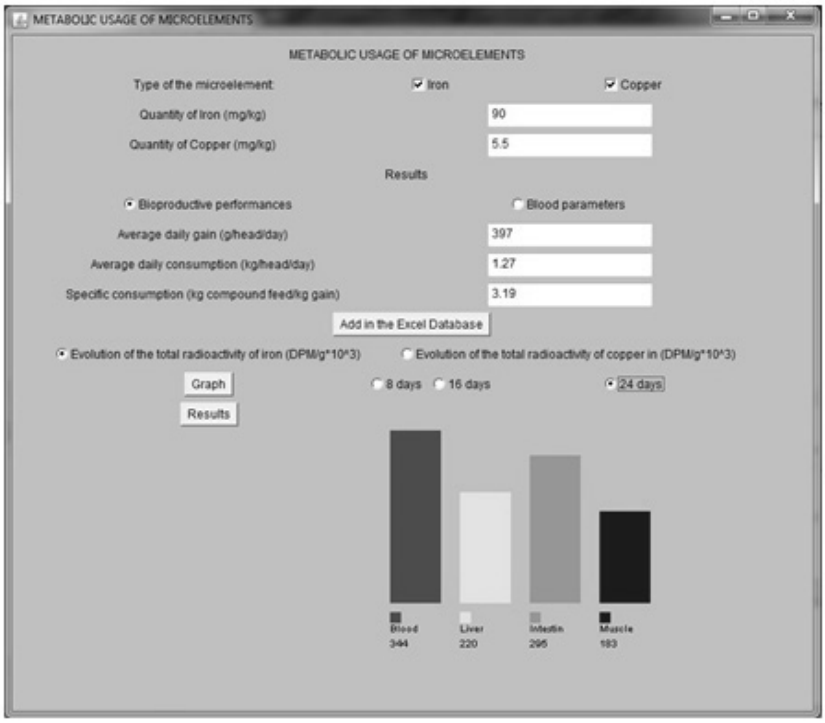

Figure 1. Simulation of metabolic usage of iron and copper in the young swine organism

CONCLUSIONS

The bioproductive performances of young swine have been positively influenced by the usage of the compound feed of a premix which assured levels of 85 mg iron/kg and 5.0 mg copper/kg feed.

The total radioactivity of iron and copper measured during the experimental period has proved an accumulation of radioactive iron and copper bigger in blood, after which the two microelements have been absorbed at the level of the small intestine.

Iron is accumulated in liver, while copper is found a higher proportion in muscle.

The intake of iron in liver and muscle is favoured by the medium values of copper, which makes that the recommended levels for the two microelements of the young swing to be of 85 mg iron/kg and 5.0 mg copper/kg feed.

Haemoglobin has obtained a slight decrease in parallel with the decrease of the iron quantity given through the compound feed.

For medium values of copper administrated in food (5 mg/kg feed) ceruplasmine has obtained a slight increase in the taken blood samples, which has shown that iron has been better valued by the young swine organism.

The metabolic usage of iron and copper in the young swine organism and the their influence over the bioproductive performances and of the blood parameters can be simulated with the help of an informatics program.

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USE OF CHROME TRACE FOR VITAL ACTIVITIES FUNCTIONS STIMULATION OF APIS MELLIFERA BEE COLONIES

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Abstract

The goal of the work was to determine the biostimulation effect of a nutritional supplement of sugar syrup enriched with biomass extract of cyanobacteria Spirulina platensis strain, grown on remedy of chromium alum and potassium, on vital activities functions of Apis mellifera bee colonies. In special experiences has been tested in bees food, an energy-mineralo-protein nutritional supplement „Apispir+Cr", composed of sugar syrup in proportion 1:1, enriched with biomass extract of cyanobacteria Spirulina platensis strain, grown in the presence of coordinate compound of chromium and potassium alum-KCr (SO4) 2·12H2O administered in nutrient medium componence, in the amount of 30...35 mg/L in the first three days of cultivation. The extract of the cyanobacteria Spirulina platensis stem biomass has been obtained by repeated extraction of biologically active substances with alcohol solution and solution of NaOH, by centrifuging, by combinig the supernatants and by making the dialysis up to pH 7.5...8.5 with getting the final extract. Nutritional supplement 'Apispir+Cr' was tested on two similar batches with bee colonies, with 10 families in each batch, including:-Ist batch-witness, where the bees were fed with sugar syrup 1:1, in amount of 100...130 mL to a frame with bees, at every 2 days during two weeks;-II batch-experimental, where bees were fed with sugar syrup 1:1, in the same amount, enriched with the 'Apispir+Cr' supplement under 500:1, and syrup with extract. In early may, before the base picking, were examined the main morphoproductive characters and qualities of bee colonies. Test results have shown that feeding the bees with a nutritional supplement 'Apispir+Cr' in early spring, during the poor harvest in nature, contributed to a significant increase in the value of the main morphoproductive characters of bee colonies. Thus, bee colonies from experimental batch, who received in food the nutritional supplement enriched with 'Apispir+Cr', exceeded their fellows: after the prolificity – with 1390 eggs/24 hours, or with 74.4% ($P < 0.001$), quantity of covered brood-with 167 hundreds of cells, or with 74.5% ($P < 0.001$), the power of the family-with 0.56 kg or 25.3% ($P < 0.001$), resistance to disease-with 10.8 percentage, or 13.4% ($P < 0.001$), the amount of wax accumulated in nest-with 1.10 kg, or with 8.,6% ($P < 0.05$), the amount of honey in the nest-2.44 kg, or 90.0% ($P < 0.001$), the quantity of pasture accumulated in the nest-with 36.4 hundreds of cells, or with 106.7% ($P < 0.001$).

Key words: *biostimulation, „Apispir+Cr", productivity, bees, families.*

INTRODUCTION

After the winter, bee colonies are usually convalescent, as a result of the various weather's action.

In Republic of Moldova, at poor periods harvest in nature, especially, early spring (March-April), before the main harvest, in feeding bees persist a deficiency of complete nutrients, such as protein, vitamins, trace elements etc. In the bees body, there is a deficiency of essential indispensable nutrients, which stagnates their vital activity and inhibit subsequently, the food accumulation processes in the nest.

A special role has feeding bees with micronutrients, such as bioactive catalysts, which determines the enzymes activity and serve as a substrate for cell regeneration at living organisms. Microelements influence refers also to digestion processes and nutrients assimilation. Particularly important are the trace elements for the oxygen transport, regulating the body hydrological regime, dissimilation products neutralizing as a result of oxidation processes.

Of trace elements, a special role has the chromium as a biologically active substance. This is contained in bigger quantity in beekeeping products, especially, in Royal Jelly and bees' bread.

Minerals, including trace elements, get into the bee body through water, pollen, nectar. The presence of trace elements in these bee foods determines the vital activity of the body, and their content in beekeeping products.

To fill the deficit of biologically active substances, including trace elements, in bee foods and unlocking their vital activity processes, during poor periods of harvest, beekeepers apply different procedures and stimulating means of bees vital functions (Tuktarav et al., 2010; Еремич, 1986; Кузин, 2003; Панин, 2001; Таранов, 1986).

It is known an accelerating method of bee colonies development, which consists of feeding them with sugar syrup, enriched with trace elements introduced in form of salts and $COCl_2$, $MnSO_4$, proteins in form of pollen collected from bees balls, calcium caseinate, made from skimmed cow's milk and medical growth stimulators, containing sulphonamides and antibiotics. The disadvantages of this technique are that it is expensive, and the milk proteins and trace elements introduced in form of salts are hard digestible for bees, and being easily oxidable, they cause disturbances in the digestive tract of their functions. Moreover, according to EU and national rules, the use of pharmaceuticals containing sulphonamides and antibiotics, in the treatment, prevention and stimulating the bees, is strictly prohibited.

The above problems can be solved in part, through the use in bees food of nutritional supplement of sugar syrup 1:1 enriched with the biomass extract of cyanobacteria Spirulina platensis stem CNB-CB-02 (Bulimaga, 2006; Rudic et al., 2004; Toderaş et al., 2003). Biomass extract of this stem has a wide spectrum of biologically active substances, but, at the same time, contains little protein and trace elements, particularly, chromium.

In this context, the development and testing of new nutritional supplements for bees food in poor harvest periods is an actual problem.

MATERIALS AND METHODS

At the experimental apiary of Zoology Institute, in collaboration with the microbiology and biotechnology Institute of Science Academy from Moldova, was developed and tested in feeding bees with a mineral energo-protein nutritional supplement, composed of sugar syrup in proportion of 1:1, enriched with the biomass extract of cyanobacteria Spirulina platensis stem, grown in the presence of coordinativ compound of chromium and potassium alum - $KCr(SO_4)_2 \cdot 12H_2O$, administred in composition of nutrient medium, in amount of 30...35 mg/L in the first three days of cultivation, as a result, the biomass gained a high content of biologically active substances, including trace elements, particularly of Cr (Toderaş et al., 2012).

The biomass extract of cyanobacteria Spirulina platensis stem has been obtained by extraction of biologically active substances with alcoholic solution of 20...30%, by its shaking and centrifugation with separation of supernatant sediment. After this, the sediment was parched at the temperature of 40...45^oC, was extracted with 0.45% NaOH solution by shaking for 60 min, centrifugation, sediment separation, and repeated extraction of biologically active substances with 0.45% NaOH solution by shaking it for 30 min, centrifugation, adding of obtained supernatants and making the dialysis up to pH 7.5...8.5 with obtaining of final supplement.

This extract, called by us 'Apispir + Cr', presents a liquid green color with a yellowish tinge, whose dry substance contains 55...65% protein, contained the entire set of essential and non essential amino acids. The extract contains as a bioactive component part, chromium in amount of 0.2...0.4%, one of the main catalysts and antioxidant element of regenerative processes of lacto sinthesing and reproductive tissues' cells of the bees.

To estimate the efficiency of this nutritional supplement 'Apispir+Cr', during the poor harvest period (early April) have been carried out comparative testing experiences on bee colonies from two similar batches, with 10 families in each batch, of which:

- Ist batch – witness, where bees was receiving as food sugar syrup 1:1, in amount of 100...130

mL at one bee comb, each 2 days**,** during 2 weeks.

- IInd batch – experimental, where the bees received as food sugar syrup 1:1 in same amount, enriched with „Apispir+Cr" supplement in proportion of 500:1, respectively syrup and extract.

In early may, before the main harvest, were studied the main morph productive characters and features of bee colonies, in accordance with zootechnic Norm regarding bee colonies evaluation, raising and certification of beekeeping genitor material.

The data obtained in the experiments were processed statistically using computer software 'STATISTICS-6' and evaluated their certainty, according to variational biometric statistics, after the methods of Плохинский Н. А., 1969.

RESULTS AND DISCUSSIONS

Test results have shown that feeding bees with the nutritional supplement 'Apispir+Cr' in early spring, during the poor harvest in nature, contributed to a significant increase in the value of the main morph productive characters of the bee families (table).

Thus, if at he beginning of experiences, the queens' prolificacy of bee colonies from both batches was at the same level, then over a month, this character at bee colonies from IInd batch, which received in food the nutritional supplement enriched with 'Apispir+Cr', has increased significantly compared to the witness batch, with 1,390 eggs/24 hours, or with 74.4% ($P < 0.001$).

Same growth regularities was observed also at the covered brood quantity. It was found that this character has increased directly proportional with the prolificacy, surpassing the witness batch with 167 hundreds of cells, or with 74.5% ($P < 0.001$).

Increased activity of the bees breeding functions from IInd batch, compared with the witness batch, has led to increasing the amount of working bees in the nest. Thus, the bee colonies from IInd batch, which received as food nutritional supplement enriched with 'Apispir+Cr', exceeded significantly the bees from witness batch by their strength with 0.56 kg or 25.3% ($P < 0.001$).

It was established that feeding bees with the nutritional supplement enriched with 'Apispir+Cr' contributes to strengthening their resistance to disease, observed from the standard test of hygienic instinct of dead brood removal. Thus, disease resistance of bee colonies fed with the nutritional supplement enriched with 'Apispir+Cr' increased compared to the witness batch, with 10.8 percentual units, or with 13.4% ($P < 0.001$).

Results of research demonstrate that the nutritional supplement enriched with 'Apispir+Cr, administered in bees' food, exerts a general cumulative influence on their vital activity functions. It was found that at worker bees from IInd batch, the wax glands significantly are increased, compared to the bees from witness batch. Thus, the wax quantity filed by the bee colonies from IInd batch, was significantly higher, compared to the witness batch, with 1.1 combs, or with 84.6% ($P < 0.05$).

Morph productive performance of bee colonies from experimental batch compared to the witness batch, are reflected more obvious in the diagram (fig.), in which the level of morph productive characters' value of bee colonies from witness batch is located on the abscissa conventionally at order 0 (zero) percent.

Table 1. Morph productive characters′ values of bee colonies from experimental batches

Specification	Ist batch $M_1 \pm m_1$	IInd batch $M_2 \pm m_2$	$M_2 - M_1$	% compared to Ist batch	td
Queens prolificacy, eggs/24 hours	1868 ± 117	3258 ± 101	1390	174.4	8.99^{***}
Quantity of covered brood, hundreds cells	224.0 ± 14.1	391.0 ± 12.1	167	174.5	8.99^{***}
Colonies′ strength, kg	2.21 ± 0.08	2.77 ± 0.08	0.56	125.3	4.95^{***}
Resistance to disease,%	80.4 ± 1.0	91.2 ± 1.0	10.8	113.4	7.66^{***}
Wax quantity, built combs	1.30 ± 0.33	2.40 ± 0.31	1.10	184.6	2.43^{*}
Quantity of honey, kg	2.71 ± 0.30	5.15 ± 0.21	2.44	190.0	6.67^{***}
Quantity of bee bread, hundreds cells	34.1 ± 4.25	70.5 ± 3.53	36.4	206.7	6.59^{***}

Remark: **B > 0.95 (P < 0.05);** *** **B > 0.999 (P < 0.001).**

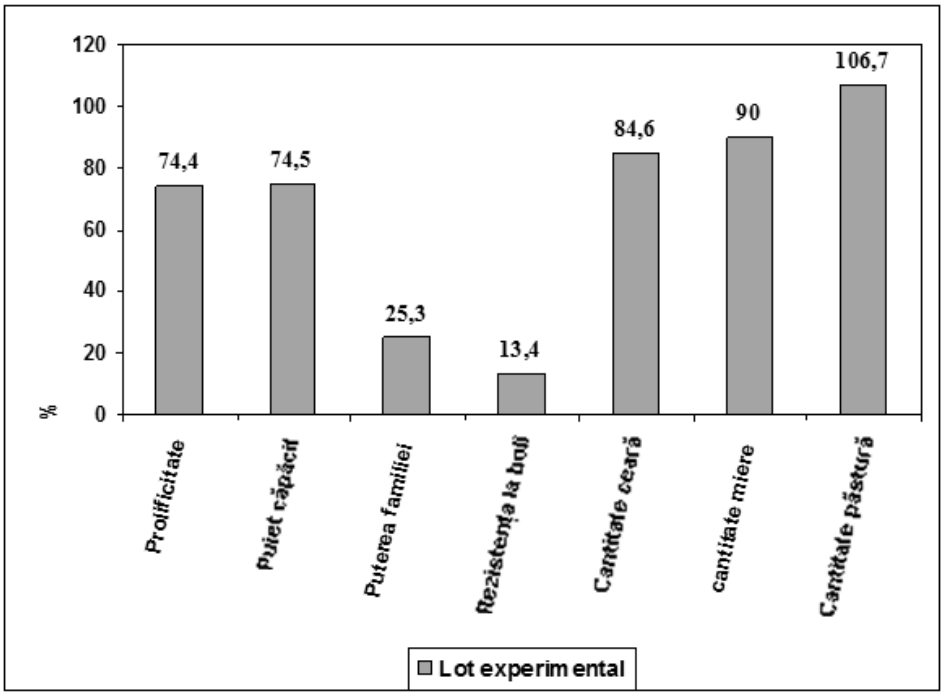

Figure 1. Morph productive performances of bee colonies from experimental batc, compared to witness batch

Due to activation of physiological functions of queens' and working bees' bodies, bee colonies from IInd batch, which received in food the nutritional supplement enriched with 'Apispir+Cr', have accumulated higher production volumes in the nest. Thus, the quantity of honey accumulated in nest by bee colonies from IInd batch was significantly higher, compared to bee colonies from witness batch, with 2.44 kg, or 90.0% ($P < 0.001$). Also, the amount of bee bread accumulated in nest by bee colonies from Iind batch was higher compared to those from witness batch, with 36.4 hundreds of cells, or with 106.7% ($P<0.001$).

Generalized result achieved, as a result of feeding bee colonies with nutritional supplement enriched with 'Apispir+Cr', is due to the presence in it, of biologically active substances, such as: amino acids in increased quantity, peptides, vitamins, pigments and trace elements, in particular, chromium in increased quantity, as catalysts of some important functions of regeneration of the queens' ovarian tissues cells, and of lactogene glands of worker bees. Chromium has high stimulating and antioxidant properties and is part of queens' milk composition, determining the quality and the level of permanent nutrition of the queen, and of brood larvae in first days.

All this has led to the intensification of the vital physiological functions of working bees, in particular, of queens prolificacy, bee colonies' resistance to disease, wax activity of working bees, which have contributed to increase of production volumes accumulated in the nest.

CONCLUSIONS

1. To fill the deficit of complete nutrients in feeding bees during poor harvest period in nature, can be used as a nutritional supplement, composed from sugar syrup in proportion of 1:1 with 'Apispir+Cr (patent MD 476 Z 2012.09.30).

2. Biologically active substances contained in the nutritional supplement 'Apispir+Cr', especially chromium, helps to stimulate the vital activity of bees, accelerating the reproduction rate of brood, increase of bee colonies strength, their resistance to disease and ability to accumulate production in the nest.

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EFFECT OF ARTIFICIAL MOLTED BROWN LAYING HENS ON PRODUCTION OF DHA ENRICHED EGGS

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Abstract

The aim of the experiment was to investigate the possibility of production docosahexaenoic acid (C22:6n-3 − DHA) enriched eggs using very old molted hens. Two groups Hisex Brown molted laying hens, 60 wks and 80 wks old, were assigned in 2 subgroups and fed diets enriched with omega 3 fatty acid (especially DHA).The source of DHA was fish oil and fish meal. The amount of omega 3 in feed offered to the experimental hens was 792 mg and 1180 mg C22:6n-3 in kg feed. Feed consumption was restricted on 120 g feed/day/hen. The intensity of egg production at 60 wks and 80 wks old molted hens was similar, 77.93% and 77.86% in subgroups fed lower amount of DHA and 79.28% and 89.16% n subgroups fed with higher amount of DHA. Younger molted hens, 60 wks old, have better egg production then older. Lower level of DHA in feed induced egg production 136,61 and 140.73 mg/egg, and higher amount of DHA in feed induced egg production richer with DHA, from 159.41 to 170.49 mg/egg. Molted older laying hens can produced richer eggs with DHA long chain polyunsaturated fatty acid.

Key words: *DHA, eggs, laying hens, molting.*

INTRODUCTION

Polyunsaturated n-3 fatty acids (PUFA n-3) have significant and various health benefits as treatment of arthtritis (Rennie et al., 2003) coronary disease (Simopoulos, 2000), blood pressure control (Holm et al, 2001), lowering triglycerides (Covington, 2004) and enhancement of immunity (Simopoulos, 2002). Consumption of enriched eggs with omega 3 fatty acids demonstrates positive effects and no negative effects on human health (Lewis et al., 2000). Enricihing the eggs with omega-3 fatty acids is the well known procedure which are related with modified laying hens diets. This procedure is challange for the scientists because using of the enriched diets with PUFA n--3 may have an undesirable effects on productive performance of laying hens, egg weight and yolk weight (Sari et al., 2002),. Laying hens strain does not have any influence on fatty acid composition of eggs yolk (Ahn et al., 1995; Grobas et al., 2001), but the hens age has an important role in deposition of n-3 PUFA in egg yolk (Nielsen, 1998; Yannakopouols et al., 2005).

The aim of this research was to evaluate the influence of the age of the molted laying hens on the production performances and content of DHA in egg yolk.

MATERIALS AND METHODS

Forty molted Hisex Brown hens (60 wks old and 80 wks old molted hens) were housed in laying cages (2 birds per cage) in standard poultry house with a light regime of 16H and 8H darkness. The hens were assigned in four experimental groups (10 birds per group). The experiment was lasting 30 days. The body weight of hens was measured at the beginning and at the end of the experiment. The egg production was controlled daily and the egg mass was controlled weekly. The feed consumption of hens was restricted to 120 g/day, but water consumption was provided ad libitum by 2 nipple waterers in every cage. The ingredients and nutrient composition of the experimental diets was presented in Table 1.

Egg samples were collected every 10th day, 6 eggs per group. The eggs were measured, cracked, the shells were discharged, the separation of the yolk from the albumen were performed manually.

Table 1. Ingredients and nutrient composition of experimental diets

Ingredients (%)	Experimental diet	
	1	2
Ground yellow corn	51.96	50.34
Wheat middlings	10.00	10.00
Sunflower meal	13.00	13.00
Soybean meal	10.18	10.86
Fish oil	1.93	2.90
Fish meal	1.71	1.69
DL methionine	0.08	0.08
L lysine	0.06	0.04
Choline chloride	0.05	0.05
Salt	0.23	0.24
Limestone	9.00	9.00
Dicalcium phosphate	1.30	1.30
Microtracer	0.50	0.50
Total	100.0	100.0
Calculated nutrient composition		
ME, Kcal/kg	2700	2722
Crude proteins,%	15.00	15.00
Crude fibre,%	4.05	4.08
Fat,%	5.48	5.40
Ash,%	12.47	13.00
Lys,%	0.80	0.80
Met,%	0.40	0.40
DHA, g/kg	0.792	1.18
Ca,%	3.80	3.89
Nonphytate P,%	0.38	0.38

The albumen residuals were eliminated from the yolk using blotting paper, viteline membrane was removed using tweezers, then mixed manually with a spatula and stored frozen and analyzed up to 7 days.

Concentrations of docosahexaenoic (DHA, C22:6n-3) fatty acid was measured in egg yolk. Six yolks were mixed, then dried with sodium sulphate, mixed with DI (deionized) water and hexane and centrifuged 2-3 minutes at 2500 rpm. Fatty acid was determined by gas chromatography (AOCS −Ce 1f − 96) adapted by Abril and Barclay (1999), with identification of fatty acids by comparing of their retention times and quantified by areas standardization.

Obtain results were analysed running f-tests on two significance level (5% $P< 0.05$ and 1% $P< 0.01$) according to Snedecor and Cochran (1989).

RESULTS AND DISCUSSIONS

Production parameters of hens were presented in Table 2.

Table 2. Production parameters

Parameter	60 wks old hens		80 wks old molted hens	
	Group 1 BF + 792 mg DHA kg^{-1}	Group 2 BF + 1180 mg DHA kg^{-1}	Group 3 BF + 792 mg DHA kg^{-1}	Group 4 BF + 1180 mg DHA kg^{-1}
Number of hens	10	10	10	10
Hen′s weight, g				
at the beginning	1910	1960	2179	2167
at the end	1900	2025	2104	2216
change in body weight	-10	65	-75	-49
Egg production				
laying intensity,%	77.93	89.16	77.86	79.28
average egg weight, g	64.74[a]	65.43[a]	70.03[b]	67.36[b]
Feed consumption				
daily consumption, g	120	120	120	120
per egg, g	154	134	145	151
per gram egg mass, g	2.38	2.05	2.07	2.24
DHA consumption				
per hen, mg/day	95	142	95	142
per egg, mg	122	158	115	179

[a.b] – Values in the same row with no common superscript differ significantly ($p< 0.05$)

During the experiment there were no health disorders, and mortality was ranged in the technological norms, while differences between groups were not significant. The hens at the beginning and the end of the experiment had similar body weight for the hybrid, and differences in body weight between groups at the beginning and the end of the experiment were not statistically significant ($p > 0.05$). The intensity of egg production at 60 wks old hens and 80 wks old molted hens was similar, 77.86% and 77.93% in groups fed with lower amount of DHA and 79.28% and 89.16% with higher amount of DHA in old molted hens and in younger 60 wks old hens, respectively. The average egg weight throughout the experiment was greater in the experimental groups 3 and 4 80 wks old molted hens (70.03 g and 67.36 g) and in group 1 and 2 60 wks old hens was 64.74 g and 65.43 g. The feed consumption was restricted (120 g feed/day), but the feed

consumption per egg was the lowest in group 2, 134 g, then in group 3, 145 g, and group 4 and 3 151 g and 154 g per egg, respectively.
The feed conversion efficiency was the lowest in group 2, (2.05 g/g egg mass), the highest was in group 1, (2.38 g/g egg), and in group 3 and 4 was 2.07 g and 2.24 g/g egg mass.
The daily consumption of DHA was 95 mg for experimental groups fed with 0.792 g DHA/kg supplemented feed and for experimental groups fed with 1.18 g DHA/ kg supplemented feed the daily consumption of DHA was 142 mg.
The highest DHA consumption per egg was recorded in groups 4 and 2 (179 mg and 158 mg) and in groups 1 and 3 was 122 and 115 mg, respectively.
The content of DHA, in gram yolk in the 60 wks old laying hens was 8.23 mg and 9.72 mg in group 1 and 2, and in gram yolk produced from 80 wks old molted laying hens was 8.13 mg and 9.77 mg in group 3 and 4, respectively.
The content of DHA, in average, was the highest in the groups 4 and 2 fed with 0.792 g DHA/kg supplemented feed (170.49 mg and 159.41 mg) and lowest in groups 3 and 1 fed with 1.18 g DHA/ kg supplemented feed (140.73 mg and 136.61 mg). The results are presented in Table 3.

Table 3. Content of DHA in egg yolk

	60 wks old hens		80 wks old molted hens	
	Group 1 BF + 792 mg DHA kg^{-1}	Group 2 BF + 1180 mg DHA kg^{-1}	Group 3 BF + 792 mg DHA kg^{-1}	Group 4 BF + 1180 mg DHA kg^{-1}
DHA in gram yolk, mg	8.23	9.72	8.13	9.77
DHA in 100 g yolk, mg	823	972	813	977
DHA in one average egg, mg	136.61	159.41	140.73	170.49

Nielsen (1998) reported that the age of laying hens had an effect on the composition of fatty acids in egg yolk. Eggs produced by white Lohmann hens were collected at the ages 21 and 51 weeks. The content of arachidonic acid (20:4 ?-6) and DHA (22:6 ?-3) was higher in egg yolk from young hens compared with older hens. An opposite conclusion was reported by Scheideler et al. (1998) who found that eggs laid by the genotypes Babcock B300, DeKalb Delta and HyLine W-36 hens at the age of 36 weeks had less DHA than at 58 weeks.
The content of DHA, in one average egg are presented in Figure 1.

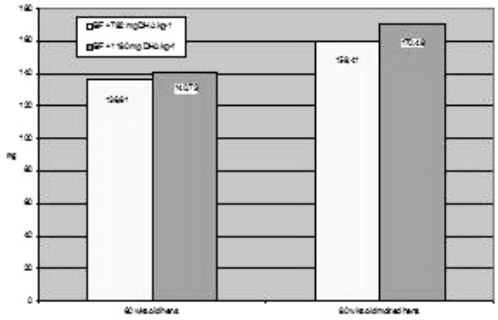

Figure 1. DHA in one average egg, mg

Younger molted hens, 60 wks old, have better egg production then older. Lower level of DHA in feed induced egg production 136,61 and 140.73 mg/egg, and higher amount of DHA in feed induced egg production richer with DHA, from 159.41 to 170.49 mg/egg. Molted older laying hens can produced richer eggs with DHA long chain polyunsaturated fatty acid.

CONCLUSIONS

There are no significant differences among investigated parameters (P> 0.05), except on the obtain results about average egg weight (p< 0.05). However, the results obtained form this investigation indicate that molted older laying hens fed with supplemented diet with fish meal and fish oil can produced richer eggs with DHA long chain polyunsaturated fatty acid.

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CHARACTERISTIC AND GROWTH PATTERN OF *BRACHIARIA HUMIDICOLA* CV. TULLY UNDERNEATH COCONUTS PLANTATION

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Abstract
The aims of the study were to determine growth characteristics and the pattern of growth of B. humidicola grown underneath coconut trees. The experiment was conducted at coconut research center area (BALITKA) Manado since April 2011 until July 2011. The variables consisted of growth and development based on the numbers of tillers, numbers of nodes, and length of stolon. Data were calculated with simple analysis of the mean and the standard deviations, and the regression equations. The results shows that number of nodes and the length of stolon steadily increased up to 10 weeks after planting, but the maximum number of tillers up to 8 weeks after planting and start to decrease at 10 weeks after planting. Furthermore, the increased of nodes, stolons and the number of tillers are positively corelated with the ages of plant and followed the normal distribution curves of growth.

Key words: *characteristic, growth pattern, humidicola, coconuts.*

INTRODUCTION

Brachiaria humidicola is one of the perennial forage grass with creeping habit growth, having stolon and rhizome, can grow well and tolerant of shaded conditions such as in coconut plantations. Understanding how grass plants grow and developed is essential to be able to manage properly a pasture. Grass like other green plants, capturing energy from the sun and stores it in the form of sugar and carbohydrates, all of which will be used along with other minerals and nutrients for cell division, growth, development and reproduction (Stichler, 2002).
The availability of light is the main ecological factors affecting plant growth. To respond the low light level plants can adapt genetic and phenotypically (Lambers et al*.,* 1998; Guenni et al., 2008). There are three responses to the shade acclimation namely a) by reducing the rate of respiration, b) increase the shoots / root ratio, and c) increasing the specific leaf area (Humphrey, 1994; Lambers et al., 1998).
Pasture development in the area of coconut plantations faced with the problem of shade, which led to the disappearance of pasture species and replaced with not edible feeds as weeds. This problem can be overcome by the introduced of shade-tolerant species, such as *Brachiaria humidicola* cv. Tully, which is recommended as pasture in the area of coconut plantations (Mullen et al., 1998).
Nevertheless, the grass is still damaged when grazed freely (free grazing) or without properly management. These results indicate that the cause of the damage is not solely just on the issue of tolerance of forage in the shade, but also a factor that is not proper grazing management, led to not able to guarantee the health of pasture for continuing to grow and reproduce. It is strongly associated with characteristic and growth patterns of plant. For that reason, the study was conducted.

MATERIALS AND METHODS

Place and Time

The research was carried out on the Coconut and Other Palma Research Institute (BALITKA) in the village of Paniki Manado, North Sulawesi, Indonesia since the beginning of April 2011 until the end of July 2011. Field experiments conducted to study the growth pattern of the grass *B. humidicola*.

Materials and Devices

Materials used in the form of grass seedlings *B. humidicola* with a length of 15 cm, with a 2.5 young leaves and secondary roots. Two seedlings were planted, then three weeks after planting (WAP) one vigor plant were selected and allowed to grow as single plant. A total of five plants were sampled and planted at a

spacing of 1.5 meters to facilitate the measurement. The variables measured in this experiment are the number of nodes and the length of stolon. Both measured on the parent plant (mother), while the number of tillers was obtained by counting all seedlings produced by observed parent plants. The equipment used small manual scales 'Ohaus', stationery, bags and oven drying samples.

Research Methods

To study the vegetative growth and development of grass the measurement was done along the vegetative periods of growth, performed every 2 weeks counted of weeks after planting (WAP) of 2, 4, 6, 8 and 10 weeks. The number of nodes is the sum of nodes in each period. The length of stolon was obtained in the last period of experiment at weeks ten, while the number of tillers was the some of tillers of each period of measurement. Furthermore, by dividing the length of stolon to the number of nodes, obtained an average length of each segment in the plant samples.

To get the growing pattern of *B. humidicola* during development phase was analyzed correlations between each of the variables measured with the age of the plant (Steel and Torrie, 1989).

RESULTS AND DISCUSSIONS

Growth Characteristics

Understanding how plants grow and the grass is very essential to be able to manage properly a pasture. Characteristics of growing *B. humidicola* in the area of coconut palm which is measured on the number of nodes, the length of stolon, and the ratio of stolon length/ number of nodes as listed in Table 1 below.

Table 1. Number of nodes (Nn), length of stolon (Ls) and the number of tillers per plant

Plant Number	Nodes Number (NN)	Stolon Length (cm) (Ls)	Ratio Ls/NN	Total Tiller
1	19.00	107.00	5.63	10.00
2	18.00	135.00	7.50	9.00
3	20.00	110.00	5.50	10.00
4	19.00	115.00	6.05	12.00
5	21.00	125.00	5.95	11.00
Mean	19.40	118.40	6.13	10.40
SD	1.1402	11.5239	0.8004	1.1402

Table 1 show that the number of nodes during the observation period of five plant samples ranged between 18 and 21 nodes. Length of stolon of the mother plant during the period of measurement varies between 107 cm to 135 cm. When calculated the ratio between the length of stolon and the number of nodes was obtained the ratio of Ls/Nn varied from 5.50 to 7.50 or the length of segment (cm) between two successive nodes on plant samples varies follow this ratio. This was probably due to the activity of photosynthetic process is more active, and is followed by more accumulation of assimilates, so the stimulation of the development of plant is bigger. Abdullah (2009) says the length of plant is supported by a number of nodes and the long of segments.

Growth Pattern

Average increase in the number of nodes, length of stolon and increasing the number of tiller is presented in Table 2.

Table 2. Average increase in the number of nodes, stolon length and the number of tiller at each age of observation

Week After Planting	Nodes Number	Stolon Length (cm)	Tiller Number
2	3.40	20.60	1.20
4	4.00	37.80	2.00
6	3.80	50.80	1.80
8	4.20	70.60	3.20
10	4.00	85.80	2.60

a. Nodes number

Growth of plant is always associated with the age of the plant. Table 2 shows that the number of nodes increased every 2 weeks during the measurement period until the age of 10 weeks. Statistical analysis showed that the number of nodes grew were strongly influenced by time or the age of the plant, and the relation follows a linear equation $y = 3.835 + 0.003750\ x$, where Y represents a variable number of nodes, and x is the time to grow the plants, with a regression coefficient $R2 = 98.7\%$. Linearly in the number of nodes really help to support the production of biomass of *B. humidicola* because in each node that touches the ground produced new tiller that allows plants to grow, spread and produce new leaves and stems, as the character of the plant growing with stolon (Wong and Stur, 1994).

b. Length of Stolon

Table 2 shows the length of stolon of mother plant by 20.60 cm at the age of 2 weeks and increased gradually from 4 weeks at 37.80 cm up to 85.80 cm at the age of 10 weeks. Length of stolon is closely related to the age of mother plant following the linear regression equation y = 4.160 x + 8.160, with a regression coefficient R2 = 99.7%. It shows one of the characters persistence of grass growing with stolon (Wong and Stur, 1994), especially those living individually where the competition of nutrients, water and sunlight are relatively low (McMaster et al., 2003).

c. Number of tiller

The highest average number of tiller of 3.20 produced in 8-week-old plants and decreased as much as 2.60 at the age of 10 weeks (Table 2). This suggests that at the age of 8 weeks the vegetative stage of growth is taking place, as well as the development of tropical grass in general (Tropical Forages). A decline in the number of tillers at the age of 10 weeks may be due to the high population density, resulting in overlap and competition for resources (Abdullah, 2009).

Statistical analysis showed a close relationship between the number of tillers and the age of the plant, following the cubic equation y = 1.240 to 0.143 x + 0.0911 x2-0.00625 x3 with regression coefficient R2 = 82.9%. Previous research demonstrated that the number of tillers always follow the pattern of cubic (Emoto and Ikeda, 2005).

Heat Unit Requirement

The needs of heat unit (^{0}C-day) for the formation of a phyllochron are different for single plants and in the community. Single plant requires less heat units to produce one phyllochron as many as of 68.19^{0}C-day, compared to 130.44^{0}C-day for grass of *B. humidicola* that grown in the community (Anis et al., 2011). This happens because there is a strong competition of resources such as water, nutrients and light occurred among the plants grown in the community. In contrast, a single plant is less than a competition, the ground surface temperature is higher because more sunlight penetration. Leaf emergence is closely linked to temperature, but was dominated by the rise of ground surface temperature as a place where there are crowns to grow new tillers (McMaster et al., 2003).

CONCLUSIONS

From these results it can be concluded that:

1. The number of nodes and length of stolon remained increased up to the age of 10 WAP, but the highest number of tillers until age 8 WAP then decreased at 10 WAP.

2. The increasing number of nodes, stolon length and number of tillers were positively correlated with the age of the plant, and still follow the normal pattern of growth and development.

3. The need of heat unit (^{0}C-day) for the establishment of a phyllochron of *B. humidicola* growing individually less than those growths in the community.

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INVESTIGATION OF *L. MONOCYTOGENES* – HEP-2 CELLS RELATIONSHIPS BY CULTURE BASED AND MICROSCOPY TOOLS

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Abstract

The first step of the infection process must triggered by virulent Listeria monocytogenes strains is the attachment to, and invasion of, the gastrointestinal epithelium. The purpose of this paper was to investigate the ability of L. monocytognes species isolated from different clinical specimens to adhere, invade and multiply in HEp-2 cells. All investigated L. monocytogenes strains exhibited the ability to adhere, invade and multiply in the eukaryotic HEp-2 cells, the internalization being followed by the activation of cellular signaling pathways, leading to the release of thin, long cytoplasmic extensions, vacuolization and apoptosis. The ability of L. monocytogenes strains to survive inside the host cell could explain their implication in chronic recurrent diseases and long-term colonization, the internalization process providing effective protectionagainst host defenses and antibiotic treatment.

Key words: *Listeria monocytogenes, apoptosis, fluorescence, invasion, HEp-2 cells.*

INTRODUCTION

Listeria monocytogenes is a fascinating bacterial pathogen able to survive in a saprophytic environment and to induce disease in mammalian hosts (Pizzaro-Cerda et al., 2012). The potential of the saprophytic *L. monocytogenes* to cause listeriosis, especially in newborns and immunocompromised individuals is correlated with its capacity to survive within macrophages, to invade nonphagocytic cells and replicate therein (Allerberger and Wagner, 2010). The first step of infection must be attachment to, and invasion of, the gastrointestinal epithelium by virulent *L. monocytogenes* (Schlech et al, 1994; Carnejo et al., 2011). The purpose of this paper was to investigate the ability of *L. monocytognes* species isolated from different clinical specimens to adhere, invade and multiply in HEp-2 cells.

MATERIALS AND METHODS

Bacterial strains

The experiment was performed on *L. monocytogenes* strains, collected from NIRDMI Cantacuzino *Zoonosis* Laboratory Collection, isolated from different clinical specimens (Table 1).

Table 1. Source of isolation and identification/confirmation of L. monocytogenes from the investigated strains (No.)

Year of isolation **Clinical specimen**	**2010**	**2011**	**2012**
Blood culture Septicemia	2		1
Cerebrospinal fluid Meningitis	1	5	1
Cerebrospinal fluid Meningo-encephalitis	1		
Total	4	5	2

The analyzed strains have been identified using classical cultural, biochemical and serological tests and were preserved at-80ºC in Brain Heart Infusion (BHI) broth (Oxoid) with 20% glycerol and next there were streaked on 7% blood agar plates at 37ºC for 24 hrs, prior the experiments (McLauchlin, 2005).

Study of the adherence and invasion capacity to the cellular substrate represented by HEp-2 cells (Cravioto's adapted method) (Cravioto et al., 1979; Lazar et al., 2002; Chifiriuc et al., 2010).

In this purpose, HEp-2 cells were routinely grown in Eagle's minimal essential medium (Eagle MEM) supplemented with 10% heat inactivated (30 min at 56°C) foetal bovine serum (Gibco BRL), 0.1 mM nonessential amino acids (Gibco BRL), and 0.5 ml of gentamycin (50 µg/ml) (Gibco BRL) and incubated in a 5% CO_2 humidified atmosphere, at 37°C for 24 hrs (Kalliomaki et al., 2001). HEp-2 cell monolayers grown in 6 multi-well plastic plates were used at 80-100% confluence. Bacterial strains from an overnight culture on 2% nutrient agar were diluted at 107 CFU/ml in Eagle MEM with no antibiotics. The HEp-2 cell monolayers were washed 3 times with Phosphate Buffered Saline (PBS) and 2 ml from the bacterial suspension were inoculated in each well. The inoculated plates were incubated for 3 hrs at 37°C. After incubation, the monolayers were washed 3 times with PBS, briefly fixed in cold ethanol (3 min), stained with Giemsa stain solution (1:20) (Merck, Darmstadt, Germany) and incubated for 30 min. The plates were washed, dried at room temperature overnight, examined microscopically (magnification, ×2500) with the immersion objective (IO) and photographed with a Contax camera (Company, City, Country) adapted for Zeiss (Axiolab 459306) microscope (Zeiss, City, Country). For the quantitative assay of adhesion and invasion capacity, the infection step was performed in duplicates for each strain, and after 3 hrs incubation of the HEp-2 monolayer in the presence of microbial strains, the first well plates were washed four times in PBS, the cells were permeabilized by Triton X 1% (Sigma) and incubated for 5 min at 37°C for the release of intracellular invasive bacteria. Thereafter, serial ten-fold dilutions in saline solution were performed and 20 µl from each dilution was spotted in triplicates on solid media; in the second plate, after 2 hrs of incubation the monolayer was washed 4 times in PBS and 1 ml of 100 mg/ml gentamycin solution was added; the plates were further incubated for 1 h, in order to kill all adherent extra-cellular bacteria. Thereafter, the second plate was treated as the first one. After incubation at 37°C for 24 hrs, we counted the bacterial colonies in each spot and the results were expressed by CFU/ml.

Fluorescent actin staining (FAS) (Knutton et al., 1991; Chifiriuc et al., 2008). Bacterial suspensions in nutrient broth prepared from cultures of 24 hrs on agar plates were used for being inoculated into the subconfluent, HEp-2 monolayers of 24 hrs cultivated in 6-multiwell plates with coverslips. After 3 hrs of incubation at 37°C, the plates were washed 3 times in PBS and the cells were briefly fixed by glutaraldehyde and permeabilized with PBS-Sap-BSA. The coverslips were removed, stained with DAPI or PI (propidium iodide), mounted in glycerol-PBS and examined by incident-light fluorescence using an Olympus Bx40 fluorescence microscope with adequate filters.

RESULTS AND DISCUSSIONS

Our results revealed that *L. monocytogenes* tested strains exhibited different adherence abilities for colonizing the HEp-2 cells, as demonstrated by different adherence patterns and rates (Table 2).

The invasion assay revealed that 27.3% of the analyzed strains were highly invasive, 27.3% were moderately invasive and 45.4% were classified as low invasive (Table 2).

The low invasion rate of some of the tested strains is reflecting a reduced ability to internalize and multiply inside the eukaryotic cells following the initial adherence step. The examination of fluorescent labeled HEp-2 cells infected with *L. monocytogenes* confirmed the results obtained in the quantitative assay of viable, internalized cells.

Table 2. The adherence and invasion percentages of the tested cells on HEp-2 cells

Tested parameter	**(%) of positive strains**
Adherence to the cellular substratum	**100%**
Localized adherence	9%
Diffuse adherence	27%
Aggregative adherence	37%
Mixed adherence patterns	27%
Invasion of the HEp-2 cells substratum	**100%**
Highly invasive 3	27.3%
Moderately invasive 3	27.3%
Low invasive 5	45.4%

The invasion ability was present in all tested strains (Figure 1).

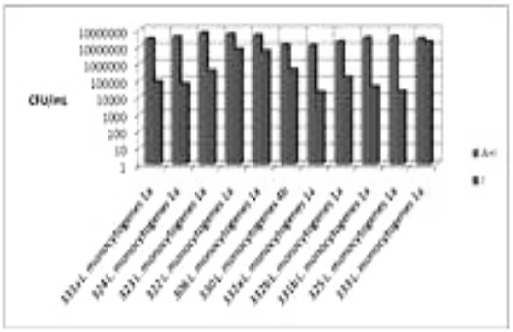


Figure 1. Graphic representation of the invasion and adherence rates of different L. monocytogenes strains

Thus the fluorescence microscopy aspects showed the presence of single bacterial cells or associated in microcolonies inside the mammalian cells, embedded in cytoplasmic vacuoles (Figure 2).

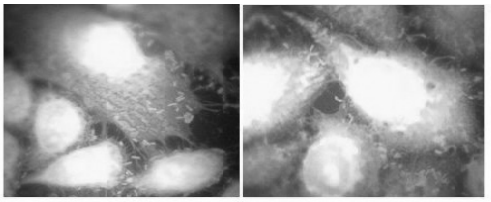


Figure 2. PI stained HEp-2 cells infected with *L. monocytogenes*, showing single bacterial cells or associated in microcolonies inside the mammalian cells. a. Bacterial cells adhered to HEp-2 cells; b. Cytoplasmic vacuoles

As the quantitative assay of the invasive ability is based on the counting of viable cells, it is possible that the internalized bacterial cells be metabolically active, playing an important role in the intracellular survival and antibiotic resistance, factors favoring the persistence of these infections.

The bacterial cell interaction with the host cell induced changes in the epithelial cell membrane, which exhibited long and thin membrane elongations, aspect demonstrating an endocytic process triggered by the bacterial cells (Figure 3).

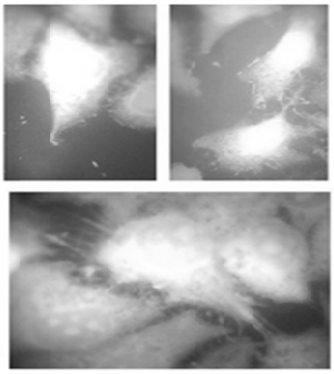
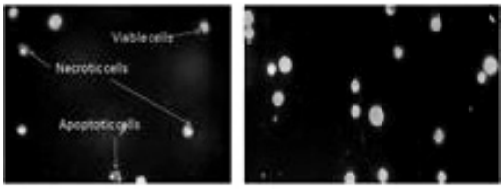


Figure 3. PI stained HEp-2 cells infected with *L. monocytogenes,* showing long and thin membrane extensions (x 100)

Once present in the cytosol of HEp-2 cells, *L. monocytogenes* could induce the apoptosis of the host cell, as revealed in Figure 4.



a. Infected HEp-2 cells; b. Control cells

Figure 2. FAS staining of HEp-2 cells infected with *L. monocytogenes* (DAPI staining, x40)

CONCLUSIONS

All investigated *L. monocytogenes* strains exhibited the ability to adhere, invade and multiply in the eukaryotic HEp-2 cells, the internalization being followed by the activation of cellular signaling pathways, leading to the release of thin, long cytoplasmic extensions, vacuolization and apoptosis. The ability of *L. monocytogenes* strains to survive inside the host cell could explain their implication in chronic recurrent diseases and long-term colonization, the internalization process providing effective protection against host defenses and antibiotic treatment.

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MICROELEMENTS AND THEIR CHELATE FORMS IN NUTRITION OF MONOGASTRIC ANIMALS: A REVIEW

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Abstract

The aim of this work was to analyze the available data concerning the characteristics and effects of micro elements and their chelate forms in swine and poultry nutrition on production parameters and cholesterol content in the tissues of broiler chickens. Usage of copper chelate forms in piglets nutrition led to increased feed consumption by 4% and daily gain weight of 4.2%. The same trend was observed in fattening pigs fed diets supplemented with 200 ppm of copper, with increased daily gain by 14.3%. The research, which aimed was to explore the possibility of significantly lower inclusion levels of minerals, the introduction of organically bound forms in broilers have not any significant differences, indicating that the much lower levels of minerals in organic form had positive impact on production parameters of broiler chickens, and improved production results of hens and parents of heavy line hybrids. The inclusion of molybdenum and sulfur in feed with high levels of copper, 100 and 200 ppm had a positive effect on the broilers growth intensity. Adding 50 ppm of molybdenum and 50 ppm of sulfur in the mixture with 100 ppm of copper, at the end of the experimental period, increased the body weight of chickens for 8.35% compared to the control group, while in the second fattening period, the group with addition of 100 ppm of copper was most effective. Also it is observed a significantly lower concentration of copper ($P< 0.01$) in the liver in the presence of molybdenum, with and without the presence of sulfur, compared to groups that contained only copper in feed. The use of chelate forms of minerals in monogastric nutrition is a newer trend that is still not fully accepted in practical production and further research in this area is needed. In deciding whether to use a more efficient, but also more expensive mineral chelate forms, environmental requirements must be taken into account.

Key words: *chelates, nutrition, poultry, swine.*

INTRODUCTION

Chelates are a special group of complex compounds known to researchers for more than eight decades (Stanacev et al., 2004). These are heterocyclic compounds in which the metal ion is bound to two or more atoms spatially oriented functional groups on the same ligand, constructing chele. The most stable structure of the complexes has five and six member rings, and the stability of the chelate in comparison to the analogous dithiocarbamate ligands, known as the chelate effect. The biological activity of the metal is determined by the physical and chemical properties, and their position in the periodic table of elements. The elements of the fourth period are biologically active, and the presence of *d*-electrons gives them a strong preference towards the formation of biologically active complexes-chelates. Chelate formation is carried in the digestive tract during digestion, between transition metals (Fe, Zn, Cu, Mn, Co, Mo) and organic substances in the structure are electron donor atoms N, O and S. Complex formation is carried out by hybrid-dization of electrons from both electronic sources (metal and ligand) in *s, p* and *d* orbitals, resulting in a new hybridized orbitals. Relation between sulfur and metals in contrast to nitrogen and oxygen can be very firm, while the ionization potential of the chelate is very small. Such compounds are ineffective in diet, and in some cases toxicosis accumulate in tissues. Protein digestion and their products are good chelators, such as amino acids, which are building with metal chelates with the structure of typical five-member rings. From the

chelating groups, especially the characteristic copper salts of amino acids, blue in color and beautifully crystallized are often used for the separation and identification of amino acids (Kessler et al., 2001; Ferketi et al., 2009; Huang et al., 2009). Feeds that are used bean in the diet, containing sufficient amounts of chelators, regardless to the specific added products. Such substances such as proteins, amino acids, peptides, starch and cellulose, citric and oxalic acids and other organic compounds of EDTA, a chelating properties and affect the metabolism of microelements. The key role of these compounds is to form soluble complexes and prevent insolubility of metals in weakly alkaline digestive tract (Donghua et al., 1995; Du et al., 1996; Hemken, 1997; Adamovic et al., 1997; Pupavac et al., 1999). Chelates are the most useful forms of metal and ligand interactions of the organism. In chelates, metal activity is increased by 10^5 to 10^7 in relation to the ionic state (Chernavina, 1970; Georgievski et al., 1982). Chelates benefits consist of greater physical stability, which reduces the separation of trace elements and vitamins in feed oxidation and increases there adoption. Transition of metal complexes are octahedral with the exception of Cu^{++} to form a twisted octahedron and with that achieves maximum stability.

MATERIALS AND METHODS

SIGNIFICANCE AND MINERALS RESORPTION

The content of microelements in feed often does not match animal needs for several reasons:

- Changing the genetic potential of animals in the direction of bigger and more meat animal productivity
- Reduced levels of microelements in feed.
- Nutrition knowledge on the importance of microelements in metabolism, reproduction, health maintenance and animal production.

Therefore, in conditions of intensive animal production it is essential to provide addition of minerals to the complete feed mixtures. The importance of microelements in addition to animal feed was observed last sixty years. Initially knowledge was limited to the application of inorganic mineral sources, and low utilization of minerals to address the increasing concentration in the diet, which are often resulted in an overdose, or waste of minerals (Manang et al., 2010; Guo et al., 2001). Today, as feed additives is used mineral chelates in a form of proteinates, minerals and amino compounds, or oligopeptides that are linked to better utilization of minerals in animals compared to inorganic sources and higher production. Normative needs of animals in microelements were determined as for most nutrients, according to the conducted experiments. In determining the need for such basic criteria are taken animal age, gender, body weight, intensity of production, type of diet, the possibility of diet consumption (Dibner, 2005; Dibner et al., 2007). But in the world today there are great differences in recommendations for individual nutrients. Review of certain recommendations by the regulations of complete feed mixtures in Serbia is given in Table 1. Recommended amounts of microelements differ according to literature sources. Deviations occur as a result of different amounts of microelements in the soil, and thus in plants.

Table 1. Microelements recommendation for complete feed mixtures, mg/kg

Microelement	Monogastric animals		
	Poultry	Pigs	Horses
Copper	6-8	20	10-19
Manganese	50-80	20-30	27-38
Zinc	30-60	100	38-43
Iron	30-40	100-120	48-62
Cobalt	-	-	0.1
Selenium	0.15	0.1	0.1
Iodine	0.5-0.8	0.5	0.1-0.5

(Ordinance on the quality and other requirements for feed, 2000).

In addition to provide minerals to animals, it is necessary to implement appropriate production technology of mineral premix that is added in the diet (Adamovic et al., 1997). This is particularly important, because of insufficient amounts of microelements, in the long run cause the deficit, and thus a negative impact on the reproductive potential animal health. Then, large amounts of certain microelements in relation to the optimal needs may have a variety of negative consequences, and ultimately fatal toxic end. This is especially related to heavy metals (lead, cadmium, and

mercury). The problem to determine the optimal concentration of microelement is their interactions with each other due to an increase in one of microelements which may cause deficit of another. Natural fertilizers are the best way to supply microelements to the animals. They are in plants in organic form, which in comparison to other sources has the highest utilization. Important microelement content in the feedstuffs is shown in Table 2.

Table 2. Concentration of microelements in feedstuffs, mg/kg

Nutrient	Copper	Manganese	Zinc	Iron	Cobalt	Selenium
Corn, grain	2.2	7.2	12.4	43.0	0.8	0.02
Wheat, grain	11.0	32.0	31.0	50.0	4.3	0.06
Barley, grain	6.0	20.0	30.0	66.0	5.4	0.35
Soybean, grain	78.0	15.0	29.0	220.0	16.0	0.13
Soybean meal	14.6	35.3	31.0	290.0	1.4	0.13
Sunflower meal	27.6	39.0	61.0	289.2	0.8	0.08
Rapeseed meal	25.0	68.0	59.0	240.0	1.2	1.0
Alfalfa hay	8.2	31.5	21.5	420.0	1.4	0.5

Adamovic et al., 1997.

Far more important is the target mineral proteinates impact on specific functions of enzyme systems in the body, because they are absorbed intact. The absorption of minerals depends on the fate of the amino acid or peptide that is bound. Since different tissues and enzyme systems have different requirements for amino acids, mineral binding to specific amino acid or peptide with synthetic chelates increases the chance that adequate micronutrient be shipped to a specific tissue or enzyme system (Table 3), according to Du et al. (1996), Pupavac et al. (1999), and Stanacev et al. (1999).

Table 3. Target tissue of some copper chelates

Microelement	Amino acid	Tissue
Copper	Tryptophan	Muscle
	Lysine	Bone
	Histidine	Liver

SYNTHETIC CHELATES

Findings have contributed to microelements in the form of inorganic compounds bean gradually suppressed from the animal diet and bean replaced with chelates, which are already on the market or as a special type of EDTA chelates, which are added to diet. The efficiency depends on the chelate stability constants (Kratzer et al., 1959; Georgievski et al., 1982; Brown and Zeringue, 1994). If the stability constant of the complex is higher than the constant of the complex formed between microelements and diet components and lower, than the constants of complexes in animal tissues chelate will be effective. This area is well known in the animal feed industry for almost 40 years, and the products obtained during this period were distinguished by their characteristics. The original chelates had very high stability constants were practically useless. Newer versions are copies of natural compounds chelate that occur in the body, and their synthesis is used as a ligand proteins, peptides and amino acids. Synthetic chelates must be compatible with the body how they could be absorbed, and their stability is maintained at a certain pH level (Reddy et al., 1992; Brown and Zeringue, 1994; Huang et al., 2009; Manang et al., 2010). The research, which aimed to explore the possibility of including significantly lower levels of minerals, the introduction of organically bound forms in broilers diet had no any significant differences, indicating that much lower levels of minerals in organic form have positive impact on production performance of broiler chickens (Table 4). Today, the most commonly used chelates are Fe, Zn, Cu, Mn and Co, and the experimental results confirm the hypothesis that the chelates in the diet increases the absorption for adoption of microelements, which reflects the increase in growth, reproductive efficiency of animals and meat quality.

In addition to chicken nutrition, organic minerals found application in the diet of laying hens and parent flocks with significant improvement of production results (Table 5 and 6).

Table 4. Influence of organic and inorganic minerals on broiler chickens performance of 42 day age

Mineral form	Body weight, kg	Feed conversion
Inorganic (Cu+Fe+Zn+Mn+Se), 75.3 ppm	2.053	1.89
Organic (Cu+Fe+Zn+Mn+Se), 25.1 ppm	2.068	1.90

Alltech Yu d.o.o (2008)

Table 5. Influence of microelements on egg shell quality and layers persistence

Production parameters	Control group	Microelements*
Egg weight, g	62.25	62.75
Shell weight, g	5.71	5.90
Shell thickness, mm	0.361	0.366
Cracked eggs,%	3.56	2.98
Persistence,%	80.13	82.10
Egg firmness, N	29.45	29.99

Alltech Yu d.o.o (2008), *-Bioplex (Cu, Mn, Zn)

Table 6. Bioplex influence on productive performance of heavy hybrid broilers parents line

Production parameters	Control group	Bioplex
Total egg production/hen	109.05	110.96
Egg for incubation/hen	98.33	101.27
Consumption (kg/hen)	24.76	25.10
Egg fertility,%	86.80	87.39
Chickens/hen	85.35	88.50

Alltech Yu d.o.o (2007)

Based on these results it can be concluded that the use of organic minerals improves the performance have a significant economic impact throughout the growing season.

RESULTS AND DISCUSSIONS

EFFECT OF COPPER, IRON AND SELENIUM CHELATES IN PIGLETS AND FATTENIG SWINE PRODUCTION

The efficiency of the chelate is manifested in several ways, primarily through better absorption, higher mineral levels in serum, increased retention of minerals and higher levels of the enzyme. The use of these compounds in animal nutrition increasing production, improving product quality and reduce production costs (Stanacev et al., 1999). Coffey et al. (1994) performed a series of experiments with piglets for a period of 28-35 days in order to determine the efficiency of Cu-lysine compared to $CuSO_4$. In all experiments it was noted the improvement of physical indicators of production. Feed consumption increased by 4%, daily gain by 4.2% and feed conversion ratio for 10.6%. That same year, Zhou et al. (1994) compared the efficacy of copper from $CuSO_4$ and Cu-lysine in weaned piglets under conditions of *ad libitum* feeding. Piglets fed with a mixture of Cu-lysine consumed more feed by 29% and achieved a higher daily weight gain by 19%. The same trend was found in fattening pig nutrition. Stimulatory effect of 200 ppm Cu from different sources ($CuSO_4$ and Cu-lysine) was studied by Apgar and Kornegay (1996). The results of these studies show positive effects of Cu-lysine. Daily weight gain with the same feed intake was higher for 14.3%. Research Close (1998) show a better effect on piglets production in the period of 10-30 kg of body weight (Table 7). Piglets on treatment with 160 ppm Cu, 60 ppm which comes from $CuSO_4$ and Bioplex 100 ppm of Cu showed a 5.5% increase in feed consumption, improved daily gain by 10.8% and the efficient use of feed for 5.1% compared to the control group with copper sulphate.

Table 7. Effect of copper source in piglets and fattening swine production

Author	Animal category	Cu source	Weight gain*	Feed conversion*	Feed consumption*
Coffey et al., 1994.	Piglets	Cu-lysine	104.2	99.4	104.0
Zhov et al., 1994.	Piglets	Cu-lysine	119.0	-	129.0
Apgar and Kornegay, 1996.	Fattening swine	Cu-lysine	114.3	-	100.0
Close, 1998.	Piglets	$CuSO_4$ 60ppm Bioplex 100 ppm	110.8	94.8	105.5

-compared to $CuSO_4$

Addition of iron chelate forms in the form of Bioplex Fe in the diet of sows, 30 days before partus, leads to a significant increase in the concentration of iron in the blood serum and colostrum. In newborn piglets, there is no significant increase in the number of red blood cells and hemoglobin, because there was a significant increase in the concentration of iron in the blood serum and liver (Table 8).

With the increasing number of sows in gestation, there is an increasing loss of selenium, and it was found that the

concentration of selenium in sows at the end of the third gestation is decreased by 10-20% compared with the approximate age of the animals, which are not farrowed, if they are fed with mixture of standard quality.

Table 8. Influence of chelated bonded iron (Bioplex Fe) in gravid sows in piglets anemia prevention

	Control	Bioplex Fe
Sows on a partus day		
Erythrocytes number x 10^{12}	5.99	5.65
Hemoglobin concentration, g/l	114.3	111.7
Fe concentration in blood serum, μmol/l	53.3	61.1
Fe concentration in colostrum, μmol/l	1.17	1.44
Piglets on a partus day		
Erythrocytes number x 10^{12}	3.97	3.97
Hemoglobin concentration, g/l	81.2	81.4
Fe concentration in blood serum, μmol/l	44.3	48.4
Fe concentration in colostrum, μmol/l	315.4	445.1

Kessler et al. (2001).

The biggest mobilization occurs during lactation when the metabolic needs are largest. Selenium plays an important role in the transfer of immunity in piglets, and therefore the maintenance of general health. From the research results of Janyk (1998), shown in Table 9, it can be seen that the piglets from sows fed with Sel-Plex had higher body weight and daily gain during the lactation period in contrast to the pigs on diet treatment with inorganic selenium.

Table 9. Influence of organic and inorganic selenium form on piglets performance

	Control	Inorganic Se (Na-Selenite)	Organic Se (Sel-Plex)
Born piglets	11.7	12.0	11.6
Live-born piglets	9.90	10.28	9.8
Mortality after weaning,%	15.49	13.08	10.54
Weaned piglets	8.37	8.94	8.77
Body weight after birth, kg	1.48	1.43	1.53
Daly bod weight gain, g	212.0	213.5	221.4

EFFECT OF ANTAGONISM BETWEEN MOLYBDENUM, SULFUR AND COPPER ON BROILER CHICKENS PERFORMANCE

Biogenic elements are chemically very active substance, so that their absorption and metabolism depend largely on the interactions that take place in the feed, the digestive tract, cells and tissues (Stanacev and Kovcin, 1998). Just a few years after establishment of stimulatory effect caused by high doses of copper, Pflander and Ellis (1960) finds that the molybdenum showed the same effect. The stimulatory effect of this element is attributed to microflora, since the component bacterial dehydrogenase molybdenum. Together copper and molybdenum may act in two ways, depending on their concentration, and the presence of mutual relations in the form of inorganic sulfate sulfur (Stanacev et al., 2001). Since these are two essential nutrients, very active chemically, absorption and metabolism of them depend heavily on the interactions that take place in the feed, the digestive tract, cells and tissues. Antagonistic elements have mutual interrelationships according to many researchers. Some of the interactions occurring at digestive tract, while the others run into the metabolism. With rough violation of element nutritional balance it can be expected heavy toll on health, due to the lack of enzymes which of they are activators (xanthine oxidase and ceruloplasmine). This violation occurs only when one of its elements exceeds the minimum toxic level of tolerance. Toxic surplus molybdenum deficiency causes secondary copper or copper-enzymes, and the consequences are anemia and depression of growth. These changes can be restored to normal physiological state supplementation with inorganic sulfur in the sulfate form, apart from increasing the copper content in the liver. To the level of copper in the liver were held constant, molybdenum and sulfur must be continually introduced into the body. In doing so, it builds insoluble molybdenum sulphide that is not absorbed and does not prevent the absorption of copper, and it is excreted from the body. Stanacev et al. (2001 and 2001a) in their investigation aimed to establish the effect of antagonism between copper, molybdenum and sulfur from sulfate on the production

parameters of broiler chickens and copper accumulation in the liver (Table 10). The introduction of molybdenum and sulfur in diet with high levels of copper, 100 and 200 ppm had a positive effect on the growth intensity of broilers. Adding 50 ppm molybdenum and 50 ppm of sulfur in the mixture with 100 ppm of copper, at the end of the trial period, increased the body weight of chickens for 8.35% compared to the control group, while in the second fattening period, the group with addition of 100 ppm copper was most effective. From the results of Stanacev et al. (2001 and 2001a), it can be concluded that the microelements copper, molybdenum and sulfur from sulphate exhibit antagonism in the digestive tract of broilers, which is manifested by interference of copper absorption and synthesis of heavy soluble coppermolybdate, which reflects the accumulation of copper in the liver (Table 10).

Table 10. Copper concentration in chicken liver, ppm

Group	**T1**	**T2**	**T3**	**T4**	**T5**	**T6**
Cu	**0**	**100**	**100**	**100**	**200**	**200**
Mo	**0**	**0**	**50**	**50**	**0**	**50**
S	**0**	**0**	**0**	**50**	**0**	**50**
Initial day	52.51	52.51	52.51	52.51	52.51	52.51
6. day	37.03	44.15	24.10	26.05	69.77	39.93
13. day	40.63	54.75	30.35	32.75	83.90	51.30
20. day	34.88	41.68	16.55	17.73	59.60	26.30
49. day	22.48	28.60	15.10	16.20	30.75	24.08

Looking at the results shown in Table 10 is observed much lower concentration of copper in the liver in the presence of molybdenum, with and without the presence of sulfur, compared to groups that contained only copper in diet. This difference was statistically significant ($P< 0.01$).

CONCLUSIONS

Based on the presented data it can be concluded that the use of chelate mineral forms in pig and poultry is recent trend with increasing tendency due to the prohibition of the usage of antibiotics. Ban on antibiotics usage in animal nutrition led to the finding of different nutritional additives that can improve performance, animal health and meat quality intended for human consumption. Findings on the use of minerals chelated forms in animal nutrition is still limited, given the wide range of their activities, there is a need for further research in this area. Decision should be made whether to use more efficient, but more expensive mineral chelate forms taking into account the increasingly stringent demands of ecology and environmental protection.

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THE EFFECT OF FEEDING INTERVALS ON THE LEVEL OF GLUCOSE, TRIGLYCERIDE, PERCENTAGEOF ABDOMINAL FAT AND CARCASS QUALITY ON BROILER CHICKENS

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Abstract

Broiler chicken has advantages in growth rate and conversion ration but these advantages are followed by increase of body fat level. This study was aimed to reduce the increase of body fat level by giving feeding interval treatment on 120 broiler chickens aged 8 days for 28 days. The study was conducted with randomized block design method. The treatment consisted of three feeding intervals (every 6, 8 and 12 hour for a day) and 2 groups of chickens which were maintained separated by sex. Commercial ration that was given contained 23% of protein and 3000 calories of metabolic energies. Observed variables are glucose, triglycerides, ration consumption, conversion ration and abdominal fat content. The result showed that the interval feeding treatment had no significant effect to glucose levels, triglycerides, ration consumption, conversion ratio and abdominal fat but the lowest percentage of carcass quality was achieved by feeding female with 12 hour interval and male 8-hour interval. In conclusion, feeding with 6, 8 and 12 hour interval did not give significant meaning physiologically while the highest percentage of carcass quality is produced in 6 and 8 hour interval for female and 6 and 12 hour interval for male.

Key words: *carcass quality, feeding interval, glucose, triglyceride.*

INTRODUCTION

Broiler chicken has a fast growth rate and good conversion ration (FCR) but along with aging process, these advantages are also followed by increase of body weight. Biologically, the male broiler chicken has greater growth rate and conversion ration (FCR) than the female, meanwhile the female has greater increase of body fat than the male (Amrullah, 2002). In Indonesia, broiler chicken is already harvested in 28-35 days old. Six week old chicken broiler that rearing in tropical area has 2.85 % of live weight (Yuniza, 2002).

One of reasons for the high level fat is the amount of ration consumption especially the energy that got in to chicken body. This energy exceeds the amount that needed for living and production so the exceeded energy will be stored as fat (Bun, 2010). The exceeded energy in broiler chicken will produce chicken carcass with high fat, meanwhile the low consumption of ration will affect the carbohydrate that stored in low glycogen form, (Tilman, et al., 1986). Not effective energy usage in body will be stored in adipose tissue (Junqueira, et al., 1986). Chicken's fat usually can be found in form of tissue in abdominal cavity, subcutaneous and abdominal fat (Klasing, 2000). Abdominal fat tissue is one of body parts that have highest potency of fat than any other parts. High level of abdominal fat will decrease the carcass percentage

Meanwhile the condition which is obtained from field shows that high level of fat on broiler chicken tends to decrease consumer's preference who wants good quality and low fat chicken carcass. One of solutions that can be used to solve that problem is by managing the feeding interval. Ration that been given in appropriate amount according to the ability of intestine to digest and absorb nutrient and also satisfy the needs for living and production will avoid excessive fat synthesis. This research's objective is to find the optimal feeding interval management in order to restrain excessive fat synthesis on chicken for a good quality chicken carcass.

MATERIALS AND METHODS

The research was done by using Block Randomize Design which the chicken sex was defined as the block. There were three treatment of

feeding intervals consist of 12, 8 and 6 hours of feeding interval. Each treatment was repeated four times. The research used 120 broiler chickens with Cobb 500 strain. Each of the chicken is 160 gram weight. Half of the chicken (60 chickens) is male while the other half is female. Ration that had been used was commercial ration with nutrient content showed in Table 1.

Table 1. Composition nutrient in ration

Nutrient	Ration (*)	Needs on Broiler Chicken (**)
Water (%)	8.66	
Crude Protein (%)	23	Min 18
Metabolic Energy (kkl/kg)	3000	Min 2900
Crude Fat (%)	6.79	Max 8
Crude Fiber (%)	6.49	Max 6
Ash (%)	4.95	Max 8
Calsium (%)	0.77	0.9 - 1.2
Phosphor (%)	0.39	0.6 - 1.0

Source : (*) Comercial Ration Label
(**) SNI 01-3931-2006 (Standard National of Indonesia)

Data were collected from 20% of samples. Recorded parameters are glucose level, triglycerides in blood, ration consumption, body weight, carcass quality and abdominal fat. The glucose level and triglycerides were measured with Trinder-GPO Enzymatic method. Body weight was measured at the end of the research while the ration consumption was measured cumulatively during the research. Abdominal fat was fat that was obtained from abdominal cavity and between internal organs. Evaluation of carcass quality was done by FDA USA standard (1998).

1. Determining Glucose Level on Plasma

Glucose level on blood can be determined by Glucose Oxidase-Phenol Amino Phenozone (GOD-PAP) method by Schmid (1971).

$$\text{Glucose Level (mg/dl)} = \frac{\text{Abs.Test}}{Abs.Std} \text{ x standard level}$$

2. Triglycerides Level on Blood

Triglycerides level on blood can be measured by GOP (Calorimetric Enzymatic Test using Glycerol-3-Phosphateoxidase). The analysis was done by using spectrophotometer.

$$\text{Triglycerides Level on Blood} = \frac{\text{Abs.Test}}{Abs.Std} \text{ x standard level}$$

RESULTS AND DISCUSSIONS

Glucose Level

Glucose is an important carbohydrate that is absorbed in large quantities and is converted in the liver (Mayes, 1999). Glucose plays an important role in the body as precursor energy, especially in the process of glycogen synthesis, fatty acid synthesis, amino acids, vitamin C and some metabolite (Klasing, 2000).

Table 2. Average Of Glucose, Triglyceride, Abdominal Fat and Ration consumption

Parameters	Male			Female		
	12 Hours	8 Hours	6 Hours	12 Hours	8 Hours	6 Hours
Glucose (mg/dL)	266.25 ± 10.21	251.27 ± 15.06	242.25 ± 24.28	232.00 ± 16.06	255.50 ± 12.89	242.25 ± 24.48
Triglycerides (mg/dL)	76.33 ± 28.43	85.67 ± 43.73	118.33 ± 14.19	184.67 ± 47.00	127.00 ± 46.94	118.33 ± 14.19
Abdominal Fat (%)	1.49	1.55	1.32	2.24	2.18	2.3
Ration Consumption	2501.5	2497.8	2502.7	2498.15	2508.65	2493.1

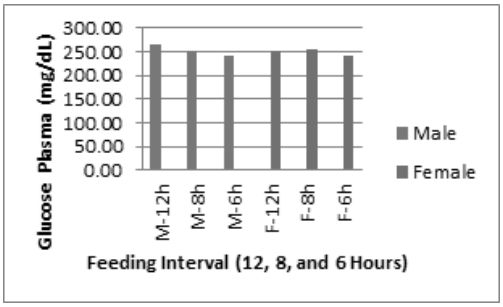

Figure 1. Average of glucose levels on Feeding interval chickens got 12.8 and 6 hours

The result of experiment can be seen in Figure 1. Male s have a range of averaging the lowest glucose levels 242.25 ± 24.28 mg / dL and the highest 266.25 ± 10.21 mg / dL, while the hen, the lowest value of 242.25 ± 24.28 mg / dL. and the highest 255.50 ± 13.89. Mean blood glucose levels in chickens tan jan 5-6% above the normal average, whereas in the range female showed a relatively normal glucose levels, which is 180-250 mg / 100 ml of blood (Hazelwood, 1986). Chickens have glucose levels that are much higher compared to mammals (Klasing, 2000).

The Chicken got feeding intervals 12, 8 and 6 hours showed a statistically not significant different (P> 0.05). Properties associated with the consumption of chicken rations, chickens will stop eating once their energy needs met (Wahyu, 1997). In this observation chickens fed with intervals and the amount of certain foods so that the chicken was forced to stop eating even though energy has not been fulfilled. Chicken with an interval of 12 hours would consume more numerous than interval 8

and 6 hours, but the difference in the amount of the consumption ration at each feeding interval had no impact on glucose levels.

Ration energy sources comes mostly from carbohydrates, which in turn will result in simpler molecules digest (glucose). High and low amount of consumption will be correlated with glucose levels, but in fact feeding interval had no impact on glucose levels. It is suspected that the chickens have a mechanism in regulating glucose homeostasis related to eating habits.In the chickens,more than a third of glucose or 37% absorb during a meal is converted to lactic in the wall intestine (Klasing, 2000, Riesenfeld et al, 1982, Reisenfeld 1985, Sturkie, 2000) before transfer into the blood circulation. Glucose will be cleared from the circulation (diclearence) by the difference between the concentration gradient in the cells of the gut and circulation.

At mealtimes lasted more than one third of the glucose or 37% or is converted to lactic acid through anaerobic gut wall epithelium (Klasing, 2000, Riesenfeld et al, 1982, Reisenfeld 1985, Sturkie, 1986) before transfer into the circulation. Glucose will be cleared from the circulation (diclearence) by the difference between the concentration gradient in the cells of the gut and circulation. Therefore happened entry into the mucosal cells. This process may explain the low level of glucose relative to mucosal glucose uptake, in addition to the conversion of glucose flux into the intestine and intestinal lactate flux sole reliance on glucose uptake of glucose turnover (Riesenfeld et al, 1982).

These circumstances reinforce a statement by Klasing (2000), although the amount of the gift rations, feeding frequency or different dietary levels but still maintained a certain extent. Production of lactate produced will be carried through the circulation to the heart and serve as the primary precursor for syntesis glucose, glycogen and fatty acids.

Triglyceride levels

Triglycerides are esterglicerol with three fatty acids that can be found in the blood circulation and an energy savings that will be used when the body lacks energy. Triglycerides come from rations and de novo process results in the body (Poedjiadi, 1994).

In Table 3 served average triglyceride levels in male s ranged from 76.33 ± 28.43-118.33 ± 14.19 mg / dL while the female have a higher average is 118.33 ± 14:19 ± 47.00-184.67 mg / dL.

Table 3. Percentage of carcass quality, meat and breast conformation in broiler chickens fed different feeding intervals

Sex	Feeding Interval (Hours)	Carcass Quality (%)		Meat and Fat Weight (gr)	Meat and Fat Percentage From Carcass (%)	Abdominal conformation (cm)	
		Grade A	Grade B			Length	Width
Male	12	100	-	864.5	70.88	17	16
	8	50	50	799.75	72.23	16	15.8
	6	100	-	864.5	70.88	17	16.25
Female	12	75	25	799.75	74.26	14.5	15.25
	8	100	-	868.75	74.26	14.33	15.33
	6	100	-	864.5	74.53	15.75	16.25

Average levels of triglyceride in male with 12 hour feeding intervals tend to be lower compared with other treatments, while the female actually feeding intervals 12 hours showed the highest triglyceride levels compared with the other two treatments. Figure 2 clarify average triglyceride levels of each treatment. On the male looks trend triglyceride levels increased with decreasing feeding interval, while in females decreased triglyceride levels in line with the decline in feeding interval

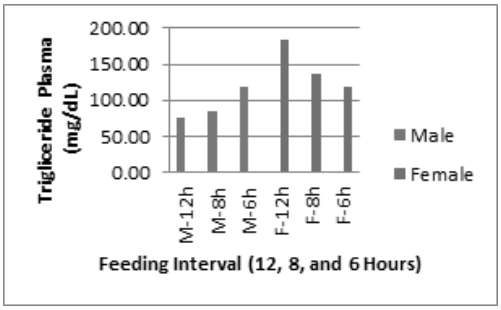

Figure 2. Average of triglyceride plasma in male and female chickens

The results of statistical analysis showed that the feeding interval, triglyceride levels did not differ significantly (P> 0.05) in chickens male or female.

The difference in feeding interval 12, 8 and 6 hours, or intervals of feeding analogy as fast, so it will change the pattern of feed intake.

In the fasted state, the metabolic processes to keep running. Broiler chicken has a high level of growth, therefore requires some energy to

supply metabolic processes. In this case, the converted savings energy due to decreased glucose availability, the liver glycogen is converted into glucose through glycogenolysis to maintain blood glucose levels and in turn through gluconeogenesis.

In order to homeostasis, decreased glucose concentration between the two feeding times will result in a decrease in circulating of insulin that will increase the glucagon hormone. With reduced use of glucose in adipose tissue and decreasing the inhibitory effects of insulin on lipolysis, fat (triglycerides) will be mobilized as free fatty acids and glycerol (Mayes, 1999), which would then be converted into energy the glucose precursors.

The dynamics of change and the synthesis of triglycerides in male and female chickens are experiencing feeding interval 12.8 and 6 hours is quite high. Shortly after feeding, a lipid that is consumed will hydrolysed into triglycerides and also some that do not oxidize glucose to be converted into triglycerides.

Therefore, there was high levels of triglyceride in the blood cirulation.It will be increased far beyond normal levels of triglyceride. So also when the two feeding time, a number of triglyceride hydrolyzed when reserves of glycogen are not sufficient.

Abdominal Fat Percentage

Average percentage of abdominal fat are presented in Table 3 male s have average from 1.32 to 1.55%, while the hen has a range of 2.18 to 3.24%. Abdominal fat percentage range of observations is lower than the proposed Summer and Lessons (2005). Abdominal Fat Percentage male and female chickens ranged respectively 3.2-2.6; 3.2-3.4%. Therefore in this case, feeding interval tends to lower of percentage of abdominal fat. The results of the statistical analysis showed feeding intervals giving 12.8 and 6 hours on a male or female chickens had no effect on levels of abdominal fat ($P > 0.05$). Fat is the body's energy savings, which are synthesized from an excess of energy that is not oxidized, especially in granivora poultry such as chickens, using carbohydrates in rations, especially glucose to fatty acid synthesis.

In this observation, the amount of abdominal fat percentage is a reflection of the deposit triglycerides in adipose tissue, particularly in the abdominal cavity. Feeding and 6-hour interval 12.8 shows the percentage of abdominal fat were not different. One of the alleged, was not different abdominal fat, it is closely related to the amount of feed intake. In the Table 3 the Average consumption ration spent during the observations ranged from 2497.8-2502.7 and 2493.1 g in males-2503.65gr for a hen. Nutritional and metabolic energy of the ration is the same, containing 23% protein and EM 3000 kkcal / kg. Rations were given according to the needs of broiler chickens by SNI 01-3930-2006, (2006). Another factor that also has a strong contribution is gender. Male s. have a high metabolic activity so that the lower energy savings, while the female have a tendency mendesposisikan fat due to low activity (Sturkie, 1986).

Carcass Quality

Carcass quality assessed based on the USDA (1998) standard criteria include fatty, meat and body conformation. Display broiler carcass quality of each treatment are presented in Table 3. In this the observation, grade A male obtained from treatment of feeding intervals of 12 and 6 hours, respectively at 100%, while the 8 hour feeding intervals resulted in 50% grade A and 50% grade B. Treated female feeding intervals 8 and 6 hours produced 100% grade A, while the 12-hour interval feeding treatment produces 75% grade A and 25% grade B.

Judging from meat and fatty, apparently male-and female including good quality or grade A.

The low quality of the carcass at 8 hour intervals feeding in males and 12 hour in females allegedly instead of meat and fat considering the average percentage meat above 70%, in accordance with the opinion Murtidjo (1987) chicken carcasses ranged from 65-75% of the live weight. Weight of meat between 50-70% of the carcass weight or approximately 40% of the live weight.

Body conformation assessment covering the sternum, back and both legs and wings (USDA, 1998), the observations showed that all samples belong to the quality of A, while the observations are focused on assessing conformation by comparing the length and width of the chest (Table 3).

Male and female chickens fed different feeding intervals (12.8 and 6 hours) showed a compact conformation. Sternum has a normal conformation, the curved one-eighth inch (Afifah, 2009). Alleged loss of quality or Grade B, male chicken with feeding intervals of 8 hours and females at feeding intervals of 12 hours, apparently not because meat and fatty or conformation of the body (chest), but from the look. Carcass looked more pale, the pale colors of carcass subcutaneous fatty allegedly uneven, or low levels of fat under the skin, subcutaneous fatty function in carotenoids absorption, especially xanthophylls (North, 1978, and Maynard et al 1979).

CONCLUSIONS

Feeding with 6, 8 and 12 hour interval did not give significant meaning physiologically while the highest percentage of carcass quality is produced in 6 and 8 hour interval for female and 6 and 12 hour interval for male.

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NUTRITIVE EVALUATION OF AMMONIATED BENGGALA GRASS AND FERMENTED SAGO WASTE

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Abstract

Ruminant feed processing technology is really needed in an attempt to increase the quality of Benggala (Panicum maximum) grass and other waste products. One of new local feed resources in North Sulawesi categorized as waste product is Sago waste which is abundantly available and has a big potential in providing animal feedstuffs. The present study was designed to evaluate the utilization of urea and optimal incubation time to produce the best nutritive quality when added to ammoniated Benggala grass. A Pleurotus ostreatus fungus was used to determine the optimal incubation time to produce the best quality of Sago waste. Research results showed that the best quality of ammoniated Benggala grass was seen at the addition of 6% urea with an incubation time of 21 days, as indicated by the increasing of crude protein, cellulose, and hemicelluloses. The best nutrient quality of Sago waste fermented with Pleurotus ostreatus was shown at incubation time of 30 days, as indicated by significantly (P< 0.01) increasing in protein content and the decreasing of lignin of Sago waste. Nutritive value evaluation of ammoniated Benggala grass and fermented Sago waste as fed showed a significant (P< 0.01) increase in dry matter and organic matter digestibility in vitro. It can be concluded that nutritive value of determined ammoniated Benggala grass and fermented Sago was increased as well asdry matter and organic matter digestibility in vitro.

Key words: *feed evaluation, benggala grass, sago waste, ammoniation, fermentation, in vitro digestibility.*

INTRODUCTION

Ruminant animals share the biggest meat supply for human need. A demand rate of cattle meat is projected to increase as the human population increases. On the other hand, land area for animal feedstuffs cultivation is getting smaller in North Sulawesi, Indonesia region due to uncontrolled human settlement and other infrastructures that occupy agricultural area and its function. One of way outs that can be taken is the utilization of locally available feedstuffs such as Benggala grass and other agricultural waste products such as Sago waste which is abundantly available, sustainable, and has a comparative value added.

The limitation of using both local feedstuffs resources for ruminant animals is their high lignin and low protein content. Weiss and Underwood (2002) stated that ammoniation of grass or straw can increase crude protein content about 6−8%, and increase in vitro organic matter digestibility. Ammoniation treatment increased reducing sugar content of rice straw (Kardaja, et al., 2006). Tuomela (2002) pointed out that *Pleurotus ostreatus* fungi is the most efficient type of microbe exists in nature that specifically breakdown lignin. The present study was design to evaluate nutritional value of ammoniated Benggala grass using urea and fermented Sago waste using *Pleurotus ostreatus* fungi.

MATERIALS AND METHODS

The present study was conducted to determine nutritive value of Benggala grass (*Panicum maximum*) by way of ammoniating using urea and fermentation of Sago waste using *Pleurotus ostreatus*. The first experiment was aimed to determine nutritive value of ammoniated Benggala grass (ABG) with different urea supplementation level of 0%, 2.0%, 4.0%, and 6.0%; and then incubated for 0, 7, 14, and 21 days. The second experiment was conducted to elaborate the nutritive value

of fermented Sago waste (FSW) using *Pleurotus ostreatus* fungi for 20, 25, and 30 day′s incubation time. Parameters measured in the second experiment were: dry matter (DM), organic matter (OM), and crude protein (CP) content. Nutrient compositions were all analyzed using AOAC (1990) standard methods. Fiber analysis for NDF, ADF, cellulose, hemicelluloses, and lignin was conducted according to Van Soest (1987) procedure. In vitro digestibility was conducted using Tilley and Terry (1963) procedure as modified by Van der Meer (1980). Both experiments using two-ways Anova and Tukey test was employed to analyze treatment differences.

RESULTS AND DISCUSSIONS

Experiment 1.*Nutritive value of Ammoniated Benggala Grass (ABG)*

Nutritive value and in vitro digestibility of Ammoniated Benggala Grass (ABG) with different urea level and incubation time is presented in Table 1. Urea level and incubation time significantly ($P< 0.05$) increased organic matter (OM) content of ABG; while ammoniating time had no significant ($P > 0.05$) effect on organic matter (OM) increase on ABG. There was an interaction effect ($P < 0.05$) between urea level and incubation time of *Pleurotus ostreatus* on dry matter (DM) content of ABG. The highest dry matter content was found in urea level of 5% with incubation time of 21 days. Level of urea used in this experiment gave a significant ($P< 0.01$) different on the increase of ABG organic matter (OM); whereas, incubation time did not give a significant ($P> 0.05$) effect on the increase of organic matter content of ABG. There was an interaction effect ($P< 0.01$) on urea level and incubation time increment. Treatment with an increase in urea level gave a significant ($P< 0.05$) increase on ABG crude protein (CP) content. The higher the urea level, the higher the crude protein content of ABG. Incubation time was also increased ($P< 0.01$) crude protein (CP) content. There was an interaction effect ($P< 0.01$) between urea level and incubation time on crude protein (CP) content and neutral detergent fiber (NDF) of ABG. There was no interaction effect ($P> 0.05$) between urea level and incubation time on acid detergent fiber (ADF) of ABG. Level of urea, incubation time, and interaction of both gave a significant ($P< 0.01$) effect on the decrease of lignin, cellulose, and hemicelluloses content of ABG.

Urea level, incubation time, and interaction of both treatments gave a significant ($P< 0.01$) effect on DM and OM digestibility in vitro of ABG. DM, OM, and CP content of ABG increased as urea level and incubation time increased in the present study. The highest DM, OM, and CP content were found in urea level of 6% and incubation time of 21 days. It is understandable because the higher the level of urea and incubation time, the higher the retention time of nitrogen (N) in ABG. At further stage, ureolytic process can take place then N is changed to NH_3 and CO_2 by urease produced by feed bacteria, and reduced sugar level increases. Kardaya, et al., (2006) reported that ammoniating treatment on rice straw using urea can increase crude protein (CP) content, dry matter and organic matter digestibility in vitro. This report was in accordance with the present study, where the highest DM and OM digestibility was found at urea level of 6% and incubation time of 21 days. The decrease in neutral detergent fiber (NDF) of ABG in this experiment might be due to the structural carbohydrate bond damage. Weiss and Underwood (2002) stated that the decrease in NDF content of foliage when treated with ammonia is due to the damage on lignin and hemicelluloses bond. Acid detergent fiber (ADF) content in this experiment was not affected much by treatment given due to the lignocelluloses bond is being hydrolyzed as was indicated by the decrease in lignin content. Indeed, the increase in cellulose content was observed at urea level of 6% with incubation time of 21 days treatment.

Table 1. Nutrient content and in vitro digestibility of ammoniated Benggala grass (ABG) with different urea level and incubation time

Parameters	Urea Level					Sig
Nutrient content (%)						
Dry matter (DM)	U0	36.34	36.34	36.34	36.34	
	U2	37.11	37.92	37.84	38.92	
	U4	38.52	38.48	38.92	38.91	
	U6	38.48	38.72	38.69	38.96	*
Organic Matter (OM)	U0	25.73	25.73	25.73	25.73	
	U2	26.63	26.87	26.81	26.75	
	U4	27.32	27.26	27.55	27.63	
	U6	28.28	28.38	27.50	28.78	**
Crude fiber (CF)	U0	4.92	4.92	4.92	4.92	
	U2	5.05	5.64	5.75	6.09	
	U4	5.27	6.85	6.91	7.03	
	U6	5.29	8.84	8.95	9.12	**
NDF	U0	77.91	77.90	77.89	77.98	
	U2	77.88	77.89	77.68	77.64	
	U4	77.89	76.45	75.44	75.49	
	U6	77.87	76.70	75.86	75.69	**
ADF	U0	55.79	55.80	55.78	55.77	
	U2	55.38	55.40	55.49	55.52	
	U4	55.44	55.43	55.09	55.47	
	U6	55.41	55.38	54.60	54.06	
LIGNIN	U0	13.52	13.53	13.51	13.52	
	U2	13.46	12.92	12.23	11.98	
	U4	13.48	12.90	11.62	11.46	
	U6	13.42	12.41	10.72	10.59	**
Celluloses	U0	51.70	51.57	51.61	51.59	
	U2	51.80	51.89	52.21	51.87	
	U4	51.68	51.04	52.87	52.97	
	U6	51.73	51.91	53.19	53.92	**
Hemicelluloses (%)	U0	25.41	25.39	25.44	25.43	
	U2	25.70	26.12	26.77	26.64	
	U4	24.98	25.08	25.88	26.94	
	U6	25.75	26.4	26.90	26.88	
In vitro digestibility (%):						
Dry matter (DM)	U0	41.40	41.39	41.43	41.42	
	U2	41.43	43.32	43.53	45.32	
	U4	41.42	47.27	48.10	48.17	
	U6	42.01	47.96	48.18	50.49	**
Organic matter (OM)	U0	39.79	39.83	40.01	39.97	
	U2	39.96	41.92	44.47	45.54	
	U4	40.02	45.34	46.81	47.18	
	U6	40.98	45.85	47.22	49.92	**

**) ($P<0.01$)
*) ($P<0.05$)

Experiment 2.*Nutritive value of fermented Sago waste (FSW)*

Nutrient content and in vitro digestibility of Sago pulp fermented with *Pleurotus ostreatus* fungi at different incubation time is presented in Table 2.

Table 2. Nutritive value and in vitro digestibility of Sago pulp at different incubation time

Parameters	Incubation (Fermentation) Time (day/s)				
	F0	F20	F25	F30	Sign
Nutrient content (%)					
Dry matter	75.10	74.21	74.10	74.09	**
Organic matter	71.98	69.78	68.52	68.53	**
Crude fiber	2.89	3.79	5.14	5.27	**
NDF	56.06	55.07	54.04	53.56	**
ADF	31.54	32.09	32.24	32.59	**
LIGNIN	9.86	6.85	5.53	5.49	**
celluloses	26.52	27.40	28.00	28.11	**
Hemicelluloses	17.92	16.10	15.06	15.11	**
In vitro Digestibility					
Dry matter	54.01	54.25	55.58	57.83	**
Organic matter	53.50	54.68	57.44	59.02	**

**) (P<0.01)
*) (P<0.05)

The results showed that fermentation using *Pleurotus ostreatus* fungi gave a significant (P< 0.01) effect on dry matter (DM), organic matter (OM), crude protein, neutral detergent fiber (NDF), acid detergent fiber (ADF), cellulose, hemicelluloses, lignin, in vitro digestibility of dry matter DM), and organic matter (OM) of Sago pulp. Fermentation using *Pleurotus ostreatus* significantly decreased dry matter (DM), organic matter (OM) content of Sago pulp. Badve et al., (1987) stated that cell wall content of sugar cane pulp (bio) converted by *pleurotus sajor caju* can breakdown hemicelluloses bond, increase fiber component solubility, and decrease cell wall component content from 88.4% to 77.2%. Besides, in fermentation processes, microbes consume carbon substances from their growth medium, so that organic matter content decreased. The increase in crude protein content is understandable because of increase in single cell protein biomass. The decrease in neutral detergent fiber (NDF) content of Sago pulp indicated that there is a breakdown of cell wall by *Pleurotus ostreatus* fungi, especially the decrease in lignin when fermentation time prolonged. In their growth, these fungi utilize hemicelluloses, so that hemicelluloses content decreases as fermentation process takes place which is then followed by fungi mycelium growth. Hydrolysis of lignocelluloses by this fungus is indeed increased Sago pulp cellulose content. In vitro digestibility of dry matter (DM) and organic matter (OM) of Sago pulp increased as fermentation time increased. Substrates that undergone fermentation becomes more digestible due to catabolic and anabolic nature of these Pleurotus fungi that enable it to breakdown complex component become more digestible.

CONCLUSIONS

It can be concluded that nutritive value of Benggala (*Panicum maximum*) grass is improved by fermentation using 6% urea for 21 days. The best nutritive value of Sago pulp is reached at fermentation time of 30 days using *Pleurotus ostreatus* fungi.

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A STUDY ON BABIRUSA (BABYROUSA BABYRUSSA CELEBENSIS) IN TROPICAL FOREST OF NORTHERN PART OF SULAWESI

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Abstract

Babirusa (Babyrousa babyrussa celebensis) is one of the endemic biodiversity of Sulawesi which is currently being very worrying population. The present study was designed to reveal habitat conditions, morphology and anatomy as well as complementary biological apparatus, feed resources, nutrition, as well as reproduction. Study was conducted in tropical rain forest of northern part of Sulawesi, where babirusa still extant. In general, species composition and community structure observed in the location illustrated that there was no particular plant species which is more dominant than others at each plant level. Babirusa is a non-ruminant foregut-fermenting frugivore (concentrate selector). Sexual maturity of either male or female babirusa is 5-10 months, with litter size 1-2 piglets. Birth weight of babirusa piglet is about 0.75 kg, suckling period of piglet is 1 month with mortality rate is 0,8%, and reproductive period after productive age is 7 years. Pangi fruit (Pangium edule) is a dominant feedstuff consumed by babirusa. It can be concluded that reproduction rate of babirusa is very low. Babirusa (Babyrousa babyrussa celebensis) habitat being a primary forest with a characteristic of Pangi fruit (Pangium edule) dominated the vegetation community in babirusa habitat.

Key words: *Babirusa (Babyrousa babyrussa celebensis), morphology, reproductive, digestive anatomy, feed.*

INTRODUCTION

Babirusa *(Babyrousa babyrussa celebensis)* is one of the endemic biodiversity of Sulawesi which is currently being very worrying population. Today, the animal is in the category of endangered species and is feared to become extinct due to habitat destruction, poaching, predators, and diseases. These animals are considered Vulnerable (VU A2cd) in the IUCN Red Data Book (IUCN, 2008). Although babirusa can be domesticated, but it is still not known whether these animals can be developed and handled in a large group. A constraint that must be addressed is the lack of scientific information on the net of babirusa life (biological). Scientific information for these constraints will be helpful in supporting conservation of babirusa in the native habitat.

The present study was designed to unveil babirusa habitat conditions, morphology, and anatomy, as well as complementary biological apparatus, feed resources and nutrition, feeding behavior including reproduction and breeding that support babirusa adaptive life as one trophic level in the food chain. Thus, this research can be used as the data base for wildlife conservation programs of babirusa, in relation to the welfare and safety of animals (animal welfare), either through management forests and wildlife in their natural habitat (*in situ*), or in particular habitat (artificial) for the nature conservation purposes.

MATERIALS AND METHODS

The present research was conducted in the tropical rainforest of North Sulawesi, where babirusa (*Babyrousa babyrussa celebensis*) still extant, among others Tangkoko Nature Reserve of Bitung North Sulawesi, Togid Forest of Bolaang Mongondow Regency, and Wildlife Nantu Gorontalo. The variables of observation were the general condition of babirusa habitat, identifying the source and type of feed and nutrient substances, morphology, physical character, digestive tracts, feeding behavior, and reproduction of babirusa. Nantu Wildlife Gorontalo was taken as a special location for observation of babirusa activity and behavior in

their habitat due to the babirusa population in this habitat still more (much common) compared with other forest locations. The diversity of vegetation and the data of fauna were collected from each location.

RESULTS AND DISCUSSIONS

General Conditions of Babirusa Habitat

Nantu Wildlife Reserve with an area of 31.215 ha designated for the protection of babirusa (*Babyrousa babyrussa celebensis*), anoa (*Bubalus depresicornis*), and monkey (*Macaca heckii*), also found some species of birds, including the hornbill bird (*Rhyticerus casidix*), small hornbill (*Penelopides exarhatus*) and Sulawesi parrots (*Prioniturus, sp*).

On the forest floor can be found partridges (*Gallus gallus*), birds Hirst (*Megapodius cumingii*) and some reptiles like python (*Python reticulata*) and monitor lizard or (*Varanus salvator*), and many other wildlife species.

In this area also found the Adudu forest with an area of approximately 800 ha with natural salt water as a gathering place for many kinds of wildlife. Saline water sources of Adudu as was observed being the biggest source of salt water that exist in the region, which consists of the muddy lagoon of about 20-30 cm deep, the rocky and sandy sections.

The mineral content of the saline water might be expected to fulfill mineral requirement for babirusa.

Vegetation Condition

Feed potential of vegetation composition was found in the original of babirusa habitat, as indicated by relative density, relative frequency, relative dominance, and of importance value index (IVI) data (Soerianegera and Indrawan, 1988). Two transect lines (A and B) were made for the data analysis. In each line was placed 7 plots consisting of the 20 x 20 m sample squares to analysis tree level plants, the 10 x 10 m sample squares for the analysis of pole level plants, the 5 x 5 m sample squares for the analysis of sampling *(young plants)* level plants and the 2 x 2 m for the analysis of seedling plants.

The types of plant communities in the flora constituent around observed area were approximately 66 species, which 6 of them were strongly considered as feed sources of babirusa, namely pangi (*Pangium edule*), rao (*Draconlo-mrlon dao*), leu (*Dracontomelonmangiferum*), lamuta / Namo-namo (*Genetum sp*), palm sugar trees (seho=local name) (*Arenga, sp*), and palango grass *(E. indica*). The other dominant plant species were *Dracontomelon mangiferum* (leu=local name), *Artocarpus elasticus* (tohupo=local name), *Eucaliptus degllupta* (wood, UK), *Cudrania, sp* (forest *langsat*=local name), *Maelotus floribunda, Litsea, sp.* (dongi=local name), *Caryota mitis* (seho yaki=local name) and *Livistonia rotundifolia* (woka=local name).

The results of the abundance analysis of the community composer species to the tree level were identified about 35 plants species. Based on the calculated importance value index (IVI) there were 12 species of among plants (34.29%) as the main constituent of habitat vegetation community of babirusa. There were also 11 species of plants (31.43%) including feed plants for babirusa, namely pangi (*Pangiumedule)*, leu, roa, seho, tohupo, dongi, nantu, banyan, bohulo and gora forest and bugis wood (a local name given for each plants).

Species abundance of community composer pole level plants was identified 31 species of plants. Ten species (32.26%) as the plants feed of babirusa, rao, lamuta, leu, langsat, dongi, tohupo, gora, white nantu, red nantu and huhito/wood bugis.

Levels vegetation were obtained 24 species of plants as a constituent vegetation community, which rattan plants was the highest index value important (IVI=38.11%), and the lowest one was Boyuhu (2.58%). Of the 24 species of plants, 9 types (37.5%) were identified as the feed of babirusa, namely rattan, rao, lomuli, lamuta, forest seho, bohulo/olive woods, seho yaki; leu and bugis wood (huhito).

The analysis of seedlings types vegetation were 22 types of plants, where both biluanga (*O. sumatrana*) and laluta plants were the lowest of index value important (IVI =1.92%). There were 6 types of plants (28.57%) as primary communities, involved tongiito, palango, rattan, woka, ferns and tombito. There were 8 types (38.09%) of the 22 species plants considered as babirusa feed, vis palango (E. indica), rattan (Calanus sp), pandan forest.

Figure 1. Babirusa (babyrousa babyrussa celebensis) are found only in undisturbed rain forest

(Pandanus sp), molitoboi (Zingeber sp), bolulo (A. unifoliata), lamuta (Gnetum sp), seho yaki (C. mitis) and nantu (P. obtusifoleum).

In general, species composition and structure community, illustrated that there was no particular plant species which dominant at the level of plants. It can be said that the condition of the forest environment Wildlife Nantu suitable for the development of each plant species.

Morphology and Digestive Organ of Babirusa

Babirusa (*Babyrousa babyrussa celebensis*) has unique characteristics that can be seen from the morphology. Based on the observed data in the present study, and the previous study reported by Tulung, *et al* (2003), the typical morphology in adult males was to have both the maxillary canines, grow up through the nose and curved down toward the forehead without getting into the mouth. While the females babirusa have small tusks that grow up through the skin of the upper lip. Male babirusa has a relatively larger body size than that of female. The babirusa body is relatively long, with the front legs being shorter than that of hind legs. The fur color of the animal varies from gray, brownish gray or black.

Physical appearance and complementary organs of babirusa compared with wild pig *(sus celebensis)* in the tropical forest habitat Tangkoko and Togid of North Sulawesi is presented in Table 1.

Babirusa classified as omnivorous animals like other pigs species, such as wild pig (*Sus celebensis*) and domestic pigs. Observations of the digestive tract showed that the structure of the adult babirusa digestive tract from esophagus until the intestine is similar to the type of wild boar and domestic pigs others. However the front of the animal's stomach bigger than any domestic pigs. Microscopy observations were performed Leus (2000), reported the size of the stomach adults babirusa about 3000 cm2, where the largest area (>70%) of the stomach internal surface contains mucus producing cardiac glands (mucosal), while in domestic pigs only about of 33%. This condition makes the gastric lumen pH babirusa ranged from 5.3 to 6.4, where there is a sizeable population of microorganisms.

Refers to some researchers studies, the microorganisms were bacterial that took roles on plant structural components fermentation by its enzymes, which are unable to produce on their own babirusa (Fischer, 2012). Leus et al. (1999) indicated that babirusa is non-ruminant foregut-fermenting frugivore concentrate selector.

Social and Reproductive Behavior

Social behavior of babirusa around the habitat more often done in the morning, everyday, while doing the activity of searching for sources of feed, scavenge, and wallowing in the mud. There was also a social interaction one and another in a small group. Number of babirusa in small groups varies between two and five heads.

Table 1. Physical performance and complementary organ of adult Babirusa (Babyrousa babyrussa celebensis) and wild pig (Sus celebensis)

Organ/physical measurement (gr/kg/cm)		n	Sex	
			Male	Female
Live weight	(kg)	5 1	33.66 (30.10)*	28.70 (20.40)*
Height	(cm)	5 1	64.80 (57.90)*	58.40 (51.50)*
Length	(cm)	5 1	106.40 (98.80)*	96.80 (90.00)*
Head length	(cm)	5 1	32.60 (31.40)*	30.30 (20.40)*
Fore leg length	(cm)	5 1	58.20 (47.70)*	51.80 (42.40)*
Hind leg length	(cm)	5 1	60.88 (50.30)*	54.50 (44.90)*
Waist length	(cm)	5 1	78.70 (73.20)*	73.50 (66.00)*
Liver	(gram)	5 1	953.80 (1287.50) *	784.20 (1000.00) *
Pancreas	(gram)	5 1	28.66 (45.15)*	25.26 (30.60)*

* numbers in the brackets are comparison measurements for adult wild pigs *(Sus celebensis)*

In addition, the interaction frequency every day was low enough. In small group interactions there were more females, while males more often observed only one animal in the group, or if there is, it was only a male children (male young). Usually the adult males which already have long tusks are doing their own activity to scrap its slender tusks against the tree trunk. Sometimes, when the two adults′ males were among a group of females, there will be a fight for the females which ready to mate. Presumably, this is part of the male babirusa reproductive behavior.

Babirusa reproductive behavior that observed during the study were, respectively: the period to reach sexual maturity both females and males were approximately 5-10 months; the gestation period of babirusa is longer than domestic pigs, from 155-158 days; the litter size of babirusa is between 1-2 piglets; birth weight about 0.75 kg; period to wean is 1 month old, with a mortality rate of 0.8%; the numbers of birth per year is once. The reproductive period after production is 7 years of age, while the lifetime of babirusa is reached to about 23 years. According to Houston (1997) in Tisleric (2000) babirusa reach sexual maturity at age 1 to 2 years, while the gestation period between 150-157 days, in which pregnancy can occur 1-2 times per year.

Babirusa Feed Sources in Original Habitat

In the original habitat, there were many types of plants or fruits, including fungi and insects as well as other materials identified, and considered to be a source of feed for babirusa. There are 7 types of them most considered to be consumed by babirusa (*Babyrousa babyrussa celebensis*) in their natural habitat as shown in Table 2.

Observations showed that fruit of pangi (*Pangium edule*) is the most dominant type of feed plants consumed by babirusa. The analysis of plants feed types showed varying nutrient content. All kinds of feed were contained a relatively high crude fiber and can be a limiting factor for babirusa as non-ruminant animals. However, according to Langer (1988) in Clayton (1996) argued that the digestive system is more complex than other types of pigs. Presumably, with the system of digestive tracts, the animal can be able to have benefit from that feed sources. Although the comparison of the babirusa stomach to a ruminant stomach is not justified yet, the microorganisms have been found in the mucus gel adhering to the stomach surface and within the stomach lumen (Leus, 1994). This is thought to be a beneficial factor for babirusa in dealing with coarser feed materials compared with other pig or non ruminant species.

Table 2. Nutrient composition of several feed sources of Babirusa (Babyrousa babyrussa celebensis) in their habitat (on a Dry-Matter Basis)

Feedstuffs	Nutrients, assayed						
	Protein (%)	Fat (%)	Fiber (%)	CH (%)	Energy (Cal)	Ca (%)	P (%)
Pangi :							
Pulp	17.70	52.08	31.50	12.33	165.84	0.40	3.15
Seed	18.05	43.86	30.02	13.06	520.98	0.20	6.43
Rao/Dao	6.24	3.34	28.12	28.34	168.38	0.74	1.06
Loyo/Leu	5.77	4.07	31.48	14.46	74.17	0.73	--
Seho hutan	14.81	3.06	22.55	18.85	162.18	0.85	1.44
Lamuta	6.54	4.75	46.14	25.85	172.31	0.20	0.34
Palango	14.82	3.86	23.16	14.44	151.78	0.57	2.78

DM = dry matter; CH = carbohydrate; Ca = calcium; P = phosphorus

Nutrients Sources of Babirusa

The feed analysis showed that pangi fruit (*Pangium edule*) has 26.2% of protein content (total seed protein and pulp), indicates that pangi fruit was a very good source of protein for babirusa. Besides, pangi fruit was also as a source of energy. In addition, seho fruit (*Arenga, sp*) has a pretty good protein content, as well as palango grass (*Eleusine indica*) and the types of grass field, although the frequencies level of babirusa in eating grass palango were relatively less as observed.

It was also identified that babirusa have a habit to scavenge for food around the rotten timbers of trees / plants. It was also proved that in the vicinity of rooting timber there were many insects′ larvae, and other types of wood mushrooms. Allegedly, babirusa also eats insect larvae and wood fungi in order to satisfy the protein requirement for babirusa.

The most important energy source for babirusa was also come from pangi fruit. Table 2 shows pangi fruit has a fat content around 43%; followed by rao fruit (*Dractomelon dao*), seho fruit (*Arenga, sp*.) and lamuta fruit (*Gnetum sp*.

The results were in agreement with other research information. Inquiry entrails and excrement babirusa in North Sulawesi by Clayton (1996) indicates that the diet of wild babirusa is mainly composed of fruits and/or seeds and some animal material, leaves, grass, soil and rock fragments. According to Leus (2000) and MacDonald (2005) as in most other species of suidae, babirusa seems as omnivorous animals. Observations made on both wild and captive individuals revealed that babirusa consume a variety of leaves, roots, fruits and other ingredients. The present study is still at the stage of identification and characterization of food resources and has not reached the stage of experimental measurement of the biological value of feed as the basis for setting up standards of nutrition and feed formulation needs for babirusa. However, Leus, *et al.* (1999) and Leus (2000) in an experiment that feed on babirusa in captivity at the zoo, using predictions equation of domestic pigs nutrient requirement, estimated that adult male babirusa needs for energy and protein 11.3 MJ or 2.7 Mcal and 88 g of protein per day, respectively. While the female babirusa needs for energy and protein 8.5 MJ or 2.03 Mcal and 59 g of protein per day, respectively.

Mineral Sources for babirusa

Field observations and laboratory analysis revealed that salt water of 'Adudu' is the main mineral source for babirusa besides minerals obtained from feedstuffs consumed. There was a tendency of a babirusa or a herd of babirusa to really engross in consuming salt water of 'Adudu'. Adudu saline water contains relatively high levels of macro minerals, as shown in Table 3.

Table 3. Macro mineral composition of Adudu saline water

Minerals	Content (ppm)
Calcium (Ca)	61.10
Magnesium (Mg)	0.92
Phosphorus (P)	46.00
Natrium (Na)	147.40
Kalium (K)	4.86

Data (Table 3) showed that sodium has the highest level amongst minerals in Adudu saline water, followed by Ca, P, K, and Mg.

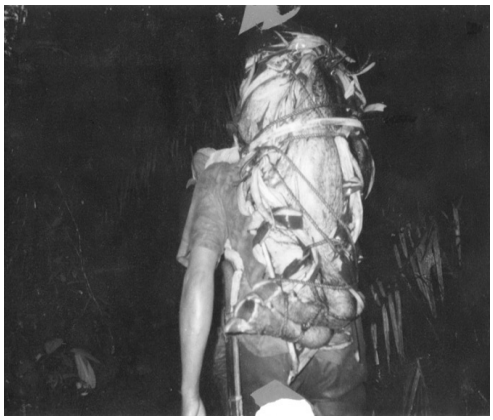

Figure 2. Poachers are now being the most dangerous predators of babirusa

CONCLUSIONS

It can be concluded that reproduction rate of babirusa is very low. It is therefore important to control the population in the habitat by propagating females′ babirusa and considering female : male ratio. Babirusa (*Babyrousa babyrussa celebensis*) habitat being a primary forest with a characteristic of Pangi fruit (*Pangium edule*) dominated the vegetation community in babirusa habitat.

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EFFECT OF FREE CHOICE FEEDING BASED ON EMMER, TRITICALE AND WHEAT TO JAPANESE QUAIL (*COTURNIX COTURNIX JAPONICA*) ON PERFORMANCE, INNER ORGANS AND INTESTINAL VISCOSITY

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Abstract

When choose feeding applied in poultry breeding birds can adjust their ration and this may be considered in terms of animal welfare and performance and carcass quality improvement. The complete ration costs may be higher than individual feedstuffs. When breeders achieved satisfactory results with choice feeding based on cereals they can obtain economical advantages. This study was conducted to investigate the effects of free choice feeding based on emmer (Triticum dicoccon), triticale (Triticale) and wheat (Triticum sp.) on Japanese quail (Coturnix coturnix japonica) growth performance, inner organ traits and intestinal viscosity. In the experiment, basal diet and ground emmer, triticale and wheat were offered separately in two different feeders. The treatments were: (1) Control (C, basal diet), (2) C and emmer (CE) (3) C and triticale (CT) and (4) C and wheat (CW). A total of 240 three-day-old Japanese quail were randomly distributed to 4 equal groups with 6 replicate and fed for 35 d. Body weight (BW) and feed intake (FI) were determined at 3, 10, 24 and 38 d of age. Carcass and inner organ weights were determined at end of the experiment. Intestinal viscosity was measured. There was no difference among the group in terms of BW at 3 d ($p>0.05$), but on d 10, 24 BW of C group was higher than other groups and BW of wheat group was higher than CE and CT group at 10 d ($p<0.01$). The BW of CE group was higher than C and CW group at 38 d ($p<0.01$). The body weight gain (BWG) in the C group was higher than CE and CT groups at 3 to 10 days but C group's BWG was lower than CE and CW groups at 24 to 38 days ($p<0.01$). The BWG of CE group's was higher than C and CW groups at 3 to 38 d ($p<0.01$). The FI and feed conversion ratio (FCR) of quail were not statistically influenced ($p>0.05$) by the treatments. There was no difference among the groups in terms of mortality. The carcass yield, liver, small intestine, heart and total gastrointestinal tract ratio were not statistically influenced by the free choice feeding based on triticale emmer and wheat ($p>0.05$). However, in the wheat group's gizzard ratio was higher than triticale and emmer groups and in this group abdominal fat pad was higher than other groups ($p<0.01$). Intestinal viscosity of quail was not influenced by choice feeding ($p>0.05$). These results show that ground emmer, triticale and wheat may be given separately together as basal ration and some advantageous in performance may gain with choice feeding based on emmer.

Key words: *carcass, choice feeding, emmer, quail, performance, triticale, viscosity, wheat.*

INTRODUCTION

Choice feeding based on grain has been considered for economic and health reasons in poultry feeding (Forbes and Covasa, 1995; Erener et al., 2006; Amerah and Ravindran, 2008; Gabriel et al., 2008). When whole or ground grains were given to chicks, many handling procedures are eliminated. Acceptable growth rate can be achieved when poultry allowed free access both basal diet and grain cereals such as wheat (Leeson and Caston, 1993) and triticale (Korver et al., 2004; Konca et al., 2012; Özek et al., 2012). Furthermore, beneficial effects of choice feeding method were also observed on digestive system activity and microflora (Engberg et al., 2004). However, different cereal cultivars vary with respect to their nutrient content, which may cause difference in broiler growth rate (Austin et al., 1999).

Triticale is a hybrid of wheat and rye and it has been proposed as an alternative cereal in animal feeding because of its potential combination of wheat feeding characteristics and rye winter hardness and disease resistance (Vieira et al., 1995). Boros (1999) reported that triticale is genetically close to wheat than rye and properties more similar to wheat; therefore it might be successfully replaced instead of wheat in poultry diets. Konca et al. (2012) reported that feeding with triticale in a separate feeder increased BW of turkeys.

Emmer is one of the primitive cereals and they are locally grown by the farmers, called “siyez” “gacer” “gernik” by Turkish producers. Emmer is popular among the local producers for making pasta, pilaw and soup. It is difficult to find emmer in public or global markets. However, its harvest is very difficult due to cover shells (hulls) remained with grain by machine, therefore hulls is separated by hand. In addition to this, there is growing interest by consumers due to organic nutrition and it is assumed that local traditions and ancient foods are close to organic production. In our field observations emmer is suitable for organic production due to original genetic species and may be desirable by the consumers. On the other hand, emmer has high protein and antioxidant capacity compared to durum wheat (Giuliani et al., 2009). Wild emmer has high concentrations of zinc, iron and protein in seeds (Peleg et al., 2008).

Wheat is well known grain used for human and animal nutrition. Wheat mostly used in choice feeding trials and positive results were obtained related to performance and health status (Plavnik et al., 2002; Bennet et al., 2002; Preston et al., 2002; Erener et al., 2003; Gabriel et al., 2003; Amerah and Ravindran, 2008). The aim of the present study was to determine the effects of choice feeding based on ground emmer, triticale and wheat on the performance, carcass yield, inner organs/carcass indices, abdominal fat and intestinal viscosity in Japanese quail. It was hypothesized that in a free choice feeding system based on cereals; they may consume appropriate levels of basal diet, emmer, triticale and wheat and satisfactory growth rate could be achieved. Therefore quail meat might be produced at lower cost compared to a complete basal diet.

MATERIALS AND METHODS

Animal and diets

A total of 240 unsexed quail chicks with three-day-old were individually weighed, wing banded and then ranked for minimal differences and distributed into 4 treatment groups with 6 replicates, 10 chicks each. The each cage was furnished with a heater, two waterier and two feeders. The rearing cage dimensions were 50 × 90 × 20 cm. The replicates were designated as the experimental units, and randomized with respect to the dietary treatments. The treatments were: (1) Control (C, basal diet), (2) C and triticale (CT), (3) C and emmer (CE) and (4) C and wheat (CW). The experimental diets were offered to respective quail for 5 weeks. Maize-soybean based diets were utilized and all formulated on similar level of nutrient composition. The emmer has been obtained from villagers in Develi-Kayseri, and triticale and wheat have been obtained from a commercial company. All experimental diets nutrient compositions were prepared according to NRC (1994) recommendations. The compositions of experimental diets used in this study were given in Table 1.

Measurements

Determination of performance traits

Individual body weight (BW) was measured on days 3, 10, 24 and 38. Body weight gain was calculated for 3 to 10, 10 to 24, 24 to 38 and 3 to 38 days. Daily feed consumption (DFC) was measured on pen bases on the same days. Leftovers of feed were reweighed. Total feed consumption for each period determined and daily feed intake were calculated from this data. In case of mortality, feed intake rates were corrected for number of animals in the pen. Feed conversion ratio was calculated as the ratio DFC:BWG of all birds in each pen.

Determination of carcass, inner organs and viscosity traits

For carcass evaluation 24 birds (12 male and 12 female) in each group were randomly selected at 38 d of age and slaughtered. Their feathers were plucked, and the carcasses were eviscerated by hand. The small intestine, large intestine, and gizzard were removed, the contents were expelled. The carcass, liver, heart, proventriculus, gizzard, empty intestine (duodenum+ileum+ jejunum+cecum+colon), total gastro-intestinal system (proventriculus+gizzard+intestine) and abdominal fat were recorded individually and part yields were obtained as part weight: carcass weight × 100. Cold carcass weight was recorded after the carcasses had been stored at +4°C for 18 h.

The total intestinal content (duodenum and jejunum) was collected for viscosity determination. Several microtubes were filled with each sample, labelled and centrifuged (4300 g, for 10 minutes at room temperature).

The centrifuge tubes with fresh digesta were immediately placed on ice in an isolated box until viscosity measurements were performed within 1 h following slaughter. The supernatant was withdrawn and the viscosity of a 0.5 mL aliquot measured using a Brookfield Digital Viscometer (Model DVII+ PRO, Brookfield Engineering Laboratories, Stoughton, MA) maintained at 40°C.

Statistical analysis

The data were subjected to one-way Anova using General Linear Models in SPSS computer program (SPSS, 1998). The model included hempseed level of diets. The means were separated using Duncan′s multiple range tests. The results of statistical analysis were shown as mean values and standard error of means (SEM) in the tables. Statistical significance was considered at $p<0.05$.

Table 1. Diet′s feedstuff and nutrient composition

Feedstuffs	Amount, kg/ton
Corn	450.0
Wheat	63.75
Soybean meal	342.99
Sunflower meal	100.0
Sodium chloride	3.37
Limestone	14.07
Vegetable oil	14.99
DL-Methionine	1.24
L-Lysine	0.70
Vitamin-mineral premix[1]	2.00
Dicalcium phosphate	6.89
Total	1000
The calculated values	
Dry matter, %	88.29
Crude protein, %	24.00
Crude cellulose, %	4.92
Metabolisable energy, kcal/kg	2900
Crude fat, %	4.45
Calcium, %	0.80
Available phosphorus, %	0.30
Lysine, %	1.30
Methionine, %	0.50

[1]Vitamin-mineral premix per kilogram of the diet, Vitamin A, 15,000 IU; Vitamin D3, 2000 IU; Vitamin E, 40.0 mg; Vitamin K, 5.0 mg; Vitamin B1 (thiamine), 3.0 mg; Vitamin B2 (riboflavin), 6.0 mg; Vitamin B6, 5.0 mg; Vitamin B12, 0.03 mg; Niacin, 30.0 mg; Biotin, 0.1 mg; Calcium D-pantothenate, 12 mg; Folic acid, 1.0 mg, Choline chloride, 400 mg, Manganese, 80.0 mg; Iron, 35.0 mg; Zinc, 50.0 mg; Copper, 5.0 mg; Iodine, 2.0 mg; Cobalt, 0.4 mg; Selenium, 0.15 mg assures.

RESULTS AND DISCUSSIONS

The BW, BWG, FC and FCR values were given in the Table 2. Day 3 body weights of quail was not significantly differ ($p>0.05$) but on d 10, 24 BW of C group was higher than other groups and BW of wheat group was higher than CE and CT group at 10 days ($p<0.01$). However, on d 38 BW of CE group was higher than C and CW group ($p<0.01$). The BWG in C group was higher than CE and CT groups at 3 to 10 days but C group′s BWG was lower than CE and CW groups at 24 to 38 days ($p<0.01$). At 3 to 38 days BWG of CE group′s was higher than C and CW groups ($p<0.01$). Over the 3 to 38 day experimental period, no statistically differences were found the FC and FCR values by the C and choice feeding with triticale, emmer and wheat ($p> 0.05$). The cereal consumption share for emmer, triticale and wheat groups were; 32.48, 30.69 and 29.04 % respectively. The mortality rate was not influenced by the treatments ($p>0.05$).

Table 2. Effects of treatments on the body weight (BW), weight gain (BWG), feed consumption (FC) and feed conversion ratio (FCR) in quail

Days	Treatments					
	Control	Emmer	Triticale	Wheat	SEM	P
Body weigh						
3	10.17	9.91	9.97	9.77	0.09	NS
10	36.79^{a}	30.14^{c}	30.42^{c}	32.81^{b}	0.81	*
24	117.89^{a}	105.48^{b}	106.08^{b}	106.99^{b}	1.77	*
38	157.70^{bc}	169.7^{a}	164.8^{ab}	154.9^{c}	2.83	*
Body weight gain, g/day						
3 to 10	3.80^{a}	2.90^{b}	2.92^{b}	3.28^{ab}	0.11	*
10 to 24	5.81	5.42	5.57	5.35	0.15	NS
24 to 38	2.87^{c}	4.54^{a}	4.01^{ab}	3.58^{b}	0.23	*
3 to 38	4.21^{bc}	4.57^{a}	4.42^{ab}	4.14^{c}	0.08	*
Daily feed consumption, g**						
3 to 10	6.56	6.72 (1.88)	5.66 (1.39)	6.18 (1.68)	0.24	NS
10 to 24	14.28	16.06 (4.95)	14.50 (3.86)	14.60 (3.55)	0.69	NS
24 to 38	25.00	23.38 (8.17)	21.16 (7.24)	21.59 (7.02)	0.76	NS
3 to 38	16.99	17.12 (5.62)	15.39 (4.72)	15.71 (4.57)	0.48	NS
Average consumed cereal ratio, %	-	32.48	30.69	29.04	-	-
Feed conversion ratio, g feed/g BWG						
3 to 10	1.72	2.32	1.94	1.88	0.23	NS
10 to 24	2.46	2.97	2.60	2.73	0.10	NS
24 to 38	8.71	5.15	5.28	6.02	0.43	NS
3 to 38	4.03	3.75	3.48	3.79	0.10	NS
Mortality, %						
3 to 38	1.33	3.17	2.00	2.33	0.78	NS

SEM: pooled standard error of the means; P: probability, NS: non significant; $^{a, b}$: Means within a row different alphabet are significant; *(P<0.01). ** Cereal consumptions were given in the parenthesis.

The effect of choice feeding based on cereals on carcass yield and intestinal organ traits and their relative incidence and viscosity were shown in Table 3. The carcass yield was not affected from choice feeding treatment (p>0.05). Similarly, the liver, heart, empty small intestine and total digestive system ratios were not significantly different among the groups (p>0.05). However, the gizzard ratio in the choice feeding group based on wheat was higher than emmer and triticale groups and this group′s abdominal fat ratio significantly higher than other groups (p<0.01). On the other hand intestinal viscosity was not influenced by the choice feeding based on cereals.

Table 3. Effect of hempseed in quail diet on carcass yield and intestinal organ traits and small intestinal viscosity

Traits	Treatments					
	Control	Emmer	Triticale	Wheat	SEM	P
Carcass ratio, %	66.55	65.58	65.64	65.17	1.69	NS
Liver, %	4.81	5.01	4.95	4.84	0.25	NS
Proventriculus, %	0.63	0.70	0.61	0.67	0.128	NS
Gizzard, %	3.04^{ab}	2.61^{c}	2.90^{bc}	3.42^{a}	0.14	*
Small intestine, %	2.67	2.89	2.68	3.06	0.11	NS
Heart, %	1.64	1.54	1.52	1.70	2.69	NS
Abdominal fat, %	1.24^{b}	1.20^{b}	1.23^{b}	1.44^{a}	0.10	*
Total gastrointestinal system, %	6.13	6.20	6.11	6.98	3.80	NS
Viscosity	2.27	2.06	2.37	2.24	0.13	NS

SEM: pooled standard error of the means; NS: non significant; $^{a, b}$: Means within a row different alphabet are significant; *(P<0.01).

We couldn′t reach research about effect of choice feeding based on cereals in quail. However, in broilers, choice feeding applications based on whole cereals very attractive for animal health and economic reasons (Forbes and Covasa, 1995; Ferket, 2000; Gabriel et al., 2008). However, one week old broiler chicks have some difficulties to eat whole cereals at initial of growth period and whole cereal consumption lower compared to older ages (Özek et al., 2012). Quail chicks have smaller body weight than broilers chicks and they may not able to eat whole cereals. Therefore in this experiment control rations and ground cereals were offered in two separate feeders. Initial of the experiment we observed that quail went to a feeder (control or cereal) and long time consumed only ones. According to our observations they didn't choice appropriate feed type, therefore in the first week quails consumed unbalanced diets and BWG of quail with choice feeding based on emmer, triticale and wheat was lower than control group. However, C group BW in the end of the experiment was lower than choice feeding groups. But FC and FCR of quail did not significantly influenced by the treatments. However, at the end of experiment, cereal consumption ratios in choice feeding groups were about one third of total feed consumption (32.48, 30.69 and 29.04 % for emmer, triticale and wheat, respectively). Therefore, free choice feeding groups quail consumed lower percentage of protein, minerals and vitamins but similar or higher level of energy (the protein and energy content of basal diet: 24%, 2900 kcal ME/kg, and cereals about 12%, 3000 to 3150 kcal ME/kg respectively, according to standard nutrient content of emmer, triticale and wheat).Protein consumption of choice feeding groups was lesser than C group, however, their BW higher than C group at the end of experiment. Compared to cost of complete diet and single cereal, this situation may get advantageous. There is no research on emmer usage in quail or poultry diets and effect on performance traits. However, triticale usage in poultry diets in general had no effect (Rao, et al., 1976; Vieira et al., 1995) or improved (Karaalp et al., 2003) in BW and BWG. Also feed efficiency was better (Ruiz et al., 1987) or not influenced (Vieira et al., 1995) with triticale diets than corn based diets. Most of the choice feeding trials have been performed with whole wheat (Bennett and Classen, 2003; Gabriel et al., 2008), and maize (Erener et al., 2006) but a little triticale (Korver et al., 2004; Konca et al., 2012; Özek et al., 2012) in poultry.

In the present study carcass yield, proventriculus, liver, small intestine, heart and total gastrointestinal system ratio (%) were not affected by choice feeding treatment. Previous researches indicated that choice feeding with cereals didn't influence carcass yield in broilers (Olver and Jonker, 1977; Golian et al., 2008; Özek et al., 2012). On the other hand, Leeson and Caston (1993) reported that choice feeding lowered carcass yield in broilers. Canoğulları et al. (2004) stated that carcass, heart and liver weights were not affected by the ground wheat compared to control group. Also, Özek et al. (2012) reported that choice feeding with triticale didn't affect liver and small intestine ratios but affected proventriculus. However, the gizzard ratio in the CW group was higher than CE and CT groups. Similarly, it is found that free choice feeding with whole or ground wheat (Canoğulları et al., 2004; Amerah and Ravindran (2008) and triticale (Özek et al., 2012) caused increase in gizzard weight and ratio. In the present experiment cereals were ground due to eating difficulty. However, whole wheat stimulated larger gizzard and pancreas in broilers (Svihus et al., 2002). The abdominal fat ratio in the CW group was higher than others. Wheat one of these three cereals, others didn't increase abdominal fat. Erener et al. (2003) expressed that whole wheat feeding increased abdominal fat in broilers. It may be related to their nutrient content. Austin et al. (1999) claimed that different cereal cultivars nutrient content are varying which may cause difference in broiler performance.

Intestinal (ileum+jejunum content) viscosity was not influenced by the feeding style. In this experiment consumed cereals ratio in total about 30 % compared to C group. Cereals contain β-glucans and they may create a viscous environment within the intestinal lumen and decreased nutrient utilization (Choct and Annison, 1992; Smits and Annison, 1996). Özek et al. (2012) noted that duodenal viscosity was not affected by the free choice feeding based on triticale. In contrast to this, J´ozefiak

et al. (2007) found that cereals decreased intestinal viscosity in broilers. Konca et al. (2012) reported that triticale and barley (mixed with C diet or separate in two feeders) didn′t affect jejunal viscosity but separate feeding with triticale decreased ileal viscosity in turkeys.

CONCLUSIONS

In conclusion, our results showed that, quail performance and carcass yield, inner organs and intestinal viscosity traits were not negatively influenced by the choice feeding based on emmer, triticale and wheat. However, in the CW group′s gizzard ratio was higher than C, EC and CT groups and this group′s abdominal fat ratio was higher than other groups. Taken together, quail may be fed with triticale, emmer and wheat as choice feeding and could provide economic advantages. However, there is no enough number of experiments about the effects of choice feeding with different cereals. Further experiments are needed to determine the effect of choice feeding with cereals in quails.

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PROTEAN NUTRITION OPTIMIZATION FOR COWS WITH HIGH MILK PRODUCTION BY USING AN UNPROTEIC NATRIUM SOURCE ASSOCIATED WITH ENERGY AND MINERAL SUPPLEMENTS

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Abstract

To ensure the necessary amount of protein for dairy cows with high milk production represents for farmers a permanent challenge directed both to supply and store raw protean materials, as well as to search for the means to exploit the ruminant digestive peculiarities in terms of producing microbial endogenous protein with high biological value of non-protean nitrogen sources in terms of energy and mineral optimization. Starting from these considerations, we have achieved a formula optimization during the "stable" period, meant for this category of animals by means of total or partial substitution of the main raw protein material in compound feed structure (soya meal) with an alternative source of nitrogen (urea) in four experimental variants of formula. For this operation, we approached the three basic levels necessary for the replacement of an organic protean source with an inorganic one, such as: adjusting the speed of decomposition at ruminating level, speed and length of nitrogen release ; to ensure a suitable level of energy support, speed and duration of release of nitrogen; creating a suitable spectrum for some mineral elements involved in microbial protein synthesis. In these circumstances, we managed the formulation of some compound feed in where the share of total provided non-protein nitrogen varied between 18.3 and 29.6%.

Key words: *mineral supplement, mollasses, protein, soya meal, urea, vegetal fats.*

INTRODUCTION

The permanent concern of specialists in the field of bulls breeding to get ever higher milk production (35 l/cow/day) requires a faster resolution to the issues related to identifying and securing protein sources which have to support at an adequate level the quantity and quality of the product, at the same time keeping the animals in a proper state of maintenance. Taking into account the ruminants digestive peculiarities and also of the difficulty in purchasing classical protean raw materials (mainly meals) livestock nutritionists recommend the use of alternative protein sources to substitute a larger percentage of the total protein in the formula (Kleen, 2010).

An industrial product which can be used for this purpose is the urea $CO(NH_2)_2$, whose high nitrogen content (42-46%) provides this uneffective element presence in the rumen, but with a major role in micro-organisms growth and multiplication, which in turn represents both an important link in the process of formula digestion, as well as a valuable source of protein (450-650 g/day), volorized at the level of the other segments of the digestive tract (Dragomir and al., 2010).

High nitrogen content of urea and its release in the rumen is done quickly, (nitrogen split is about 4 times more intense than the use of microbial protein synthesis) (Hutiens, 2010), it requires finding processes by means of which the urea decomposition to be done gradually, so that the risk of accidents caused by excess ammonia to be removed (Chase and al., 2007). But on the other hand, protein is the most complex organic substance, so that nitrogen provision is not the only missing link in the complex process of microbial multiplication, whole series of mineral elements being also necessary (in particular, sulphur, phosphorus, cobalt, zinc, manganese) which form the amino acids structure (Stoica and al., 2010). To this, it is added the fact that any process of synthesis also requires a power source corresponding to the intensity and length of the respective activity. All this process (of microbial

synthesis) should be unfolded in ruminal pH optimum limits to maintain favorable fermentative processes for the production of volatile fatty acids involved in milk synthesis (Velea and Marginean, 2012).
This simultaneous coordination necessary for the optimum unfoldement of physiological processes comes to livestock nutritionists who have to provide to the breeder an energy-protein-mineral 'package' easy to use and to exploit the maximum potential of the protean endogenous synthesis of animals (Beever and Doyle, 2007).

MATERIALS AND METHODS

In the context of the elements outlined above and taking into account the recommendations concerning the non-nitrogen doses that can be used for maintenance and health of the animals status, the conditions to be observed in case of using an inorganic sources of nitrogen, we have made two steps of optimizing the report protean nitrogen - non-protean nitrogen. This was carried out through partial or total replacement, in the structure of the combined feeds, of soya meal with a non-nitrogen source (urea) in two combinations with a variety of substances (mineral and energetic ones) which have an increasing role of ruminal microorganisms activity.
The two combinations where urea is found differ among themselves by the way of fixing it, such as:
- in Rumagen product, urea is fixed on a support of gelly starch, which gives a delay of ammonia release at a ruminal level, and for the energetic support there were also incorporated oils (sunflower and rapeseed). The structure of this product can be synthesized as follows: crude protein, crude protein equivalent of nitrogen 85.5%, oils 5%, crude fiber 2%;
- a mixture of urea + an easy fermentable glucid (molasses) which involves the inclusion of urea in the compound feeds production flow and the spray of energy supplement on the fodder grains.
To adjust the intake of essential minerals in the microbial synthesis process of microbial capable, there was conceived a formula of premix where macro and micro-elements have been supplemented, depending on their degree of involvement in this complex physiological process.
Optimization activity of formula for lactation cows was achieved through the use of the main pillars that control the formula, namely:
1. Energy necessary calculus and protein for maintenance and production (taking into account the average values of animals weight and milk production), the calculus relations being the following:
- for energy (milk nutritive units MNL)
a. maintenance MNL = 1.8 + 0.006 x GV
b. production MNL = 0.47 x PL
where : GV = live weight (kg);
PL = milk production (l/day).
- for protein
a. PDI (g) maintenance = 3.25 g x $G^{0,75}$ (kg)
b. PDI (g) production = 50 g x PL (l/day)
where: $G^{0,75}$ = metabolic weight
2. Identify sources and obtain the necessary fodders for the production of formulae, the determination of chemical composition and their nutritive value, using the calculus basis for UNL and PDI
3. Ccompliance with the basic principles for the formulae elaboration in lactating cows, namely ensuring adequate physical structure conditions, optimal loading and fermentability coefficient.
4. Using an alternative source of nitrogen (urea) for the total or partial replacement of soya meal, at the same time with the ensurance of a mineral and energy support corresponding to the nitrogen contribution.

RESULTS AND DISCUSSIONS

1. The obtained results for the calculus of the energy and nutritive substances.
Adjusting the calculus relations of the energy and protean necessary to the production characteristics (361/day) and animals weight (600 kg), there were obtained the following values:
- for energy (milk nutritive units UNL)
a. UNL maintenance 1.8 + 0.006 x 600 (kg) = 5,4
b. UNL production = 0.47 x 36 (l/day) = 17
Total = 22.4 UNL.
- for protein
a. PDI (g) maintenance 3.25 g x 121.23 (kg) = 394

b. PDI (g) production = 50 g x 36 (l/zi) = 1800
Total = 2194 g PDI

2. Results obtained in the identification and the determination of the fodder nutritional value necessary to formulae elaboration.

The main feed to be used in the formulae elaboration for the winter period and their nutritive value expressed in MNL, PDI, crude protein and metabolisable energy are presented in tables 1 and 2.

Note that for these feeds (except beer residues) there were achieved stocks necessary to feed 35 animals (5 livestock x 7 cows/livestock) over a period of 180 days, respectively from December 2012 – April 2013.

For the beer residues, there is a strict contract of supply which is unfolded without difficulty all over the year.

Rumagen product is designed and delivered by Alltech Romania and the corresponding quantities of urea and molasses were purchased and stored in the warehouse IBNA-Balotesti which ensures also the production of compound feeds.

3. Results obtained in the field of formulae elaboration.

Formulae intended for cows in lactation were comprised so that as to comply with the conditions mentioned above, they can be characterized as a tile which has 2 distinct branches, namely:

a. basic formula made only of volume fodders (Table 3) which should ensure the necessay for the maintenance and achievement of 10 l milk production;

b. complementary formula, represented by compound feed (tables 4 and 5). Regarding the compound feed, please note that the experimental variants are presented in comparison with the control group only at the level of crude protein-metabolisable energy and, without holding onto account of the MNL and PDI, as this mode of expression to be achieved after testing the product.

Using the structures foreseen in the experimental variants, we notice that the highest amounts of urea is registered with the experimental version no.4 (18 g urea/kg /combined feed), which is the corresponding amount of urea/day, 198, g a dose 33 g100 kg AW being followed by the experimental version no. 3 with 16 g of urea/kg of combined fodder, 176 g urea/day of combined fodder,. the dosage being of 29 g urea /100 kg AW ,the other two variants being limited to 22 g urea /100 AW for the experimental version no. 2, respectively 20 g urea/ 100 kgAW for the experimental version no.1.

Table 1. Fodders used for formulae elaboration meant to lactating cows

Fodder	SU (g/kg)	UNL	PDIN (g/kg)	PDIE (g/kg)	Ca (g/kg)	P (g/kg)
Alfa-alfa hay	880	0,60	75	65	10	1,9
Sudan grass hay	850	0,66	43	62	7	1,2
Pickled corn, early milk stage	260	0,22	13	17	1,2	0,5
Beer residues	225	0,19	30	18	1	0,6
Corn	880	1,27	73	110	0,3	2,3
Wheat	870	1,20	79	101	0,7	3,5
Wheat bran	880	0,84	101	82	1,9	10
Soya meal	900	1,14	317	229	2,2	7
Rapeseed meal	890	0,99	227	131	5,5	9

Table 2. Raw materials used in formulae elaboration –PB expression (g/kg) si EM (kcal/kg)

Specifi-cation	Corn	Wheat	Wheat Bran	Soya meal	Rapessed meal	Mollasses	Urea	Rumagen	Alfa-alfa hay	Sudan grass hay	Pickled corn	Beer residues
PB	82	120	140	450	320	90	2600	1120	106	74	23	50
EM	3360	3190	2840	3130	3100	2925	-	3060	2413	2230	695	520

Table 3. Formula structure meant for lactating cows

Fodder/ Norm	kg	DM (kg)	UNL Total=22.4 Maintenance+10 l=10.1	PDIN (g) Total=2194 Maintenance +10 l=894	PDIE (g) Total=2194 Maintenance+10 l=894	Ca (g) 140	P (g) 100
Alfa-alfa hay	3	2.64	1.80	225	195	30	5.7
Sudan grass hay	3	2.55	1.98	129	186	21	3.6
Pickled corn	21	5.46	4.62	273	357	25.2	10.5
Beer residues	9	2.02	1.71	270	162	9	5.4
Basic formula total		12.67	10.11	897	900	85.2	25.2
Loss		-	12.3	1297	1294	54.8	74.8
Compound meal	11	9.68	12.32	1298	1298	76	76
TOTAL		22.35	22.43	2195	2198	161.2	101.1

Table 4. Structure of compound feed formulated for lactation cows during experimental period

Specification/ Fodders	VM			VE1			VE2			VE3			VE4		
	Quantity (kg)	PB (g /kg)	EM (kcal /kg)	Can-titate (kg)	PB (g /kg)	EM (kcal /kg)	Can-titate (kg)	PB (g /kg)	EM (kcal /kg)	Can-titate (kg)	PB (g /kg)	EM (kcal /kg)	Can-titate (kg)	PB (g /kg)	EM (kcal /kg)
Corn	47	39	1579	47	39	1579	47	39	1579	47	39	1579	47	39	1579
Wheat	15	18	480	20,8	25	663	17	20	542	23.4	28	746	20.9	25	668
Wheat bran	15	21	426	15	21	426	15	21	426	15	21	426	15	21	426
Rapeseed meal	7	22	217	7	22	217	7	22	217	7	22	217	7	22	217
Soya meal	13	58	406	4	18	126	5	22	157	-	-	-	-	-	-
Rumagen	-	-	-	3	33	92	-	-	-	4.3	48	132	-	-	-
Urea	-	-	-	-	-	-	1.2	31	-	-	-	-	1.8	47	-
Mollasses	-	-	-	-	-	-	3.6	3	105	-	-	-	5	4	145
Premix VL I	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Premix VL II	-	-	-	3,2	-	-	3.2	-	-	3.3	-	-	3.3	-	-
Total	100	158	3108	100	158	3103	100	158	3026	100	158	3100	100	158	3035

Table 5. Compound meal structure - control variant , expressed in UNL and PDI system

Fodder	kg	SU (kg)	UNL	PDIN (g)	PDIE (g)	Ca (g)	P (g)
Corn	47	41.36	59.69	3431	5170	14.1	108.1
Wheat	15	13.05	18.00	1185	1515	10.5	52.5
Soya meal	13	11.70	14.82	4121	2977	28.6	91.0
Rapeseed meal	7	6.23	6.93	1589	917	38.5	63.0
Wheat bran	15	13.20	12.6	1515	1230	28.5	150.0
Premix VL I	3	2.26	-	-	-	570	230.0
Total	100	87.80	112.04	11841	11809	690.2	694.6
	1	0.88	1.12	118	118	6.9	6.9

Analyzing energy and protein parameters of compound meal, we acknowledge a great uniformity at the level of metabolic energy whose values are placed around 3100 kcal, the maximum difference being of 82 kcal (2.6%) recorded between the control and experimental variant version 2, this one being considered negligible, particularly at the level of the protein, where the value of 158 g/kg meets at all experimental variants.

In these conditions, energy-protein ratio has almost imperceptible limits, variations comprised between 19.2 and 19.6 kcal E. M/g crude protein.

CONCLUSIONS

1. Formula optimization for cows is the main condition in order to obtain some milk productions quantitative and qualitative superior.

2. Among all nutritional parameters which must be ensured in order to support a high milk production, under animal optimal physiological conditions, most of the times, protein represents the limiting factor for formula.

3. The ruminant physiological peculiarities make possible to use alternative sources of

protein to serve as 'donors' of nitrogen for the development of microbial populations in the rumen.

4. When to use inorganic sources of nitrogen in dairy cows formula, we must find solutions both for ammonia release distribution and for the energy and mineral support of microbial biosynthesis.

5. By means of professional coordination of all factors involved in achieving physiological balance in the rumen, the non-protean nitrogen can provide about 20 %of the total protein in the formula, which allows obtaining some formula of combined fodder where soy meal can be totally substituted by urea.

6. The use of urea in compound feeds structure combined fed to high-dairy cattle milk production involves a lot of attention from nutritionists in terms of identification and use inside the formula of fodder ingredients (fibrous and succulent) that should allow the ensuring of physical structure conditions, fermentability and charging coefficient adequate to this animal category.

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EVALUATION OF FRUCTAN CONTENTS IN THE TAPROOTS OF PLANTS *LACTUCA SERRIOLA* L. AND *SONCHUS OLERACEUS* L.

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Abstract

The current research aimed to present the evaluation of the underground parts of two widespread plants in Bulgaria - prickly lettuce (Lactuca serriola L.*) and annual sow thistle* (*Sonchus oleraceus* L.) *as a source of inulin-type fructans. The sequential ethanol and water extractions from their dry taproots were carried out. The amount of extracted fructans was defined by the resorcinol assay. The fructooligosacharides and inulin contents of the obtained extracts were analyzed by TLC and HPLC-RID methods. The total fructan content in the weed plant Sonchus oleraceus L. (19.6% dw) is higher than the fructan level in the roots of Lactuca serriola L (9.56% dw). In the ethanol extracts were observed the presence of monosaccharide glucose and fructose, high level of sucrose and trisaccharides 1-kestose. In the result of the carried analysis, we can conclude that the roots are rich source of fructans as the fructoligosacharides fraction dominates in ethanolic extracts. These plants could not only be consider as weeds, but it have to pay attention to their future possibility to be used as a potential source of fructooligosacharides with prebiotic effect in nutrition formula for animals and human.*

Key words*: fructoligosacharide, inulin, Lactuca serriola, Sonchus oleraceus.*

INTRODUCTION

Inulin is a polydisperse plant polysaccharide, member of fructan family, consisting mainly of β-(2→1) fructofuranosyl units (F_m), and a terminal α-glycopyranose unit (1→2) (GF_n) (Van Laere et al., 2002). The degree of polymerization (DP) of inulin varies from 2 to 70 (De Leenheer et al., 1994). Molecules with DP<10 are called oligofructoses or fructooligosaccharides (FOSs) (Figure 1) and they are a subgroup of inulin (Niness, 1999).

Inulin and FOSs are classified as soluble dietary fiber. They act as prebiotics, because stimulate growth of *Bifidobacteria.* Inulin is only hydrolyzed in small amounts in the stomach. In large intestine it is fermented by intestinal microflora into short-chain fatty acid (SCFA), lactic acid and gases (Gibson, 1995, Knudsen, 1995). Inulin-type prebiotics reduce blood levels of triglycerides (Roberfroid, 2005); prevent cardiovascular disease and osteoporosis (Delzenne, 2002). Inulin is helpful in the management of diabetes and blood sugar-related illness (Rumessen, 1998). In recent issues, inulin is presented as immunomodulator and anticancer agent (Barclay et al., 2010).

Depending on the conditions of extraction and the type of used raw material, a short-chain bioactive molecules (FOSs) or long-chain ones (inulin) could be achieved. Both they have different bioactivity as no digestible oligosaccharides of long chain length are typically less biodegradable than compounds of shorter chain length. Van Loo (2007) proposed that a combination of short-chain and long-chain fructans is physiologically more active that the individual fractions.

Inulin serves as a reserve carbohydrate in underground part of the *Compositae* (*Asteraceae*) plants such as *Cichorium intybus, Inula helenium and Helianthus tuberosus* (Van Laere et al., 2002). Prickly lettuce (*Lactuca serriola*)

and annual sow thistle *Sonchus oleraceus* L. also belong to this plant family.

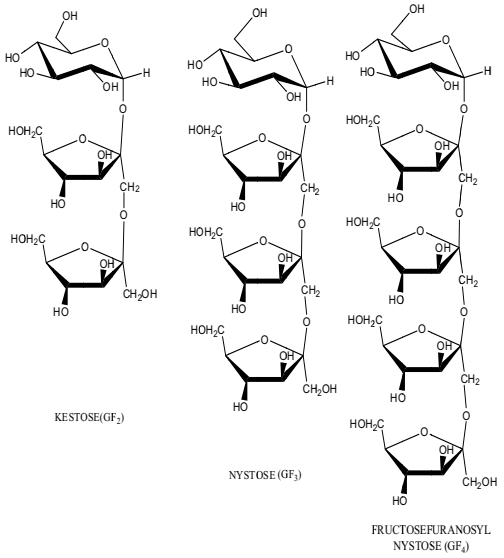

Figure 1. Chemical structure of fructooligosaccharides

Prickly lettuce (*Lactuca serriola* L.) is an annual or biennial plant, slightly foetid, that is commonly considered as a weed of orchards, roadsides and field crops. Many species in Lactuca are medical herbs, as well as wild vegetable. Scientists focused their research interest on searching for some promising compound with effectiveness and low toxicity for the benefit human`s health (Ren et al., 2004). The plant can be eaten as a salad, although it has a bitter taste. The young leaves can be eaten raw or cooked (Kleonikos, 2006)

Sonchus oleraceus L. is growing in cultivated fields and disturbed sites, ditch banks, bottomlands, city lots and alleys (Reaume, 2010). *Sonchus* wild food plants might be applicable in natural medicine and healthy food. *Sonchus oleraceus* L. and *Sonchus* sp.pl. eaten in several Italian regions, are cholagogue and laxative agents, due to their sesquiterpene lactones but also the high content of vitamin C, carotenoids and fatty acids of type ω-3 (Aliotta, 1981, Guil-Guerrero, 1998). In China, Sonchus wild vegetables are used mostly in infusion or decoction and are administered to treat acute icterohepatitis, cancer, inflammation, rheumatism, diarrhoea and snake venom poisoning (Dao et al., 2011). The underground roots of sow thistle store reserve carbohydrates, and inulin is the major storage carbohydrate in them (Lemna et al., 1990).

Figure 2. Photos of prickly lettuce *(Lactuca serriola* L.*)* and annual sow thistle (*Sonchus oleraceus* L.)

The variety *S. arvensis* can be used as a livestock feed and is considered to be highly nutritious for rabbits (Szcza-wenski et al., 1978). Boulos (1973) stated that *S. arvensis* roots can be used as a coffee substitute when is roasted.

According to Jana et al. (2010) the prebiotic effect of the *Taraxacum officinale, Sonchus oleraceus* and *Asparagus sprengeri* extracts on *L. lactis* and *L. reuteri* was higher than or equivalent to inulin - a commercial prebiotic, as *Sonchus oleraceus* exhibited the best prebiotic effect. It was the only plant to stimulate all the probiotics including *B. longum*.

In this context, the paper present an analysis of the fructooligosaccharides and inulin content in the roots of *Lactuca serriola* L. and *Sonchus oleraceus* L. from Plovdiv region of Bulgaria in order to study their inulin-type fructan content. This investigation aimed to present that these weeds can be potential and unstudied source of prebiotics.

MATERIALS AND METHODS

The roots of *Lactuca serriola* L. and *Sonchus oleraceus* L. were collected from Thracian valley near to Plovdiv (Bulgaria) during the months September and November in 2012 year. The underground parts were dried and ground into a fine powder.

All used reagents and solvents were of analytical grade scale. Carbohydrate glucose, fructose, sucrose, together with high purity 1-kestose and nystose, used as standards for the identification of low molecular weight oligomers have been purchased from Sigma-Aldrich (Steinheim, Germany). Fructooligosacchrides Frutafit®CLR, HD and inulin Frutafit®TEX were supplied by Sensus (Roosendaal, the Netherlands). Frutafit®CLR contains high level of oligofructoses with the average chain length of 7-9 monomers. Frutafit®HD - with the average chain length of 8-13 monomers. Frutafit®TEX was characterized with mean degree of polymerization DP 22. Inulin Raftiline®HP (DP~25) was purchased from Orafti (Belgium).

Moisture content of the dried ground roots were determined according to AOAC 945.32.

Dried roots of weed plants were extracted in a Soxhlet apparatus successively with hexane, $CHCl_3$, and ethyl acetate to remove phenolic and lipophilic compounds (Olennikov et al., 2009). Then the residue of roots was dried and the extraction process was carried as follows: 0.45 g dry sample (roots) was put into a round bottom flask and was extracted three times with 95% (v/v) boiling ethanol. For the first and the second extraction, 40 ml 95% (v/v) ethanol were used and 20 ml for the third one. The duration of each extraction procedure was 60 minutes. The extracts were collected in 100 ml volumetric flask. The low-molecular carbohydrate fraction composed of fructose and FOSs was obtained in the ethanol extracts. For extraction of high-molecular fraction (inulin), the residue in the flask after ethanol extraction was extracted by three following extractions (40, 40, 20 ml) with boiling water as it was described above. The content of mono-, di-, oligosaccharides and inulin in the obtained extracts was analyzed by TLC in order to observe the extraction rate of fructans.

Thin-layer chromatography (TLC) of the obtained ethanol and water extracts from roots of prickly lettuce and annual sow thistle were performed on silica gel 60 F_{254} plates (Merck, Germany) with *n*-BuOH:*i*-Pro:H_2O:CH_3COOH (7:5:4:2) (v/v/v/v) used as a mobile phase. The spots were detected by dipping the plates into the solution with detecting reagent diphenylamine-aniline-H_3PO_4–acetone (1:1:5:50) (Lingyun et al., 2007) and heating at 120 °C for 5 min. As carbohydrate standards were used glucose, fructose, sucrose, 1-kestose, nystose, fructooligosaccharides (Frutafit CLR and HD) and inulin (Frutafit TEX and Raftiline HP) all of them in concentration 2 mg/ml. Thin-layer chromatograms were generated by densitometry measurement of obtained spots with QuantiScan Version 3.0 software(Biosoft).

The fructan contents in ethanol and water extracts were analysed spectrophotometrically at wavelength 480 nm by resorcinol-thiourea reagent (Pencheva et al., 2012). The experiments were carried out on a Camspec M107 Vis spectrophotometer (UK).

The sugars and FOSs content in ethanol extracts was analyzed by HPLC. Chromatographic separations were performed on HPLC Shimadzu, coupled with LC-20AD pump, refractive index detector Shimadzu RID-10A, a column Supelcosil LC-NH2 (Supelco®, Sigma-Aldrich, Bellefonte, PA, USA) with pore size 5 μm and degasser Waters In-Line –IF (Milfrd, MA, USA). The separations were performed on an analytical aminopropyl silica column SUPELCOSIL LC-NH2 (250 x 4.6 mm i.d.) equipped with a guard column (2.5 x 4.6 mm i.d.) of the same filling. The mobile phase used for separation of glucose, fructose, sucrose and FOSs was acetonitrile/water (83/17 v/v). The column was placed into a temperature-controlled unit LCO 102 (ECOM spol. s.r.o., Czech Republic) maintained at 40 °C. All samples were filtered through a 0.45 μm filter. Injection volume of the sample was 20 μL and the flow rate of the eluent was 1.5 $ml.min^{-1}$ with an isocratic mobile phase. Detection and identification of sugars and fructooligosaccharides were performed using RID detector that operated at 40 °C. The control of the system, data acquisition, and data analysis were under the control of the software program LC solution

version 1.24 SP1 (Shimadzu Corporation, Kyoto, Japan).

RESULTS AND DISCUSSIONS

The moisture content in the taproots of plants prickly lettuce was 8.46% and 10.41 % in the roots of annual sow thistle, respectively.

The results from determination of fructan content in the extracts from the underground parts of prickly lettuce and annual sow thistle were obtained by our developed ketose-specific spectrophotometric method with resorcinol reagent (Pencheva et al., 2012). On the base of our previous investigations of the extracts from dandelion, elecampane and topinambour, our observation during analysis have been shown high levels of low moleculecular fraction in ethanol extracts. Therefore, after ethanol pretreatment of the samples in water extracts have been remained FOSs with longer chain length and inulin. The ethanol and water extracts obtained from *Sonchus oleraceus* L. (8.26 ±0.22 g/100 g dw and 11.30±0.09 g/100 g dw) contained big quantity of low molecular fraction than the same extracts obtained from *Lactuca serriola* L. The ratio between fructans in the ethanol and water extracts from roots of prickly lettuce is almost equal. Therefore, the low and high molecular fractions have been extracted at the same extent. In the result of our study we can conclude that from both plants *Sonchus oleraceus* is richer source of FOSs and inulin than prickly lettuce (Table 1 and Figure 3).

Table 1. Fructan content in the extracts obtained from the taproots of prickly lettuce and sow thistle (g/100 g dw[1])

Plant type	Low molecular fraction (fructose, sucrose & FOS[1])	High molecular fraction (inulin)	Total fructants
	mean ± SD[3]		
prickly lettuce (*Lactuca serriola* L.)	5.39±0.22	4.17±0.50	9.6±0.86
annual sow thistle (*Sonchus oleraceus* L.)	8.26±0.22	11.30±0.09	19.56±0.14

[1]dw – dry weight; [2]FOS – fructooligosaccharides; [3]SD – standard deviation

The obtained results from TLC analysis of the ethanol and water extracts from the roots of prickly lettuce and annual sow thistle showed that extraction process in triplicate was efficient. Almost all carbohydrates presented in the samples have been successively extracted during these sequential extractions with ethanol and water used as solvents. All ethanol extracts (from 8 to 11 and from 16 to 19) contained fructose (R_f = 0.55), sucrose (R_f = 0.48) and FOSs which are equivalent to standards Frutafit CLR (7-9 oligomers) and HD (8-13 oligomers). The TLC analysis of the water extracts from the roots (12, 13, 14, 15, 20, 21, 22, 23) showed the presence not only of mentioned above FOSs, but also these extracts contained high molecular fraction of inulin with DP, similar to these of used as standards Frutafit TEX and Raftiline HP (DP 22-25). The water extracts obtained from the roots of annual sow thistle contained also and sucrose (R_f= 0.48) (Figure 3).

The results obtained from densitometry analysis of the thin-layer chromatograms showed presence of high level of trisaccharides 1-kestose (R_f = 0.37) and tetrasaccharide nystose (R_f = 0.34) in ethanol and water extracts from the roots of prickly lettuce (*Lactuca serriola* L.) and annual sow thistle (*Sonchus oleraceus* L.). Except sugars fructose and sucrose, the extracts contained FOSs like commercial FOSs or inulin, used as standards. Solvent ethanol have been extracted FOSs until 9 monomer units (from GF3 to GF8). In the water extracts except FOSs with GF9 also dominate and high molecular inulin (Figure 4).

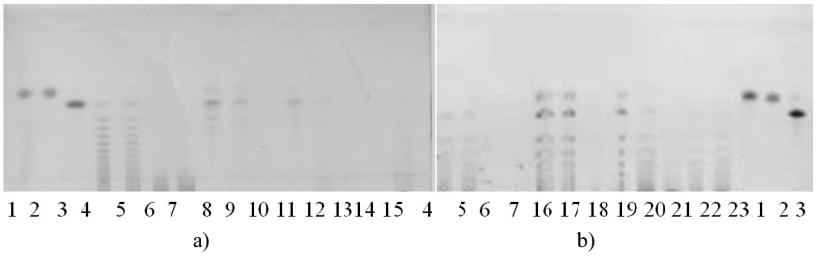

Figure 3. Thin-layer chromatography of fructans in 5 µl ethanol and 5 µl water extracts obtained from plants a) prickly lettuce (*Lactuca serriola* L.) and b) annual sow thistle (*Sonchus oleraceus* L.), standards 1-glucose, 2-fructose, 3-sucrose, 4 and 5-FOSs Frutafit CLR and HD, 6 and 7 - inulin Frutafit, TEX and Raftiline HP; 8, 9, 10, 11 – first, second, third and common ethanol extract from prickly lettuce; 12, 13, 14, 15 - first, second, third and common water extracts; 16,17,18 and 19 - first, second, third and common ethanol extracts; 20, 21, 22, 23 first, second, third and common water extracts from annual sow thistle.

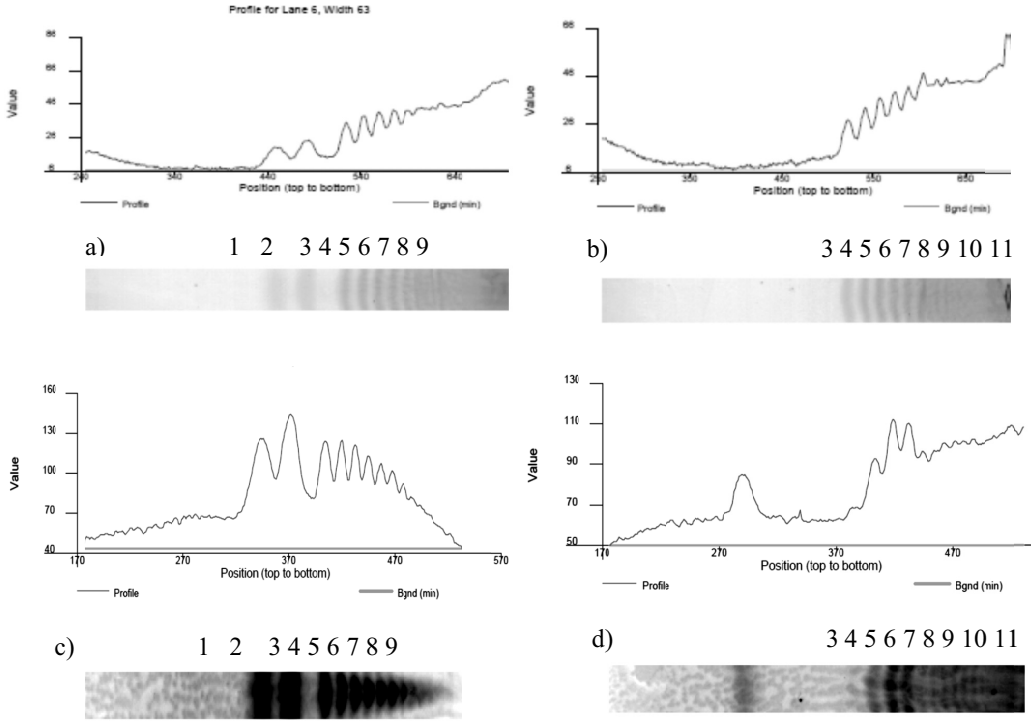

Figure 4. Thin-layer chromatograms of extracts from 5 µl a) ethanol and b) water extracts from roots of *Sonchus oleraceus* L. and 10 µl c) ethanol and d) water extracts from roots of *Lactuca serriola* L., where 1. fructose, 2. sucrose, 3.1-kestose (GF2), 4.nystose (GF3), 5.pentafructooligosaccharide (GF4), 6,7,8,9,10. fructooligosaccharides (respectively GF5, GF6, GF7, GF8, GF9) and 11. inulin

High-performance liquid chromatography with refractive index detection (HPLC-RID) has been widely used for determination of sugars and small oligosaccharides. After the ethanol extracts have been obtained from roots *Lactuca serriola* L. and *Sonchus oleraceus* L. these

xtracts have been analysed by the HPLC coupled with refractive index detector. These analyses help us to determinate the quantity of sugars and FOSs in their roots. The HPLC analysis proved the results obtained from the TLC analysis. The obtained chromatograms showed the presence of fructose (t_R=3,9 min), sucrose (t_R=6,1 min), 1-kestose (t_R=14,1 min) and nystose (t_R=20,9 min) in the ethanol extracts and also showed the presence of glucose (t_R=4,7 min) in them (The HPLC chromatogram of *Lactuca serriola* was not shown) (Figure 5).
The obtained results from HPLC analysis showed that the ethanol extract from roots of *Sonchus oleraceus* L. contained more 1-kestose and nystose (1.25 and 1.28 % dw, respectively) than prickly lettuce (Table 2).

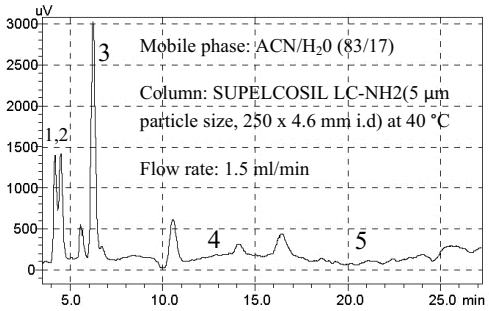

Figure 5. HPLC chromatograms of 95% (v/v) ethanol extracts of a) prickly lettuce *(Lactuca serriola* L.*)* and b) annual sow thistle (*Sonchus oleraceus* L.): 1.fructose, 2.glucose, 3.sucrose, 4.kestose and 5.nystose.

Table 2. Mono- and oligosaccharides content (% d.w) in the ethanol extracts obtained from the roots of *Lactuca serriola* L. and *Sonchus oleraceus* L.

Plant	fructose	glucose	sucrose	1-kestose	nystose
prickly lettuce (*Lactuca serriola* L.)	1.78	0.91	2.23	0.80	0.65
sow thistle (*Sonchus oleraceus* L.)	2.03	1.31	3.92	1.25	1.28

Lactuca serriola L. and *Sonchus oleraceus* L. contains in their roots high amount of fructooligosaccharides. The results of our research showed that the underground parts of annual sow thistle is rich source of trisaccharide kestose, tetrasaccharide nystose, FOSs and inulin. All these inulin-type fructans possess well-pronounced prebiotic effect. These taproots could be used in feed and foods to increase the dietary fiber content in them. Our research explained and proved the statement of Jana et al. (2010) that *Sonthus oleraceus* L. possess the best prebiotic effect and stimulate growth of *B. longum*.

CONCLUSIONS

The results from our analysis of the ethanol and water extracts obtained from the roots of *Lactuca serriola* L. and *Sonchus oleraceus* L. showed that these plants contain inulin-type fructan. Because of the absence of information in literature about the fructooligosaccharides and inulin contents in their underground parts for us it was a challenge to investigate these weed plants eaten as a salad in some countries in the world. The roots of annual sow thistle (*Sonchus oleraceus* L.) contains much more total inulin-type fructans (19.6 g/100g dw) than the roots of *Lactuca serriola* L. *(*9.56% dw) The levels of 1-kestose and nystose are higher in the ethanol extract of the annual sow thistle. The both plants are rich source of fructooligosaccharides that are in much more content in the ethanol extracts. The water extracts contain high molecular fructooligosaccharides and inulin. The findings of the current study showed that these two widespread weed plants are potential source of fructooligosaccharides (DP 3-5) and can be used as a new source of prebiotics that can find application in human or animal nutrition.

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STUDIES ON IMPROVING LOCAL SHEEP BREEDS FERTILITY IN EGYPT

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Abstract

Egyptian local sheep breeds are subtropical fat-tailed ,sheep characterized by satisfactory fertility and ability to breed all year around, but have low prolificacy and growth rate. The breeding objectives were to increase the prolificacy of Ossimi and Rahmani breeds usually in the small holders and in small farm conditions. There was no formal study to set the breeding objectives. The three main breeds of sheep in Egypt are Rahmani, Ossimi and Barki. It was recommended for farmers to exchange rams and increase crossing among the three local breeds. Crossing with outside breeds such as Awassi, Cyprus and finn. The first cross was back-crossed to the local to produce1/2 Finn ¾ local from each breed group. The cross animals were mated for some generation, and involved in a selection programmed to establish a new breed type with better lamb production. The first cross ewes had a slightly better fertility conception rate than the local ewes. Crossed ram lambs performed better in pubertal age than purebred parents. Semen quality at first ejaculation had poor quality, while it was good at 80% semen motility score. Different research experiments were conducted and many additives were used to improve preserved semen quality such as dyes, Caffeine, Sugars, aromatic compounds chelating agents, antioxidants, selenium, seminal plasma, Soybean and injecting different types of Hormone. Licorice contains many photochemical which have ameliorating effects on semen quality and considered as a versatile additive. Fish oil was also used as supplementation and could improve semen quality and the conception rate and enhanced ram fertility.

Effect of different dietary energy levels were also investigated on some blood and seminal constituents and semen quality of Rahmani rams. High and low dietary energy were 2615, 3138 and 2092 Kcal. ME/Kg on DM basis. Most semen physical characteristics were high LE fed rams but did not differ significantly. Effect of (50 ug IM of) GnRH injection/week on semen ejaculate characteristics in rams collected in breeding and non-breeding seasons. GnRH treatment improved libido, semen volume, sperm numbers, but not viability. More over the administration of 80 mg (Recombination Bovine somatotropin rbST), at 14 day interval starting at 2 month of age, improved growth performance puberty characteristics and semen physical characteristics of male rams.

In another trail, in the hot season of the year, animals were treated with selenium (0.1 mg/DM as sodium salient) orally ,injection with melatonin 25 ug/Kg body weight daily at sunrise, and prostaglandin F2 (PG F2,3 mg/head one hour before collection of semen. Ram injected with prostaglandin F2 surpassed ($p< 0.05$) the control in sperm motility, Rams treated with selenium had lower significantly ($P< 0.05$) semen PH. Blood components in ram, were insignificant affected by selenium ,melatonin or PGF2 treatments. Research plans, programs and factors influencing the Egyptian local sheep breeds fertility of stored semen and methods used for improvements are discussed.

Key words: *local breeds , semen additives, Diluents.*

INTRODUCTION

Sheep with its multi facet utility (for meet, wool, skin, manure and to some extent milk) play an important role in Egyptian animal meet production.

They are better adapted to arid and semi-arid tropics with marginal and sub-marginal lands, otherwise unfit for crop production (Mousa, 1991; Osman, 1985) and (FAO, 2000).

The three main breeds of sheep in Egypt are Rahmani, Osseimi and Barki. Rahmani is the largest breed, easily identifiable by its red wool and small ears.

The Osseimi is slightly smaller, with white wool. Barki is the smallest breed, with white wool and a brown neck. Purebred Barki is the breed of choice for Bedouins in the desert.

All are fat-tailed sheep. What distinguishes fat-tailed sheep from other sheep is their long tails, filled with fat and having a function similar to the camel's hump.

Figure 1. Rahmani Sheep

Fat-tailed sheep are hardy and adaptable, able to withstand the tough challenges of desert life. When feed is ample and parasites not burdensome, fat-tailed sheep can be impressive in size, growth, and conformation. Carcass quality is good, with most of the fat concentrated in the tail region. The carcass and meat are preferred by Moslems.

The wool from fat-tailed breeds is coarse and frequently has colored fibers. It would be of limited value in world markets. It is used primarily for rug making and other cottage-type industries. Some shepherds sell their wool clip, while others give it away to the shearer. The Bedouin women make beautiful rugs and blankets from the wool.

Some of their handiwork can be purchased in the villages (Shoenlan, 1996).

Shearing is done once or twice a year with hand clippers. There is a reluctance to use electric shears because of wool quality and the difficulty in getting replacement combs and cutters. With the tropical climate, sheep and goats breed year-round, typically producing three crops in two years. Twinning is common in goats, but quite variable in sheep, with considerable room for improvement (Marai et al., 2008; Marai et al., 2003; Mousa, 1991).

Some shepherds have only a few sets of twins, while others claim to have a majority of twin births. Limited selection is practiced for reproductive rate. Given the difficult environmental conditions, not all farmers are convinced they want multiple births. Nonetheless, we encourage selection for twins, as reproductive rate is one of the most important factors affecting profitability (Osman, 1985).

Rams typically run with ewes all year round, making it difficult for farmers to plan breedings, flush ewes and feed according to stage of production. Some of the better farmers have started to separate rams and have defined breeding seasons. Sheep are inbred, which depresses performance and fixes negative traits in the flock (FAO, 2000; Ferial et al., 2000).

It was recommended for farmers to exchange rams and increase crossing among the three local, fat-tailed breeds. Crossing with outside breeds such as the Awassi, but only if the breed is adapted to the desert. Other breeds have been tried and failed in this environment (Aboul Naga, 1995).

The Mediterranean subtropical Chios (C) sheep are known for their high prolificacy, early sexual maturity and good milk production. Their usefulness for improving sheep production in the subtropics where prolific temperate breeds performed poorly as purebreds has been reported (Aboul Naga, 1995; Aboul Naga et al., 1997; FAO, 2000). A crossbreeding program was carried out by the Ministry of Agriculture in Egypt between the imported C sheep from Cyprus and both Ossimi (O) and Awassi (A) sheep. Production performance of these breeds and their crosses has been described by (Aboul-Naga and Abdoul-Ela, 1985; Ferial et al., 2000).

Animal breeding and reproduction have a great contribution to make to a future sustainable animal agriculture.

Figure 2. Crossbred Barki Lambs

Many opportunities are open to the animal breeding and reproduction sector for improving the biological and economic efficiency of food production and increasing food supply. These

opportunities are dietary energy is the most important factor which affects semen quality in farm animals (Lysandrides, 1981). The deficiency of dietary energy causes delay of animal puberty, suppresses libido and sperm production (Yassien, 2009). Also, the amount of the mutton depends mainly on the reproductive performance of the rams.

Alleviation of heat stress may be achieved by ameliorating the environment, reducing the animal′s heat production and/or helping the animals to dissipate the heat load. The latter includes physical, physiological and nutritional techniques (Marai et al., 2003). In that respect, melatonin hormone and prostanglandins can be used as physiological techiniques and selenium can be used as nutritional technique. This is attributed to that selenium acts as a component of the enzyme catalyzes the degradation of organic hydroperoxdase. Regarding the melatonin hormone, it can be used ton increase fecundity. The prostaglandins have a role in increase of testicular contraction with consequentpro motion in the release and progression on spermatozoa towards the epididyms (Marai et al., 2003).

Figure 3. Barki sheep

Many additives were used to improve preserved semen quality such as: dyes, caffeine, sugars, and aromatic compounds chelating agents, antioxidants, selenium, seminal plasma and soybean (Leeuw et al., 2000; Mohamed El - Sharawy et al., 2003).

Licorice contains many photochemical which may have ameliorating effects on semen quality , so this study was designed to investigate the possible effects of licorice extract addition to the diluter on sperm motility of chilled stored ram Licorice is the name applied to the roots and rhizomes of *Glycyrrhiza* sp ecies and has been used for medicinal purposes for at least 4000 years (Ibrahim, 2010).

Glycyrrhiza glabra L. is one of the very important nutraceuticals, contains some 400 bioactive phytochemicals and has manydocumented bioactivities such as: steroid like activity , powerful antioxidants activity, antibacterial activity and antiviral activity (Ibrahim, 2010).

The biggest obstacle in the exploitation of frozen ram semen is that freezing and thawing reduces motility and membrane integrity, which leads to poor fertility following cervical AI. Extensive research has been conducted in the last few decades on ram semen diluents, semen processing, freezing and thawing methods for improving the post-thaw viability and membrane integrity of motile sperm cells (Mohamed El − Sharawy et al., 2012). Techniques such as administration of recombinant bovine somatotropin (rbST) could lower the cost of production in farms. Bovine somatotropin (bST) is a growth hormone (GH) produced by cow pituitary gland and of importance to growth, metabolism, lactation and reproduction of all animals (El-Gohary et al., 2011). The effects of using bST have been studied in sheep (Shahin et al., 2004), dairy goats and and dairy cattle (Yassien, 2009).

Many experiments have been performed to study the effects of somatotropin (GH) on the reproductive functions of cattle, but few were carried out to study its effects on sheep and goat. Somatotropin (ST) plays an important role in the reproductive process (spermatogenesis and steroidgenesis), where its receptors were found in leyding and sertoli cells, vas deference, prostate gland, epididymis and seminal vesicles (Marai et al., 2003). The effects of rbST on reproduction was related to rbST dose, time of starting treatment, breed and other factors such as nutritional status and milk production (Shahin et al., 2004). GnRH has been used in rams, bulls, boars and stallions to increase sperm numbers in the ejaculate (Azawi et al., 2012).

In addition to increased sperm numbers in the ejaculate following GnRH administration, some researchers noted that treated animals had a greater libido at the time of semen collection (Azawi et al., 2012). Libido was assessed using

quantifiable observations, such as time to initial false mount and time to ejaculation in buffalo, and time for collection in rams (Leeuw et al., 2000).

Initially, the effects on reaction time and collection time were attributed to hypothalamic-pituitary-testicular effect following GnRH administration. A great deal of attention also has recently been given to the essential roles of polyunsaturated fatty acids of sperm membrane.

Semen from all domestic species contains high levels of polyunsaturated fatty acids, in particular, docosahexaenoic acid (DHA) and docosapentaenoic acid (Awad and Graham, 2004). It was claimed that DHA is an essential component of healthy sperm cells, enhancing membrane integrity and tail flexibility, as well as increasing output.

Moreover, Awad and Graham, 2004, reported that ram spermatozoa are especially rich in DHA, which competes with arachidonic acid for the sn-2 position in membrane phospholipids. There is also evidence that the lipid and fatty acid compositions of chicken sperm play important roles in maintaining semen quality (Shahin et al., 2004).

The studies of specific requirement for DHA by sperm cells has focused attention on the required physical structure that promotes fertility and their potential association with tissue DHA content e.g. a positive correlation between the state of polyunsaturation and membrane fluidity and function (Azawi et al., 2012). DHA is the predominant fatty acid in the sperm and was highly correlated with sperm motility (Chinoy, 1972) and other semen characteristics and freezability (El-Darawany, 1999).

As Fish oil is rich in polyunsaturated fatty acids mainly DHA (Abd El-Razek, 2009), this study was to investigate the effect of oral fish oil supplementation on the fresh and frozen semen. A number of commercially available extenders containing a substitute for egg yolk have been used for the preservation of bovine, ovine, ram and caprine semen by a number of workers. Ram spermatozo diluted in soya lecithin based extender Bioxcell maintained the sperm quality and produced acceptable fertility rates (Abd El-Razek, 2009).

MATERIALS AND METHODS

Experiment I

Plan was to cross the local ewes with the imported Finn rams. The first cross was back-crossed to the local to produce 1/4 Finn 3/4 local (1/4 F 3/4 L) from each breed group. The 1/4 F 3/4 L cross was either inter se mated, for some generations, and involved in a selection programme to establish a new breed type with better lamb production, or utilized as a dam breed to be mated to terminal size (Suffolk cross) to produce fat lambs. The 1/4 F 3/4 L was thought to be more suitable as a crossbred group for the prevailing conditions based on the following criteria:

- Their prolificacy would not be too high and ewes could be managed easily by the farmers.
- Ewes could stand the prevailing environmental conditions better than the crosses with higher Finn blood.
- The ewes' ability to breed at different times of the year was expected to be closer to the local sheep.
- Sheep have a reasonable size fat tail which is a determinant factor in consumer preference and price in the market.
- The genotype could be easily produced by using the 1/2 Finn rams, produced on state farms, on the breeders flock.

Experiment II.

Two types of diluters were used; egg yolk- tris (EYT) (Shahin et al., 2004) and yolk- glucose-citrate (Shoenlan, 1996). Licorice extract powder (levels of 1, 5, 10, 50 and 100 µg per ml. of diluter. Diluter containing no licorice extracts served as control (0). Diluters were prepared the day prior to use, allowing large participle to settle overnight at 5˚C, so that the supernatant could be used. Before use, each diluter was warmed to 37 ˚C.

Experiment III.

Four experimental extenders, i.e., tris-egg yolk (TEY) and 3.0 gr.tris; 80 ml distilled water; fructose 0.2% wt/vol; egg yolk 20%), egg yolk-citrate (EYC) (2.9 g sodium citrate 80 ml distilled water; fructose 0.2% wt/vol; egg yolk 20% v/v), milk extender (MILK) (10 % skim milk and Bioxcell were used in this study.

Antibiotics including gentamycin sulphate (500 µg/mltylosin tartrate (100 µg/ml; lincomycin

hydrochloride (300 µg/ml; and spectinomycin hydrochloride (600 µg/ml; were added to TEY, EYC and MILK extenders.

Using artificial vagina (42°C) semen was collected (two consecutive ejaculates/raml/ week) for a period of 3 weeks. The semen was transferred to laboratory within minutes of collection. Visual motility was assessed microscopically (at 200x) with and sperm concentration was determined by Neubauer haemocytometer. sperm/ml of the ejaculate were selected for further processing., the qualifying ejaculates were pooled and held for 15 min at 37°C in a water bath before dilution. Pooled semen was split into four equal aliquots for dilution in four different experimental extenders.

Semen aliquots were diluted at 37°C with one of the four experimental extenders. Sperm quality assays including sperm progressive motility, sperm viability (Live/dead percentage), sperm plasma membrane integrity, sperm acrosomal integrity and sperm abnormalities were unstained as live. Sperm plasma membrane integrity (PMI) was assessed by hypo-osmotic swelling (HOS), assay abnormalities semen samples (100µl) was fixed in 500µl of 1% formal citrate (2.9 g tri-sodium citrate dehydrate, 1 ml of 37% solution of formaldehyde, dissolved in 100 ml of distilled water) and one hundred spermatozoa were examined with a phase contrast microscope (X 1000) under oil immersion. Normal acrosome was characterized.

Experiment IV

Six rams aged 2 years were used. The experiments were performed in October and November in the breeding season. Semen was collected, samples showing less than 70% motility were discarded. After the spermatological characteristics of each ram were determined, the ejaculates were pooled and diluted at a 1:4 ratio (semen:diluent) at +37 oC with Tris extender. The diluent contained tris (hydroxymethyl) aminomethane (3.63 g), glucose (0.50 g), citric acid (1.99 g) and egg yolk (15%).

Diluted semen was cooled gradually to +4^{o}C within 2 h. Cooled semen was split into 6 parts and different amounts of ascorbic acid (0 (control), 0.5,1, 2, 5 and 10 mg/ml) were added to each group. Motility and pH were evaluated 0, 2, 4, 8, 16 and 24 h after dilution.

Diluted and cooled semen was split into 20 parts. Each part was diluted at a 1:1 ratio with one of the extender groups containing different proportions of glycerol (0%, 1%, 3%, 5% and 7%) and ascorbic acid (0, 0.5, 1, 2 mg/ml). Extended semen was packaged in 0.25 ml French straws. The semen was allowed to equilibrate for 4 h before freezing. The semen in straws was frozen in liquid nitrogen vapor. They were thawed in a water bath at +38^{o}C for 25 s.

Motility, dead spermatozoa rates, damaged acrosome rates and total abnormal spermatozoa rates were determined following cooling to +4 oC, glycerolizatione equilibration and freezing-thawing.

Experiment V.

Six rams of local breed (Rahmani and Ossimi) 2–4 years old were used for semen collection by artificial vaginaOn each day, ejaculates, from three different rams, containing greater than 70% progressive motility were pooled together and considered to be one sperm sample. For this experiment 10 sperm samples were extended, frozen, thawed and analyzed. Sample was split into three aliquots for freezing: 0.25 ml straws(control or in pellets on the cold surface of paraffin wax or on the cold surface of cattle fat. Pellet blocks were cooled by immersing the aluminum boxes in liquid nitrogen for 30 s and placing the block horizontally in liquid nitrogen vapor 3 cm above the surface of liquid nitrogen. Volumes of 0.1 ml, of equilibrated spermatozoa, were dropped into the depressions on the surface of each pellet block. After 10 min in the liquid nitrogen vapor, the pellet blocks were immersed in liquid nitrogen and the pellets packaged in small goblets for storage at -196^{o}C.

The surface temperature of the pellet blocks was determined by making a 1mm deep hole in the wax or fat using a blunt 22 gauge needle. A thermocouple was inserted into the hole and sealed in place with 10 _l of melted wax or fat, respectively. The pellet blocks were then immersed into liquid nitrogen for 30 s, and then placed in the freezing apparatus, 3 cm above the liquid nitrogen surface and the temperature of the blocks measured at 1 min intervals for 15

min. For these experiments, no spermatozoa were placed onto blocks, and block temperatures were monitored 5 min longer than blocks containing spermatozoa would have been plunged into the liquid nitrogen. In a second experiment, paraffin wax and cattle fat blocks were immersed in liquid nitrogen for 5 min prior to being placed in the freezing apparatus, and the temperature of the blocks measured for 15 min at 1 min intervals.

Experiment VI

A total number of 20 rams of Egyptian Suffolk sheep were used, during May-July months. The rams were 1.5-2.5 years of age and 60-70 kg body weight. The animals were divided into four groups of nearly equal average weights. Each group was of 5 rams. The first group was kept without treatment as control. The first second group was treated with selenium (0.1 mg/ kg DM as sodium selenite) orally. The third group was injected with melatonin (25 µg/ kg body weight. Daily at sunrise; melatonin was dissolved in a minimum of absolute ethanol and diluted in 0.9 NaCl 1:9) and the fourth was injected with Prostaglandin F2α (PGF2α; 3mg/head, one hour before collection of semen).

RESULTS AND DISCUSSIONS

I-Reproductive performance of the Finn ewes

The first results on the reproductive performance of the Finn crosses with either 0 or R local ewes were reported by[4] Prolificacy, expressed as number of lambs born/ewe lambed, increased by 0.68 and 0.70 in the Finn-Rahmani (FR) and Finn-Osimi (FO) first cross, respectively, and by 0.17 and 0.27 lambs in 1/4 F 3/4 R and 1/4 F 3/4 0, respectively, over the local ewes It should be noted that the latter group were 2-3 years old and 2-5 years in the first cross and 2-9 years for the local ewes. Although age of ewe was included in the model adopted for analyzing the data there could however be a confounding effect between age and breed groups.

The most interesting result is that the Finn crossbred ewes showed better fertility than the local ewes at different seasons of mating which resulted in a higher figure for number of lambings/ewe/year. Such performance resulted in a detectable improvement in annual number of lambs produced/ewe in the Finn crosses over the local ewes; 1.25 and 0.80 lamb for FR and FO and 0.19-0.44 and 0.34-0.55 lamb for 1/4 F 3/4 R and 1/4 F 3/4 0, respectively rebreed each 8 months and that 1/4 Finn ewes are expected to show better performance when they have attained maturity.

- Cross-breeding programs involving crosses with specific breed combination are difficult to sustain at the farmer level. A range of combinations should be envisaged, e.g. in the present program a 12-37 percent range would be allowed and probably investigated rather than the 25 percent F genetic;
- A structure must be established to guarantee the flow of the desired genotypes. In the present case, non-sustainability evolved as it depends mainly on state institutions to provide the exotic genotype;
- Enhancement of improved cross-breeding genetic material should be accompanied by access of breeders to inputs, e.g. regular availability of feed stuff;
- Phenotypic characters of local breeds involved in the consumer preference and consequently in market price, should be taken into consideration in the cross-breeding programs with exotic breeds;
- A lower portion of the exotic temperate blood seems more suitable for crosses in subtropical conditions.

II.

Progressive sperm motility, sperm viability, sperm plasma membrane integrity and NAR were significantly ($P < 0.05$) higher for BIOX, MILK, and TEY extenders at 1st, 3rd and 5th day of storage compared to EYC extender. Moreover, progressive sperm motility, sperm viability and sperm plasma membrane integrity were not affected up to third day of storage in BIOX extender and at 5th day of storage the values for these parameters remained significantly ($P < 0.05$) higher in BIOX compared to other extenders. Sperm abnormalities (head, mid piece and tail) did not differ among the different extenders

III.

Motility increased significantly ($p < 0.01$) in levels of licorice extract 1, 5, 10,50 and 100 µg / ml in both diluters, during all storage periods. The means of progressive motility were 72.5 ±

1.02 %, 72.08 ± 1.05, 70.90 ± 2.05 % and 66.25 ± 3.15 % respectively, compared to the control (0) 61.45 ± 16.2 %. (fig1). Levels 1, 5 and 10 µg /ml were superior ($p< 0.01$) to levels 50 and 100 µg /ml (fig1). Diluter type had a significant effect ($p < 0.01$) on sperm motility. Overall the percentage of motile sperm in EYT diluter (66.48 ± 1.21 %) was higher than that in yolk-glucose citrate diluter (64.37± 1.44 %).

Sperm motility tended to decline significantly ($p < 0.01$) as the length of storage period increased. The means of progressive motility were 80.00 ± 2.04 % after dilution (0h), 68.75±3.15 % 61.25 ± 4.27% and 50.62 ±4.61 %, at 24, 48 and 72 h after cooling, respectively. The study findings may contribute to the recent attempts to design defined semen diluter and move away from animal-based cryoprotectants, which may pose hygienic risks and are difficult to standardize..Finally there are several factors affecting the phytochemistry of the licorice root such as geographical location, soil condition, time of harvesting and the environmental factors and this should be considered when applying such treatment widely.

In conclusion, the addition of licorice extract to the diluter improved ram sperm progressive motility during cooled storage at 5˚C.

IV.

In the preliminary study, ascorbic acid at concentrations of 0.5, 1 and 2 mg/ml in diluent during the storage of semen at +4^{o}C did not affect the motility of spermatozoa or pH ($P > 0.05$) compared to the control group. However, ascorbic acid at concentrations of 5 and 10 mg/ml in the diluted semen significantly decreased ($P < 0.05$) motility and pH. In the main experiment there was no significant difference in motility, acrosomal integrity, total abnormal spermatozoa rate or dead spermatozoa rate depending on the increase in the proportions of ascorbic acid in the diluted semen groups containing the same glycerol levels after equilibration.

The percentage of progressively motile spermatozoa in the A1 control group (without glycerol and ascorbic acid) was 79.0 ± 0.77% after equilibration, and the increase in the glycerol level significantly decreased motility in the C4, D4 and E4 groups compared to the A1 control group.

V.

Fish oil supplementation with the different doses affected the semen physical parameters (The ejaculate volume, sperm cell concentration, sperm motility and live sperm percentages of rams in all fish oil groups were significantly ($P<0.05$) increased during treatment period than that of the control rams. However, the percentage of sperm abnormalities was significantly reduced in fish oil treated groups compared to that for control one. Treatment with fish oil led to increase seminal plasma proteins which adsorbed into the cold-shocked ram sperm surface and that this adsorption is able to reverse the membrane alterations induced by cold-shock and maintain high percentage of frozen thawed ram sperm motility.

VI.

Semen physical characteristics:

Semen physical characteristics of ram lambs in control and treated groups during different stages of puberty are presented in Table 1. Ejaculate volume, percentages of initial gross motility, sperm livability and abnormality percentage improved ($P<0.05$) by injection of 80 mg rbST.

On the other hand, sperm cell concentration was not affected by rbST treatment. Results agree with Fukui, 2008, who reported an increase ($P<0.05$) of semen ejaculate volume, percentage of live sperm and total sperm output and decrease ($P<0.05$) in abnormal spermatozoa in mature rams injected with 100 mg rbST five times with 14 days gap. El-Gohary et al., 2011, reported a decrease ($P<0.05$) in sperm abnormalities percentage and an increase of sperm output from Simmental sires injected with 640 mg rbST seven times every 14 days gap. Moreover, injection with rbST (5 injections, 100 mg/ male at 14 day-intervals) improved semen quality and ejaculate volume of rams (Azawi et al., 2012) and goats (El-Darawany, 1999) reported that all physical characteristics of Friesian bull semen improved ($P<0.05$) by rbST treatment (Table 1).

Table 1. Semen characteristics of first ejaculate containing spermatozoa of ram lambs in rbST and control groups

Characteristics	G1	G2	Sign.
Ejaculate volume (ml)	0.26±0.12	0.32±0.11	*
Initial gross motility (%)	43.6±0.13	50.6±0.10	*
Live sperm (%)	41.3±0.12	50.2±0.12	*
Abnormal sperm (%)	17.2±0.12	10.6±0.10	*
Sperm concentration (x109/ml)	1.23±0.12	1.42±0.12	NS

Significant at P<0.05. NS: non-significant.

The use of GnRH treatment in conjunction with semen collection has been shown to optimize the number of spermatozoa/ejaculate in the ejaculate of the Awassi ram semen. This result is in agreement with the findings of Shahin et al., 2004, who found to optimize the number of spermatozoa in the ejaculate of the bull.

CONCLUSIONS

Reproduction is directly affected by various management related factors. Manipulation of these factors can cause changes in reproductive performance. The control and manipulation of the sheep reproduction has been the objective of scientists around the world for many years.

The crossbred ram lambs reached puberty at younger ages and heavier weights than local ram lambs. Lambs seem to be adversely affected by the environment in Upper Egypt, resulting in slower growth rates than those reported in their home country. The evidence does not show crossbreeds having better semen properties at puberty. However, by the time the sperm had reached 80% motility, ejaculation volumes in the two crossbreeds and C ram lambs were significantly higher (P<0.05) than in both O and A ram lambs. These differences may be due to the variation in testicular size. This study shows that crossbreeding with the highly fertile Chios subtropical breed can considerably improve the early breeding performances

Artificial insemination (A.I.) is a reproductive method of great influence in the improvement of productivity. The advantage of A.I. has to be coupled, however, with an appropriate reproductive rate. That means that the A.I. must not have a detrimental effect on conception and lambing rates. The effort of the research centers has been focused on increasing the viability of the semen doses, especially testing different diluents.

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DYNAMIC STUDIES IN BROILER CHICKEN NATURAL IMMUNE FACTORS

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Abstract

Natural immune factors are the first defense mechanism against variety of pathogenic agents. Both the blood serum lysozyme and alternative pathway of complement activation play significant role in stock animals′ welfare. In this study we decided to investigate the dynamics of the aforementioned humoral factors among two of the most popular broiler chicken hybrids – Ross and Cobb. Both hybrids show relatively high concentrations of blood serum lysozyme during the first week of life (Ross-7,79 and Cobb-11,06 mg/L). Which could be explained by the small amounts of yolk sack, left from the egg embryo. During the second week the levels of blood serum lysozyme lowers dramatically. Through the next weeks the concentration of blood lysozyme increases gradually among both hybrids. The comparative analysis show faster and higher levels in favor of the Ross hybrid. The dynamics of the alternative pathway of complement activation (APCA) were relatively similar. Both hybrids have moderate levels of APCA activity through the first week (Ross-325,32 CH50 and Cobb – 346,94 CH50), which lowers in the second week of live and grows gradually trough the life of the bird. Compared with the Cobb, the Ross hybrid exhibited higher levels of APCA, which gives these birds better protection against pathogens. Similar results for both factors of interest, were obtained among the hybrids′ parent flocks. The results of our experiment unambiguously show better humoral immunity protection in favor of the Ross hybrid.

Key words: *Chicken hybrids, Complement system, Lysozyme.*

INTRODUCTION

Nowadays stock animals are bred for fast growth and maximal production. The huge populations reared at relatively small spaces increase the risk of spontaneous infections. Despite the use of different vaccine programs, the mortality rates in chicken farms are relatively high. Innate immune factors such as phagocytosis, complement, beta lysins, lysozyme, interferon etc. play significant role in animal′s protection against variety of pathogens. Blood serum lysozyme is an important factor of the humoral immune response. The serum lysozyme protects the host organism against variety of Gram negative bacteria and some big viruses, such as the *Avipoxvirus* (Nguyen-Huu, 1979; Zyczko and Zyczko, 1998).

Evolutionary the complement system is one of the oldest defense mechanisms against pathogenic bacteria. The alternative pathway of complement activation (APCA) does not require the complex antigen-antibody, so it plays significant role in the first minutes after infection. It is quite efficient against Gram negative bacteria, viruses, neoplastic cells etc. hence it plays significant role in animals′ protection mechanisms (Mueller-Ortiz, et al., 2004; Paape and Capuco, 1997; Zhu et al., 2005).

It has been discovered that both serum lysozyme concentrations and APCA activity play significant role in chicken defense mechanism against *Eimeria tenella* infections. This data combined with the protective effect against bacterial and viral agents defines the two factors of innate immune response as critical for livestock animals (Koinarski and Sotirov, 2005).

Breed, species, age and sex have been evaluated as factors with huge impacts on the levels of blood serum lysozyme and APCA activity (Koynarski and Sotirov, 2012; Sotirov et al., 2011; Sotirov et al., 2007).

The importance of these two innate immune factors motivated us to investigate their variations and dynamics among two of the most common broiler chicken hybrids – Ross and Cobb. Additionally to the broiler flocks, we decided to investigate both factors of humoral immunity among hybrids′ parent flocks.

MATERIALS AND METHODS

Blood serum complement and lysozyme concentrations were analyzed in broiler chicken from the popular Ross and Cobb hybrids, reared in private farms. Samples were collected during the six week growing period of the broiler chicken, where 25 samples were collected for every week of life of the birds. Additionally we analyzed the target immune factors among both hybrids parent flocks between the 45th and 55th week of age. Twenty five samples from each parent flock were collected every five weeks from the aforementioned period of time. The total number of investigated samples was 450. Blood for analysis was collected aseptically from *v. ulnaris* with disposable needles in plain vacuum tubes after fixation. Blood was transported in cool bags at 6^{o}C.

The activity of the alternative pathway of complement activation (APCA) was assayed by the method of Sotirov (1991). Each serum sample was first diluted by mixing 100µl serum with 350 µl veronal-veronal Na buffer (in final concentrations: 146 mM NaCI, 1,8 mM 5,5-diethylbarbituric acid sodium salt; 3,2 mM 5,5-diethylbarbituric acid; 1 mM EGTA and 0,8 mM MgCl2). In U bottomed plates (Flow Laboratories, UK), 7 other dilutions from each diluted serum were again prepared in veronal-veronal Na buffer: 80 µl diluted serum + 20 µl buffer, 70 µl diluted serum + 30 µl buffer, 60 µl diluted serum + 40 µl buffer, 50 µl diluted serum + 50 µl buffer, 40 µl diluted serum + 60 µl buffer, 30 µl diluted serum + 70 µl buffer and 20 µl diluted serum + 80 µl buffer. The final serum dilutions were, respectively, 8/45, 7/45, 6/45, 5/45, 4/45, 3/45 and 2/45. Then 50 µl buffer and 100 µl of 1% rabbit erythrocyte suspension were added to each well. After incubation for 1 hour at 37°C, samples were centrifuged at 150 g for 3 minutes at room temperature (23°C). Thereafter, 150 µl of each supernatant was removed and placed in flat bottomed plates for measurement of optical density at 540 nm using 'Sumal-PE2' ELISA reader (Karl Zeiss, Germany). The final APCA activity was calculated using special computer programs developed in the Trakia University, and expressed as CH50 units (CH50 units correspond to 50% of complement induced haemolysis of applied erythrocytes).

Blood serum lysozyme concentrations were assayed by the method of Lie (1985). Twenty ml of 2% agarose dissolved in phosphate buffer (0.07 M $NaHPO_4$ and NaH_2PO_4) was mixed with 20 mililiters suspension of 24-hour culture of Micrococcus lysodeicticus at 67°C. The mixture was poured out in 14-cm Petri dish. After solidifying at room temperature, thirty-two 5-mm wells were made with a special device. Fifty microliters of undiluted sera were pipetted in each well. Eight standard lysozyme dilutions (from 0.025 to 3.125 µg/ml) were prepared and pipetted in weight wells. The samples were incubated for 20 hours at 37°C and lytic zone diameters were measured. The final lysozyme concentrations were calculated by special software developed at the Trakia University.

Data were processed by one-way analysis of variance (ANOVA) with the fixed effect model using Data analysis tool pack, Microsoft Excel 2010, Microsoft Corporation Ltd.

RESULTS AND DISCUSSIONS

The analyzed data for the blood serum lysozyme concentrations among the broiler hybrids is presented on table 1. As seen from the table, both hybrids show extremely high lysozyme concentrations through the first day after hatching. These results unambiguously exhibit the effect of the remaining small amounts of yolk in the first few days of life. The concentration of serum lysozyme was much higher among the Cobb hybrids ($P < 0.01$), compared with the Ross flock. Through the next two weeks of life, both hybrids show decrease of the lysozyme concentrations (about 50%), compared with the first week ($P < 0.05$). Significant differences between the hybrids at this stage were not detected. From the forth to sixth week both hybrids exhibit growth in the lysozyme levels, which indicates activation of animals′ genetic resources. In the fourth week of life (day 22) the Ross hybrid shows significant increase of the serum lysozyme levels (15.72 mg/L). The Cobb flock also exhibited higher concentrations (10.03 mg/L), but levels were in favor of the Ross hybrid ($P < 0.01$). For the last two weeks of life the Ross

flocks show almost constant levels of lysozyme concentrations, while the Cobb hybrid exhibited significant reduction in the fifth week of life, where the lysozyme levels were also in favor of the Ross hybrids ($P < 0.01$).

Table 1. Dynamic analysis of blood serum lysozyme concentrations (mg/L) in broiler chicken hybrids for six week growing period

Hybrid	Ross	Cobb
Age	X ± Sx	X ± Sx
1 day	7.79±0.24^{abc1}	11.06±0.12^{jmq1}
8 days	4.27±0.21^{def}	4.54±0.18^{jkl}
15 days	6.24±0.08^{ghi}	6.04±0.11^{mno}
22 days	15.72±0.14^{cdg2}	10.03±0.22^{knp2}
29 days	10.33±0.13^{aeh3}	6.66±0.06^{pqr3}
36 days	12.37±0.07^{bfi}	12.89±0.10^{lor}

$^{a-r}$ – $P < 0.05$ – significant differences between different ages among each hybrid population.
$^{1-3}$ – $P < 0.01$ – significant differences between hybrids for the same age.

The results from our study exhibit higher and faster increase of serum lysozyme concentrations for the Ross hybrids, which gives these birds better protection against pathogens through the growth period. Although not investigated we suggest that the better lysozyme concentrations will result in better mortality and growth rates in favor of the Ross hybrid.

The obtained results for the APCA activity trough growth period and its variations over time and hybrid type are shown on table 2. Both hybrids show gradual increase of complement activity over time.

Soon after hatching both hybrids show moderate levels of APCA activity, but during the next weeks the values get close to the ones seen in adult birds.

Comparing the data from the first two weeks of life with the last two weeks (week 5 and 6) we determined significantly different results among both hybrid populations ($P < 0.01$). Both broiler types exhibit increase of the APCA activity between week 5 and week 6 ($P < 0.05$), which indicates the enhancement of birds′ immune system. Although not significant, the outcome indicates slightly better APCA activity for the Ross hybrid.

Table 2. Dynamic analysis of blood serum APCA activity (CH50) in broiler chicken hybrids for six week growing period

Hybrid	Ross	Cobb
Age	X ± Sx	X ± Sx
1 day	325.32±4.15^{ab}	346.94±5.12^{h}
8 days	339.27±8.20^{cd}	312.88±6.51^{ij}
15 days	321.34±4.12^{ef}	320.79±5.02^{kl}
22 days	357.36±11.22^{g}	336.79±9.50^{m}
29 days	415.02±4.30^{ace}	398.32±6.21^{ikn}
36 days	522.14±9.25^{bdfg}	518.93±6.61^{hjlmn}

^{a,b,i,j,k,l} – $P < 0.01$; ^{c,d,e,f,g,h,m,n} – $P < .,05$ – significant differences between different ages among each hybrid population.

The results for the dynamics of APCA activity and blood serum lysozyme among the parent flocks are shown on table 3. Similarly to the broiler flocks, the Ross hybrid parent stock exhibited significantly higher results for the blood serum lysozyme concentration ($P < 0.01$), compared with the Cobb hybrids. The established results for the APCA activity among parent stock were again not significant, but with a tendency for higher levels among the Ross hybrids.

Table 3. Blood serum lysozyme (mg/L) and APCA activity (CH50) among broiler chicken parent flocks

Hybrid Age	Lysozyme concentration		APCA	
	Ross	Cobb	Ross	Cobb
	X ± Sx	X ± Sx	X ± Sx	X ± Sx
45 weeks	6.46±0.21^{1}	3.4±0.14^{1}	431.04±7.20	424.65±9.12
50 weeks	8.25±0.48^{2}	3.90±0.30^{2}	438.94±9.02	411.36±6.22
55 weeks	9.19±0.61^{3}	4.39±0.27^{3}	440.19±6.30	428.15±8.32

$^{1-3}$ – $P < 0.01$ – significant differences between hybrids for the same age.

The overall results for both parameters indicate significant advantage for the Ross hybrid. The data concerning the serum lysozyme levels among the stock animals, backed up by the results obtained from the parent flocks, unambiguously show the better genetic potential of the Ross hybrid. According to Lie (1985) the serum lysozyme is under polygenic control.

Irwin (2004) suggests that some individuals carry out some major genes coding higher serum lysozyme levels. We could suggest that somehow the Ross hybrid breeding was able to

detect and maintain some of the lysozyme major genes, which give these birds huge advantage compared with the other hybrid. Koinarski and Sotirov (2005) demonstrate the crucial role of both serum lysozyme concentrations and APCA activity in chickens' protection against *Eimeria* infections. Authors detect less damage in intestinal tract and overall lower mortality rates among chicken with high concentrations of serum lysozyme, compared with those exhibiting low levels. Authors suggest that potential selection of animals towards high lysozyme concentrations will lead to substantial reduction of losses caused by Coccidiosis. Breed related differences were detected among other species too. In sheep Sotirov et al., (2011) established significantly higher lysozyme concentrations in Milk cross sheep, compared with breeds like Mouton Charolaise and Ile de France. Similar differences in lysozyme concentrations were observed in cattle by Semerdjiev et al., (2006).

The results for the other important factor of the innate immune response were not that considerable. Although not significant the higher APCA activity for both parent and stock animals was shown from the Ross hybrid. We should point out the relatively low variation for this indicator among both hybrids. This phenomenon could be explained by the extremely important role of the complement system in the fight against pathogenic agents.

Breed related differences in APCA activity was observed by Sutherland et al., (2005) in pigs. Authors evidenced higher complement activity in pigs from the Duroc breed, compared with the Landrace and Yorkshire breeds.

The absolute superiority for the serum lysozyme concentrations and high levels of complement activity shows that the Ross hybrid has great genetic potential for the intensive ways of rearing in livestock farming.

CONCLUSIONS

The higher concentrations of serum lysozyme and APCA activity suggests better protection for the Ross hybrid against pathogenic bacteria and some viral infectious agents. The present study exhibit better natural immunity of the Ross hybrid, which probably will have effect on the performance traits of the birds.

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MAINTENANCE OF REPRODUCTIVE HEALTH WHEN USING MEXIDOLIN THE COURSE OF A SPERM CRYOPRESERVATION

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Abstract

Maintenance and strengthening of reproductive health is defined by need of the solution of problems in the field of veterinary and human medicine. In the conducted researches were used physiological, cryobiological and statistical methods. Their use allowed to establish that mexidol as the substance from a class 3-oxipurin, with concentration of 0,026 mg % in composition of the cryoprotective medium promotes increase of mobility, life expectancy and an absolute index of survival of spermatozoa after thawing of sperm of a bull, a ram and the man. These indicators substantially determines the reproductive health of posterity. The optimal concentration of mexidol has no specific meaning and is common for all of the studied species. Its positive effect is determined by the antioxidant properties, thanks to which it is possible to recover the structure and function of biological membranes after cryopreservation.

Key words: *sanogenic sperm, mexidol, reproductive health, sanogenity, freezing, defrosting.*

INTRODUCTION

In the case of infertility couples, reproduction is performed through the method of transfer of embryos, *in vitro* fertilization and artificial insemination with the native or frozen sperm (Дунаевская, 2000).

Cryopreservation of sperm causes a decrease of its quality. It is necessary to increase period of storage of frozen sperm and improve their methods of freezing.

It is known, that for increase the fertilization of frozen sperm are used physical factors (ultrasound, laser, permanent magnetic field), biologically active substances (follicular fluid, blood serum) and pharmaceuticals (prostaglandins, pentoxifyllins, progesterone, caffeine, antioxidants) (Борончук, 1999; Евдокимов et al., 2010; Казакова, 1992; Меркуриева, 1964; Наук, 1987; Родионова et al., 2008).

It is necessary to explore new conditions and protectors to improve indices of sperm after freezing and defrosting through the study of antioxidants, which causing inhibition of lipid peroxidation processes and increasing the antioxidant effect and activity of antioxidant system, providing increased sanogenity of spermatozoa.

Hence the objective of the study was to determine the effect of different concentrations of mexidol on physiological indicators of sperm of human and animals which characterize the sanogenic sperm.

MATERIALS AND METHODS

The material of study was ejaculate from donors with a normal sperm. The sperm of breeding bulls were collected in "vivarium" conditions. The study was conducted in the laboratory of Cryosanocreatology 'V.Nauc'. Mobility of a spermatozoa was determined by the usual method, and the concentration by means of the camera Goreaev (hemocytometer) or photoelectric colorimeter.

The sperm was diluted in proportion of 1:3. The freeze was held in the form of granules on fluoroplastic plate in the volume of 0.1 ml, in pairs of the nitrogen at a temperature of -110 − -120°C with the subsequent diving in liquid nitrogen. Defrosting of the sperm of ram is carried out with the help of a special device to thaw at a temperature of 60°C with the separation of the thawed fractions.

Defrosting of the human sperm and the bull was carried out with the help of a special device at a temperature of 38°C.

The survival of sperm in hours was defined in the conditions of incubation of diluted sperm at a temperature of 38°C, and the absolute survival rate was determined through the method of V. Milovanov.

The obtained data were processed with the help of Student's t-test.

RESULTS AND DISCUSSIONS

It was determined the antioxidant effect of mexidol. This substance belongs to the class of 3-oxypyridine and it is synthesized in the Institute of Physical Chemistry of the Academy of Sciences of Russia.

Taking into account, chemical and biological properties of the mexidol we investigated its effect in the composition of medium ─ lactose-glycerol-yolk (LGY) in different concentrations for dilution and freezing of the sperm of ram (table 1).

Table 1. The effect of mexidol in the medium lactose-glycerol-yolk on physiological indicators after freezing and defrosting of the sperm of ram, n=8

Variant of the medium	The concentration of mexidol in the medium LGY, mg/%	Physiological indicators which characterize the sanogenic sperm after freezing and defrosting		
		Mobility in points	Survival at 38°C, hours	Absolute survival rate at 38°C, c.u. (conventional units)
	0 (control)	2.6±0.19	6.3±0.27	11.22±1.17
	$2.55 \cdot 10^{-4}$	2.7±0.17	6.4±0.20	13.22±1.46
	$2.55 \cdot 10^{-3}$	2.5±0.20	6.6±0.28	14.70±1.67
	$2.55 \cdot 10^{-2}$	2.7±0.10	7.0±0.29	14.75±1.50
	$2.55 \cdot 10^{-1}$	3.1±0.10*	7.3±0.17*	16.65±0.80**
	2.55	2.8±0.10	7.4±0.2*	14.63±1.30
	25.5	2.8±0.14	6.8±0.27	13.21±1.27
	255.0	2.3±0.20	6.1±0.24	7.25±2.10

The difference is statistically authentic, *P <0.05; **P< 0.01.

The analysis of the obtained experimental data (tab. 1) shows that mexidol in the concentration of $2.55 \cdot 10^{-2}$mg/%, in the medium of lactose-glycerol-yolk for dilution and freezing of the sperm of ram increases physiological indicators which characterize the sanogenic sperm, such as survivability, mobility and absolute survival rate after freezing and defrosting.

Next, we investigated the effect of mexidol in the medium of lactose-glycerol-yolk (LGY) at the frozen sperm of bull (table 2).

Table 2. The action of mexidol in the composition of medium lactose-glycerol-yolk on physiological indicators after freezing-defrosting of the sperm of bull, n=8

Variant of the medium	The concentration of mexidol in the medium LGY, mg/%	Physiological indicators which characterize the sanogenic sperm after freezing and defrosting		
		Mobility in points	Survival at 38°C, hours	Absolute survival rate at 38°C, c.u. (conventional units)
	0 (control)	3,8±0,14	8,8±0,17	18,70±1,18
	$2{,}55 \cdot 10^{-4}$	3,9±0,26	9,3±0,26	24,21±2,50
	$2{,}55 \cdot 10^{-3}$	3,8±0,14	8,6±0,49	20,40±2,05
	$2{,}55 \cdot 10^{-2}$	3,9±0,22	9,9±0,37*	25,43±1,67*
	$2{,}55 \cdot 10^{-1}$	3,6±0,13	9,6±0,49	28,60±2,60
	2,55	3,6±0,22	10,0±0,49	25,80±2,76
	25,5	3,4±0,22	9,3±0,17	18,30±1,54
	255,0	2,2±0,28**	6,9±0,98	9,28±1,67**

The difference is statistically authentic, *P <0,05; **P< 0,01.

Experimental data of table 2 show that mexidol possessing antioxidant and membrane active properties in the composition of medium lactose-glycerol-yolk, depending on the concentration acts differently on the restoration of physiological indicators which provide sanogenity of sperm. Mexidol concentration of $2.55 \cdot 10^{-2}$mg /% in medium lactose-glycerol-yolk (LGY) provides increase of physiological indicators which characterize the sanogenic sperm.

The concentration of the mexidol used in the above experiments were extrapolated on the human sperm. It was studied the influence of mexidol in the composition of medium ─ sucrose-glycerol-yolk, which can be more beneficial for the freezing of human sperm.

Table 3. The action of mexidol in the composition of medium sucrose-glycerol-yolk on the physiological indicators, which characterize the sanogenic sperm after freezing-defrosting of the human sperm, n = 5

Variant of the medium	The concentration of mexidol in the medium SGY, mg/%	Physiological indicators which characterize the sanogenic sperm after freezing and defrosting		
		Mobility in points	Survival at 38°C, hours	Absolute survival rate at 38°C, c.u. (conventional units)
1	0 (control)	2.3±0.16	6.0±0	8.95±0.31
2	$2.55 \cdot 10^{-4}$	2.5±0	6.6±0.27	10.75±0.85
3	$2.55 \cdot 10^{-3}$	2.8±0.14	7.0±0.35	13.05±1.23
4	$2.55 \cdot 10^{-2}$	3.2±0.14	7.6±0.27	14.5±1.38*
5	$2.55 \cdot 10^{-1}$	3.1±0.11	7.8±0.22	14.35±1.35
6	2.55	2.3±0.14	6.6±0.45	10.45±1.27
7	25.55	2.1±0.11	5.8±0.22	7.45±0.59
8	255.0	2.0±0	5.4±0.27	7.0±0.31

The difference is statistically authentic, *P <0.05.

As a result of research it was concluded that the concentration of mexidol of $2.55 \cdot 10^{-2}$ mg/% allows to improve the physiological indicators, which characterize the sanogenic sperm (table 3). A further increase in the concentration of mexidol in the composition of medium leads to decrease of physiological parameters.

The positive impact of mexidol on cryopreservation of sperm of different species may be explained by the fact that this substance possesses properties which are similar to vitamin B6. This preparation is not toxic and has a wide spectrum of biological action. It is used in medicine for the incubation *in vitro* of the human spermatozoa. Mexidol causes the slowdown of the reactions of peroxidation of lipids, activates the synthesis of proteins, nucleic acids and processes of fermentation of the Krebs cycle, restores the structure and function of membranes (Евдокимов et al., 2010; Казакова, 1992).

Thus the inclusion of mexidol in the composition of medium contributes to the maintenance of the sanogenity indices.

CONCLUSIONS

The researches allow to make the following conclusions:

1. Mexidol has a positive effect on physiological parameters, which characterize the sanogenic sperm.

2. Among all of the studied variants of concentrations of mexidol for the sperm of all species (ram, bull and human) the priority has a concentration of $2{,}55 \cdot 10^{-2}$ mg/% in the composition of medium for freezing.

3. We suggest for future studying and other representatives from class 3-oxipurin in the composition of medium for freezing of sperm of human and animals.

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CERVICAL INSEMINATION IN KARYA SHEEP

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Abstract

The objective of this study was to investigate the feasibility of use of cervical artificial insemination (CAI) method in Karya sheep breed. Planning the mating plans and controlling the inbreeding may be possible with the use of effective artificial insemination techniques. In the present study, 89, 16 and 85 ewes showing estrus were cervically inseminated with diluted fresh semen from 13, 4 and 9 head rams at 2005, 2007 and 2009 mating seasons, respectively. Data were evaluated with respect to gestation length, litter size and AI success rate. The least square means for mentioned traits were found as 147.61 days, 1.73 and 57.30% for 2005 lambing season and 148.57 days, 1.71 and 43.80% for 2007 lambing season and 147.53 days, 1.47 and 50.59% for 2009 lambing season respectively. The difference between means of years were not statistically significant (P> 0.05) for all the evaluated variables except gestation length and litter size. These results have been close to the values indicated in the literature. Our results show that cervical insemination will be a useful tool in field conditions.

Key words: *Cervical insemination, Karya sheep, reproductive performance, sheep.*

INTRODUCTION

Artificial insemination (AI) is probably the most important technique devised to facilitate the genetic improvement of farm animals. The widespread use of AI in cattle has allowed accurate genetic evaluation and rapid dissemination of genetic merit on a national and international basis to the benefit of both breeders and consumers. For sheep producers, the technology for the control of reproduction, including control of estrus, fixed-time AI, early pregnancy diagnosis and synchronization of lambing are now available and offers possibilities in allowing lamb production to be planned in a way which is not feasible under nature (Halbert, 1990; Donavan et al., 2001; Eppleston and Maxwell, 1993). The use of AI can greatly increase the number of offspring produced per sire per year because a ram has the potential to produce enough spermatozoa to inseminate thousands of ewes. So artificial insemination is probably the most important and the first great biotechnology devised to facilitate the genetic and reproduction improvement of sheep. Insemination outcome is affected by many factors (intrinsic and extrinsic) related to female (handling, seasonality, genital morphology, etc.), male (seasonality, sperm quality, sperm conservation, etc.), farm (environmental conditions, sanitary status, handling, etc.) and the technique itself (route of application, spermatozoa/dose, technician, etc.) (Shackell et al 1990; Donovan et al 2004; Paulenz et al. 2005).

Most of insemination procedures in sheep are performed after oestrus syncronization. Penetration of the cervix is a major problem in sheep (Verberckmoes et al, 2001). Conception rates with fresh semen are good (65 to 75%). While cervical artificial insemination with fresh semen yields acceptable conception rates, the short shelf life of fresh semen coupled with a natural limitation on the number of semen doses achievable per unit time restricts the widespread use of individual sires (Gordon, 1997).

But many research reported that conception rate of artificial insemination of sheep is low in comparison with that of the cow. It is reported that the conception rates are %45 on average (Anca et al 2007).

Cervical insemination is a cheap and relatively easy method of artificial insemination. Cervical artificial insemination, using a speculum and pipette, deposits semen directly into the cervix, through the vagina. Using fresh diluted semen, it has the advantage of being a relatively simple operation and potentially large numbers of

animals can be inseminated during a short period.

In the last two decades, fat-tailed sheep breeds at western part of the Turkey were backcrossed with rams of thin-tailed breeds by farmers to form a thin tailed genotype (namely Karya) that have high reproductive and growth performance. The breeds used for crossbreeding of local genotypes are prolific Chios breed that a common breed of Greece and Turkey (known as Sakiz in Turkey) and Kivircik breed that have a good growth performance. In 1994, an open nucleus flock was established to improve performance traits in synthetic Karya sheep. Karya sheep which have high reproductive performance has been preferred by breeders in the region (Karaca et al 2009a; Karaca et al 1998, Cemal et al 2009). A breeding scheme named as Adnan Menderes University Group Sheep Breeding Program (ADÜ-GKYP) was established to improve performance traits of Karya sheep at extensive management conditions.

One of the most important problem in Aydin finding stud ram for breeding season. If this technique become available in a widespread manner can be overcome a shortage of stud rams. Hereby farms will be able to record keeping more accurate pedigree records. This study aims to investigate the feasibility of use of cervical insemination technique in Karya Sheep under open nucleus breeding program.

MATERIALS AND METHODS

The research was made 2005, 2007 and 2009 mating season at Karya sheep elite flock, Adnan Menderes University Group Sheep Breeding Program (ADU-GKYP), Aydin, Turkey. Its objective was the sheep artificial insemination with fresh diluted semen, by the cervical method. In the present study, 89, 16 and 85 ewes showing estrus were cervically inseminated with diluted fresh semen from 13, 4 and 9 head rams at 2005, 2007 and 2009 mating seasons, respectively. The age of rams are ranged from 3 to 5 years. Animals were fed with concentrate mix and had access to a shelter, where water and salt stone complement were available.

Oestrus cycles of all ewes was synchronised with progestagen sponges for 14-day treatment of progestagen vaginal sponges (40 mg FGA, Chronogest CR, Intervet) with an 500 IU pregnant mare serum gonadotrophin (Chronogest CR) given (i.m.) at the time of sponge removal to stimulate follicular development.

Semen was collected from each ram using an artificial vagina before artificial insemination. Immediate after semen collection, the semen was examined. Semen samples were evaluated for volume (SV, ml), mass motility (MM), percentage of dead sperm cells (PDSC,%), sperm concentration (SC) and total number of spermatozoa per ejaculate (TNSPE). Simple statistics for some semen characteristics of Karya rams used for cervical inseminations were given in Table 1. One part of evaluated semen were diluted with three part of a commercial semen extender (Laiciphos, IMV, Sark Kemikal, Istanbul, Turkey). The semen was kept in water bath at 30°C until inseminations.

Inseminations were carried out 50-51 h after sponge removal. Only ewes that showing estrus was inseminated. After cervix making evident with a speculum which was equipped with a light source, the fresh diluted semen was inoculated inside of cervix, in 0.5 ml. doses.

Data were analyzed using GLM (General Linear Model) procedure and phenotypic correlations between variables were obtained using the CORR procedure of the SAS (1999) statistical software.

RESULTS AND DISCUSSIONS

Semen characteristics are important variables considered in the most studies of artificial insemination (Colas, 1980; Gordon, 1997; Kaymakçi, 2002; Ollero et al, 1996). Simple statistics for some semen characteristics of Karya rams was given in Table 1. In the present study all ejaculate characteristics had similar to previous studies (Yilmaz and Karaca, 2004, Yilmaz et al., 2009).

Table 1. Simple statistics for some semen characteristics of Karya rams used for cervical inseminations

Years	Variable	N	Mean±SD	Min.	Max.	CV (%)
2005	SV	13	0.95±0.360	0.50	1.50	38.01
	MM	13	4.62±0.650	3.00	5.00	14.09
	PDSC (%)	13	12.6±3.032	9.75	20.50	24.07
	SC	13	1.42±0.206	1.04	1.77	14.54
	TNSPE	13	1.38±0.625	0.52	2.65	45.44
2007	SV	4	1.13±0.250	1.00	1.50	22.22
	MM	4	5.00±0.000	5.00	5.00	0.00
	PDSC (%)	4	2.81±0.774	1.75	3.50	27.52
	SC	4	1.82±0.093	1.72	1.94	5.12
	TNSPE	4	2.06±0.571	1.72	2.91	27.75
2009	SV	9	0.96±0.288	0.50	1.30	30.11
	MM	9	4.67±0.500	4.00	5.00	10.71
	PDSC (%)	9	3.31±0.527	2.75	4.25	15.94
	SC	9	1.60±0.153	1.37	1.81	9.55
	TNSPE	9	1.51±0.442	0.82	2.26	29.23

SV: Semen volume (cm^3). **MM:** Mass motility**. PDSC (%) :** Percentage of dead sperm cells. **SC:** Semen concentration ($x10^6/mm^3$).
TNSPE: Total number of spermatozoa per ejaculate ($x10^9$/ejaculate volume)

The general mean values for SV, MM, PDSC and TNSPE were found as 0.98, 4.69, 7.88, 1.54 and 1.53, respectively.

SV, PDSC and TNSPE had lower value and MM and SC had higher value than Yilmaz and Karaca 2004, who working in the same genotype, in this study. Although SV, MM, PDSC and TNSPE were greater than Yilmaz et al, 2009, SC value was less than the same literature. Semen volume and sperm concentration are comparable to those reported by Saleh (1997) who established 0.67 ml and 4.3 × 109. However in the present study had higher volume and lower concentration to those reported by Saleh (1997). It might be due to the breed and environmental differences.

Phenotypic correlation coefficients for some semen characteristics were given Table 2. The correlation between SV with TNSPE, and SC with MM and SC with TNSPE were found to be positive and significant. But the correlation between SC with PDSC and TNSPE with PDSC were found to be negative and significant. The correlation between semen volume with total number of sperm per ejaculate and sperm concentration with total number of sperm per ejaculate were found to be positive and significant, in agreement with previous reports (Yilmaz and Karaca, 2004, Karagiannidis, 2000).

Table 2. Phenotypic Correlations among semen characteristics (N=26)

	SV	MM	PDSC (%)	SC
MM	-0.04^{NS}			
PDSC (%)	-0.27^{NS}	-0.34^{NS}		
SC	0.29^{NS}	0.45^{*}	-0.72^{**}	
TNSPE	0.93^{**}	0.11^{NS}	-0.45*	0.59^{**}

NS=Non-significant, **=**P< 0.05, ****=**P< 0.001

The correlation coefficient between semen volumes with sperm concentration did not differ significantly in this work in agreement with Yilmaz et al. 2009, but this result are not consistent with previous studies (Karagiannidis, 2000, Yilmaz and Karaca, 2004).

Least square means and standard errors for some semen characteristics of Karya rams were given in Table 3. The mean values for PDSC and SC except the other semen characteristics were significantly differed between years (P< 0.01). All semen characteristics were not affected ram age.

Although there are statistically significant changes in SC, TNSPE is an interesting result that there is appears to be a statistically insignificant. These results are not consistent with previous studies (Yilmaz et al, 2009, Yilmaz and Karaca 2004, Saleh, 1997)

Table 3. Least square means of semen characteristics

Variable	N	SV	MM	PDSC (%)	SC	TNSPE
Years		**P=0.614**	**P=0.344**	**P=0.000**	**P=0.002**	**P=0.126**
2005	13	0.95±0.090	4.54±0.15	12.6±0.618	1.42±0.049	1.37±0.155
2007	4	1.13±0.162	5.00±0.27	2.81±1.115	1.81±0.089	2.06±0.280
2009	9	0.96±0.108	4.67±0.18	3.31±0.743	1.60±0.059	1.51±0.187
General	**26**	**1.01±0.071**	**4.74±0.119**	**6.24±0.492**	**1.61±0.039**	**1.65±0.124**

SV: Semen volume (cm^3). **MM:** Mass motility**. PDSC (%) :** Percentage of dead sperm cells. **SC:** Semen concentration ($x10^6/mm^3$).
TNSPE: Total number of spermatozoa per ejaculate ($x10^9$/ejaculate volume).

The success of cervical insemination according to years was summarized in Figure 1. Success rates were found 57.30%, 43.80% and 50.59% in 2005, 2007 and 2009 respectively.

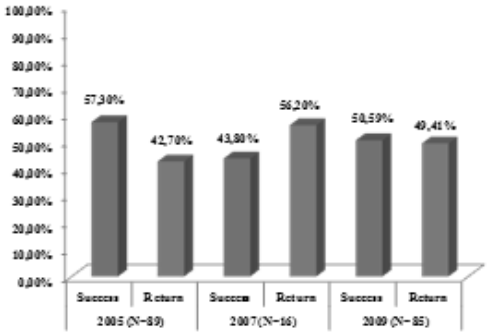

Figure 1. Success of cervical insemination according to years

Cervical insemination using fresh semen gives a higher pregnancy rate than cervical insemination using frozen-thawed semen (Donovan et al., 2004); 76% compared with 46% (Irish breed) and 36% (Norwegian breed). The pregnancy rate obtained following cervical insemination with fresh diluted semen is less than Evans and Maxwell, 1987 (65-75%). However in the present study had higher insemination success than reported previous studies (Rojero et al. 2009; Anca et al., 2007). These differences might be due to the breed and environmental differences.

Simple statistics for gestation length and litter size were given in Table 4.

Table 4. Simple statistics for gestation length and litter size from successful insemination

Year	Variable	N	Mean±SD	CV (%)	Min	Max
2005	GL	51	147.61±3.75	2.54	133.00	153.00
	LS	51	1.73±0.666	38.58	1.00	3.00
2007	GL	7	148.57±1.27	0.86	147.00	150.00
	LS	7	1.71±0.756	44.10	1.00	3.00
2009	GL	43	147.53±3.91	2.65	131.00	152.00
	LS	43	1.47±0.592	40.38	1.00	3.00

GL: Gestation length, **LS**:Litter size

General mean of litter size was 1.64 for all the years. The highest litter size was 1.73 in 2005. Least square means and standard errors for gestation length and litter size for Karya ewes which were cervically inseminated in 2005, 2007 and 2009 years were given Table 5.

Table 5. Least square means of gestation lenght and litter size from successful insemination

	N	GL	LS
Year		**P=0.457**	**P=0.357**
2005	51	147.52±0.564	1.76±0.095
2007	7	149.17±1.419	1.60±0.238
2009	43	147.23±0.626	1.55±0.105
Dam Age		**P=0.116**	**P=0.013**
2	34	148.68±0.829	1.40±0.139
3	24	149.17±0.885	1.45±0.149
4	17	146.87±1.006	1.95±0.169
5>	26	147.18±0.784	1.72±0.132
General		**147.97±0.536**	**1.63±0.090**

GL: Gestation length, **LS**:Litter size

The difference between means of years for gestation length were not statistically significant (P>0.05). The results show that years have a significant effect on litter size (P< 0.05). The gestation length obtained following cervical insemination with diluted fresh semen is similar to that previously reported under same conditions in Çine Çapari Sheep in Turkey (Karaca et al., 2009b). Litter size obtained from this study had higher value than Karaca et al., 2009a. The results show that litter size is higher than the other Turkish sheep breeds.

CONCLUSIONS

Artificial insemination (AI) of sheep is an advantageous management practice aimed at the genetic improvement at farm level and a programme of genetic selection. Furthermore AI has the potential for a significant impact on the sheep breeding industry. The main role of AI in sheep production is to increase the rate of genetic improvement and AI also contributes to achieving other goals, e.g. allowing extensive use of the best available rams, therefore increasing selection pressure and the rate of response to selection.

In conclusion, the very low level of fertility obtained when frozen-thawed semen is used for cervical insemination in sheep has stemmed widespread interest/uptake of AI by the sheep sector. It is an effective, easy and cheap method of cervical insemination with diluted fresh semen.

It is important to determine the feasibility of use of cervical insemination technique in field conditions. The use of reproductive technologies such as artificial insemination in

animal breeding programs is very important tools. Our results show that cervical insemination can easily use in field conditions, while not a very high success planning the mating plans and controlling the inbreeding may be possible with the effective use of this technique.

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MOLECULAR BIOLOGY STUDIES OF PROTEIN SYNTHESIS AND ^{45}CA TRANSPORT IN STRIATED AND CARDIAC MUSCLE OF RABBITS WITH EXPERIMENTAL GLANDULARDYS FUNCTIONS

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Abstract

The aim of our study was related with investigation of hormone excess treatment (Thyroxine and Hydrocortisone) upon heart and skeletal muscle metabolism, looking for modifications in nucleic acid and protein synthesis by means of radioisotope methods of 45Ca,3H Tryptohane and 3H Uridine uptake. In young heart rabbits treated with 0.625mg Hydrocortisone, a reduction in 45Ca uptake has been recorded ,while in old rabbits there is a progressive increase in 45Ca uptake as a function of dose of hormone used. As far as skeletal muscle is concerned, both in young and old rabbits treated with Hydrocortisone there is a progressive decline in cellular receptors for 45Ca affinity. An increase in the uptake of 3H Uridine for 0.15 and 1.50 mg thyroxine in rabbit heart has been recorded while at 0.75mg there is a decrease in the uptake. In skeletal muscle, there is a progressive increase in RNA synthesis under hormone impulse as a function of admitted dose in comparison with Controls.

Key words: *aging rabbit heart,aorta, thyroxine, hydrocortisone,45 Ca, skeletal muscle.*

INTRODUCTION

Hypertrophy is an adaptive response of ventricular myocardium to a variety of physiological and pathological stimuli. Removal of the stimulus responsible for the growth process usually results in a return of cardiac mass to normal. For example, regression of thyroxine administration is a reproducible and extensively studied model for the development of cardiac hypertrophy. We have previously shown that thyroxine-induced cardiac hypertrophy is associated with both increased fractional and absolute rates of in vivo protein synthesis, as well as increases in the efficiency and capacity for protein synthesis (Parmacek et al., 1986). Measurements of protein degradative rates in this hormone induced model of hypertrophy vary, but in all cases, hypertrophy occurs because the accelerated rate of new protein synthesis exceeds the rate of degradation (Morgan et al., 1987).

Corticosteroids also may increase vascular tone by trophic effects, i.e., hypertrophy or hyperplasia of VSMCs. More VSMCs or larger VSMCs in a given vessel may allow enhanced contractile responses to angiotensin II or norepinephrine (Michael, 1999).

The study of molecular basis of hormone action represents an important place in fundamental and applicative research. A great interest is represented by this study in Gerontology field, taking into account the fact that ageing process evolves with a series of modifications at endocrine level (Peeters, 2008).

Many hormones affect transmembranar transport of ions and molecules modifying target tissue metabolisms (Peeters, 2008).

Thyroid hormones affect muscle metabolism, the ionic content of muscle fiber as well as

blood irrigation at the level of cardiac and skeletal muscle (Moriun et al., 1983).
Adrenal steroids modify muscle metabolism and Na/K balance (Bonnin et al., 1983)
These hormones not only modulates molecular processes at the level of cardiac and skeletal muscle but they play a decisive role in human pathology of these tissues (Carter et al., 1982).
The aim of study was related with the investigation of the effects of hormone excess (hydrocortisone and thyroxine) upon skeletal and cardiac muscle metabolism from rabbits of different ages, following modifications in cellular metabolism(nucleic acid and protein synthesis with radioisotope methods of uptake ^{3}H Uridine and ^{3}H Tryptophan.

MATERIALS AND METHODS

Our study has been done on fresh striated and cardiac muscle from rabbits treated in excess with hormones and also, from age matched controls as well as in glycerinated muscle.

Animal groups:

Our study has been done on 36 male rabbits (24-37 months old) out of these 18 have been treated in excess with: Thyroxine (9 treated and 9 controls) and 18 with Hydrocortisone (9 treated and 9 controls).

Methods: Two weeks treatment with Thyroxine has been done with 0.25 ug/kg body weight, 0.50 ug/kg body weight, 1 ug/kg body weight.
Hydrocortisone treatment has been applied for two weeks in the following doses: 0.125 mg, 0.625 mg, 1.25 mg.

The biology of cardiac and striated muscle tissue have been investigated with radioisotope methods using ^{3}H Tryptophane and ^{45}Ca uptake.

Experimental protocol

For experiments, ^{3}H Tryptophane uptake and ^{45}Ca transport, tissue fragments between 50-100 mg each have been used. Tissue fragments have been introduced in Hanks medium (1 ml in a test tube) and left for preincubation at 37^{o}C for ½ hour according to the published technique (Revnic, 1993).
In each test tube we placed 10ul 3H Tryptophane from 500 mCi/ml solution. The specific activity was 26 mCi/mg.
The next step is related with incubation of biological samples with isotope for 1 ½ hour at 37^{o}C.
Next step was related with extraction of samples with 2N HCl. Tissue fragments have been taken out from incubation medium,being placed into test tubes with 1 ml HCl 2N.
Biological samples have been kept in extraction medium for 24 hours.
Next day the radioactivity in incubation and extraction media has been measured 96 vials with 5 ml scintillation liquid. In each vial 0.2 ml from incubation and extraction medium has been placed. The radioactivity has been estimated with a Beta Berthold Liquid Scintillation Counter for ^{3}H on three channels (Revnic, 1993).
The same procedure has been used for the uptake of ^{3}H Uridine and $^{45}CaCl_2$.

RESULTS AND DISCUSSIONS

Table 1. The values of body weight and heart from controls and thyroxin treated rabbits

Age (months)	Nr.	Thyroid status	Body weight	RV	RV/BW	LV	LV/BW
24	9	Control	531+/-23	64+/-3	0.120+/-0.08*	276+/-2	0.519
24	9	Treated	478+/-20	94+/-15	0.196+/-0.03	324+/-5	0.677+/-8

p<0.01C/T(BW)
*p<0.001 C/T (VD/BW)

Table 2. The mean values of ^{3}H Tryptophane uptake in rabbit heart from control and thyroxine treated rabbts

Heart	Age	Control	Thyroxine treated rabbits		
			0.250ug	0.50ug	1.0ug
	24	678	414	360	290

In table I are presented the values of body weight and heart from controls and thyroxin treated rabbits.

We can observe a decrease in 3H Tryptophane in rabbits treated with different doses of thyroxine versus controls, accounting for a reduction in protein synthesis. Our data are in accordance with the literature (Peeters, 2008) which have shown that thyroxine hormone administered in excess inhibits protein synthesis.

Concerning the uptake of ^{3}H Uridine an increase in ^{3}H Uridine uptake has been recorded in rabbits treated with 0.50 ug Thyroxine. We can conclude that for 0.50 ug thyroxine a stimulatory effect on mRNA synthesis.

Another objective of our study was related with pointing out of modifications in ^{45}Ca transport in rabbits heart of treated rabbits with different doses of Thyroxine.

It is known that Ca^{++} together with other ions are implicated in regulation of neuromuscular sensitivity and in transmission of nerve influx, triggering the contraction of atrial muscle.

Hyperthiroidism is associated with an abnormal metabolism of Ca+2 .

Our experiments of incubation of heart fragments from treated rabbits with different doses of thyroxine, with ^{45}Ca have pointed out a decrease in ^{45}Ca uptake dose dependent versus controls.

Table 3. ^{45}Ca uptake in heart of old Controls and Thyroxin treated rabbits

Heart	**Age**	**Control**	**Thyroxine treated rabbits**		
			0.250ug	0.50ug	1.0ug
	24	320	270	235	178

The reduction in the uptake of ^{45}Ca is inversely proportional with thyroxine dose administrated and it can be correlated with the fact that hypertiroidism induced with hormone excess leads to an increase in calcium in rat heart in such a way that all binding sites available for Ca^{2+} are already engaged by the existing Ca^{2+} from cardiac tissue.

Table 4. The uptake of ^{45}Ca (dpm/g) in young and old rabbits treated with hydrocortisone

Heart	**Age**	**Control**	**Thyroxine treated rabbits**		
			0.150mg	0.625mg	1.250mg
	8	2.6	2.2	1.7	2.7
	24	1.6	2.6	2.9	4.8

Table 5. The uptake of ^{45}Ca (dpm/g) in young and old rabbits treated with hydrocortisone

SKELETAL MUSCLE	**Age**	**Control**	**Thyroxine treated rabbits**		
			0.150mg	0.625mg	1.250mg
	8	5.5	4.4	5.1	2.5
	24	4.1	5.0	3.7	3.9

The administration of thyroxine in the rabbit produces cardiac hypertrophy in vivo by increasing protein synthetic rates to a greater degree than degradative rates, resulting in the net accumulation of cardiac protein (Parmacek et al., 1986). Under the treatment with thyroxine in excess, there is a reduction in ^{3}H tryptophane uptake in rabbits heart versus controls,accounting for a decrease in protein synthesis. The lowest uptake has been recorded for 0.50 mg thyroxine dose. Our results support the findings of previous investigators regarding the effect of thyroxine administration on protein synthesis during the development of cardiac hypertrophy (Morgan et al., 1987). Our data have pointed out an age dependent reduction in ^{45}Ca uptake in heart of rabbits belonging to Control group in comparison with age matched.

In young rabbits treated with treated with 0.625 mg Hydrocortisone a reduction in the uptake of 45Ca was recorded, while in old rabbits there is a progressive uptake in ^{45}Ca as a function of administrated dose. That means that with aging,

and in conditions of hormone excess a lot of disturbances in heart metabolism occurred expressed by an increase in the affinity of cellular receptors for ^{45}Ca with important consequences at the functional level. Concerning skeletal muscle, both in young and old rabbits treated with hydrocortisone there is a decline in ^{45}Ca uptake.

Corticosteroids foster hypertension not only by enhancing renal sodium reabsorption but also by augmenting vascular tone. Corticosteroids augment vascular tone by potentiating the actions of vasoconstrictor hormones and by direct actions on VSMCs that are independent of vasoconstrictor hormone.

CONCLUSIONS

Investigation of striated and cardiac muscle tissue biology have pointed out that hormones in excess administrated in rabbits lead to an increase in protein metabolism as well as in membrane permeability.

In conditions of hydrocortisone administrated in excess, there is an increase in cell receptors affinity for ^{45}Ca with important consequences on the functional level, while in young rabbits treated in excess with hydrocortisone, there is a decline in ^{45}Ca uptake.

Under the treatment with thyroxine in excess, there is a reduction in ^{3}H tryptophane uptake in rabbits heart versus controls,accounting for a decrease in protein synthesis. The lowest uptake has been recorded for 0.50 mg thyroxine dose.

In skeletal muscle, on the contrary, there is an increase in the uptake of ^{3}H tryptophane in thyroxin treated rabbits versus controls.

There is an age dependent modification in affinity of cell receptors for ^{45}Ca in rabbits treated with excess of corticosteroids.

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RESEARCHES REGARDING AGE, BREED AND COLLECTING SEASON INFLUENCE IN QUALITY AND QUANTITY BOARS SEMEN

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Abstract

Semen production from reproduction boars depends upon different factors as: age, breed, using way to natural mating or collecting, but also the environmental conditions. The present researches have in view to establish how three influence factors as breed, age and collecting season could change the quantitative and qualitative indices of the semen. There were analyzed 65 boars owing to three different genetic types: Large white (16 boars), Landrace (16 boars) and Pietrain (33 boars). At every collecting there were recorded the following sperm indices: macroscopic-sperm volume and microscopic-sperm concentration (x 10^6spz/ml), and total number of sperm cells/ejaculate (x10^9). The influence of the three factors was made along two years, studying the dynamics of the sperm volume, concentration and total number of sperm cells/ejaculate depending on boars breed (large white, Landrace and Pietrain), boars age (1 year, comparative two years) and collecting season (spring, summer, autumn and winter). The results of the research regarding the season of semen collecting revealed the fact that the value of the analyzed sperm indices was superior in the cold season.

Key words: *sperm collecting, qualitative and quantitative indices.*

INTRODUCTION

Swine breeding in industrial system supposes the applying of the most modern breeding technologies to exteriorize the swine biologic potential and also the best using of forages, shelters, working force to assure some high, rhythmic and valuable productions (Marin, 2006; Tapaloaga, 2008; Tapaloaga, 2012). The productive genetic potential breeding to increase the quantity and quality of meat production is linked to the reproduction activity setting. The Romanian experience has proved in the last decade that the artificial insemination is an up to date compound of the reproduction function increasing in swine (Tapaloaga, 2008; Tapaloaga, 2012; Tapaloaga, 2011).

MATERIALS AND METHODS

As it is known, the semen production in boar is appreciated by its volume, sperm cells concentration, and sperm motility and presents large variability depending on some factors, existing differences between different breeds, ages and collecting season. The aim of this paper was to study the three factors that influence the quantity and quality of the semen in boars used for artificial insemination in a famous breeding unit. Thus, there were analyzed 65 boars in three genetic types: 16 Large White boars, 16 Landrace boars and 33 Pietrain boars.

At every sperm collecting there were analyzed and recorded the following sperm indices:

- Macroscopic indices**:** sperm volume, measured in the collecting glass, in the moment of sperm filtering
- Microscopic indices**:** sperm cells concentration (x 10^6spz/ml) determined by the colorimeter and the total number of sperm cells per ejaculate (x10^9) calculated multiplying the sperm cell concentration and its volume.

The study was conducted during two years, having in view the evolution of the three sperm indices depending on:

- breed (Large white, Landrace, Pietrain);
- age (1 year, comparatively 2 years;)
- collecting season (spring, summer, autumn and winter).

The study regarding the breed effect upon the sperm production was made on 400 ejaculates, 100 ejaculate from Large white boars, 100

ejaculates from Landrace boars and 200 ejaculates from Pietrain. The sampling was made conformingly the using program to artificial insemination of boars.

The values obtained upon the analyses of the ejaculates from the three studied breeds (Large white, Landrace, Pietrain) were used to statistical processing including mean and standard deviations, sperm concentration and the total number of sperm cell/ ejaculate, having in view the emphasizing the breed differences. The effect of boar age upon the seminal production was studied by comparing the sperm indices recorded at one year boars, comparative the same boars at two years old.

The researches were made during 24 months. To cancel the breed effect and to be correct evaluate the effect of age upon the semen, there were analyzed equal numbers of ejaculates from the three breeds, for the both years. Thus, from Large white boars were studied 100 ejaculates, 50 of them in the first year and the others in the second years and the same for the other two breeds, Landrace and Pietrain.

The seasonal effect on the main spermatic indices was studied during 12 months, grouped by the four seasons of the year (winter, spring, summer and autumn). There were analyzed 25 ejaculates per each breed, per each season.

RESULTS AND DISCUSSIONS

Analyzing the values of the seminal material depending on the boars breed, there was noticed that there are differences regarding the sperm production among the three genetic types (table 1, charts 1, 2, 3).

Table 1. Sperm indices in the three analyzed boar breed

Genetic type		**Large white**	**Landrace**	**Pietrain**	**All breeds**
Sperm indices	**Boars number/ analysed ejaculates**	**16/ 100**	**16/ 100**	**33/ 200**	**65 400**
Volume (ml)	**x ± sx**	271±96 (2)	291±91 (1)	260±85 (3)	**270±91**
Sperm cells concentration (10^6/ml)	**x ± sx**	350±89 (2)	320±99 (3)	380±90 (1)	**347±93**
Total number of sperm cells /ejaculate (x10^9)	**x ± sx**	89±31 (1)	77±28 (2)	75±27 (3)	**80±29**

Thus, it was noticed that regarding the volume of the ejaculate, the highest value was recorded in Landrace breed (291±91 ml), the lowest value in Pietrain boars (260±85 ml), while Large white boars achieved, 271±96 ml, an intermediary value between the other two analyzed breeds, Landrace and Pietrain (table 1, chart 1).

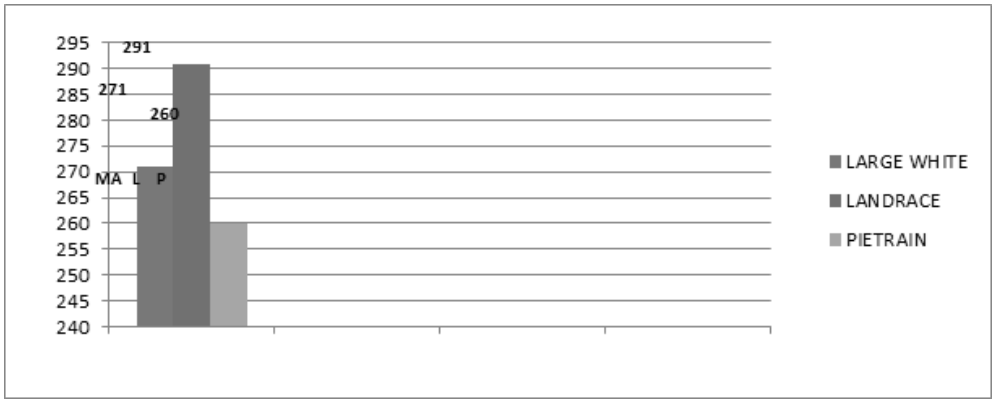

Figure 1. Ejaculate volume dynamics

Sperm concentration in sperm cells (10^6/ml) in the studied material had also different values in the analyzed values, existing practically a negative correlation to the ejaculate volume:

the highest value of the sperm concentration in sperm cells was recorded in Pietrain breed (380±90x10^6/ml), the lowest value in Landrace boars (320±99x10^6/ml), and the large white boars recording an intermediary value, as is seen in table 1 and chart 2, 350±89x10^6/ml.

Figure 2. Sperm cells concentration (106/ml)

The total number of sperm cells/ejaculate *(x10^9)* had values within 75±27*x10^9* and 89±31*x10^9*. Thus, in table 1 and chart 3 it may notice that the highest value of the total number of sperm cells/ejaculate was recorded in Large white boars (89±31x10^9), the lowest value was recorded in Pietrain boars (75±27x10^9), while Landrace boars recorded almost the same values as in Landrace boars, 77±28x10^9.

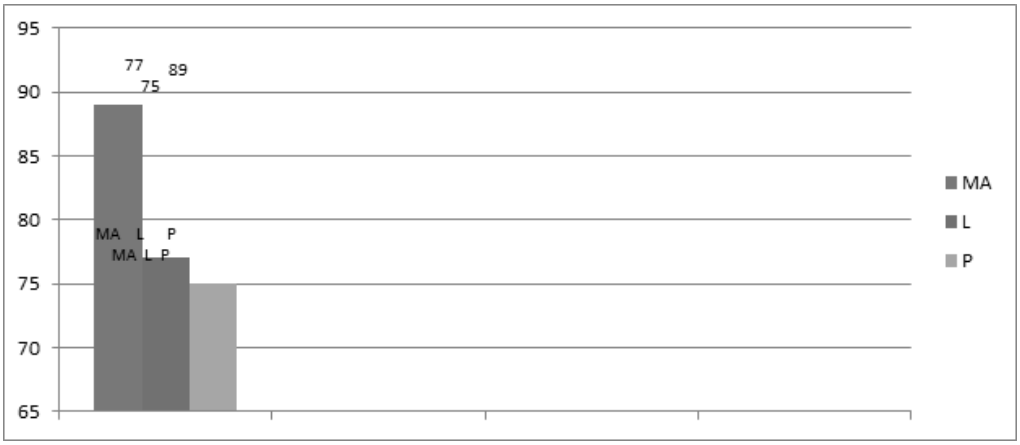

Figure 3. Total number of sperm cells /ejaculate dynamics (x109)

Analyzing the values of the sperm indices depending the age of boars, it was noticed that there were little differences regarding the sperm production between the two age studied categories. (table 2).

Table 2. Sperm indices depending on age at collecting

Age category		**1 year**	**2 years**
Sperm indices	**No of analysed ejaculates**	**150**	**150**
Volume (ml)	**x ± sx**	265±54	270±60
Sperm cells concentration (10^6/ml)	**x ± sx**	340±45	342±40
Total number of sperm cells /ejaculate ($x10^9$)	**x ± sx**	75±17	78±21

Thus, the ejaculate volume increases as the boars age from 265±54 ml at 1 year old, la 270±60 ml at two years old, while the sperm concentration in sperm cells ($x10^6$/ml), less age dependant, seems to be balanced at 1 year old. ($340±45x10^6$/ml-$342±40x10^6$/ml).

The total number of sperm cells /ejaculate ($x10^9$) had a low increasing by the boars' age from $75±17x10^9$ to$78±21$ $x10^9$. This increasing is linked to the one of volume of ejaculate after 12 years old.

The results of the researches carried out during a year having in view the influence of the season upon the sperm material are presented in table 3.

Table 3. Sperm indices depending on collecting season

Season	**Volume (ml)**	**Sperm cells concentration** (10^6/ml)	**Total number of sperm cells /ejaculate** ($x10^9$)
Spring	270±48	320±43	74±27
Summer	260±52	310±40	76±21
Autumn	280±50	325±41	85±30
Winter	272±60	338±46	88±31

Analyzing the data in table 3, we can notice the following aspects:

- the volume of the ejaculate depending on the collecting season was framed within 260±52 ml and 280±50 ml, the highest values recording as it was expected in cold seasons (autumn and winter), and the lowest value, in summer (260±52ml);
- sperm concentration in sperm cells ($x10^6$/ml), classified depending on collecting season was framed within $310±40x10^6$/ml and $338±46x10^6$/ml, the highest values being recorded in the cold seasons (autumn and winter);
- the total number of sperm cells/ejaculate (x109) is correlated with sperm concentration, so the highest values in this sperm index too are recorded in the cold seasons (autumn and winter) more exactly: in autumn, 85±30x109 and in winter, 88±31x109.

The researches regarding the effect of boars breed upon the spermatic material, carried out on three genetic types (Large white, Landrace and Pietrain), emphasized some differences. Thus, even in the volume of ejaculate the landrace boars recorded the highest values (291±91 ml), the ones in Pietrain breed had the highest value of sperm concentration in sperm cells. ($380±90x10^6$/ml).

The total number of sperm cells per ejaculate ($x10^9$) had a little increasing parallel the age of collecting from $75±17x10^9$ to $78±21$ $x10^9$. This increasing is linked to the one of the volume of ejaculate after 12 months of age.

The researches regarding the effect of collecting season on the main sperm indices revealed, as it was expected that the highest values were recorded in cold seasons.

CONCLUSIONS

There were also low differences regarding the effect of boars age, the sperm indices increased parallel the age increasing.

The effect of collecting season on sperm indices revealed high performances in the cold season.

The obtained results in the present research prove the fact that besides the best organizing of the activity in an artificial insemination center which depends on the staff professional status and correctitude, the reactive and equipment quality, there are influence factor which could modify more or less the

quantitative and qualitative index in boars spermatic material.

ACKNOWLEDGEMENTS

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MILK QUANTITY AND QUALITY IN A DAIRY UNIT-STUDY CASE

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Abstract

Milk is proved a complete food from latest scientific researchers. Milk and dairy products contain many nutrients and provide quick and easy way of supplying major vital substances in human being life. Due to their importance, their benefits to our bodies, health and mind, but not neglecting the financial aspects, many dairy units and also the processing units have to assure large amounts of good quality milk. The present study is a study case regarding the quantitative and qualitative milk amount in a dairy farm in the south of Romania. The livestock in the studied farm consisted in Holstein-Frisian cows in different stages of lactation, which were statistically analyzed from the milk quality and quantity point of view. During April 2011 and May 2012, based on the primary data recorded in the farm it was established the reproductive livestock, grouping the females in three categories depending on their lactation. There were recorded the cows in lactation and also the dry cows and the dynamic of the milk production was recorded daily and monthly, too. Due to the fact that the milk is processed in a special unit and after that goes to the market, the milk quality parameters were analyzed too. The recorded situation leads to the conclusion that the unit represents a high class unit in the Romanian dairy units.

Key words: *normal lactation, reproductive livestock, dry-period, chemical, physical, parameters.*

INTRODUCTION

In human beings nutrition, animal origin food products have a great importance assuring the energy and the basic substances necessary to metabolic processes, raising and development of organisms (Ilie et al., 2011; Tapaloaga, 2008; Tapaloaga, 2012). Milk is the most important product due to its complex chemical composition, biologic value and the high digestibility range. Thus, the present paper has as aim the study of the milk production in a modern farm located in the southern Romania.

MATERIALS AND METHODS

The study of milk production in S.C. ILYA AGRO S.R.L. was carried out having in view the quantitative and also qualitative aspects. During a whole year, since April 2011 and May 2012, we have recorded and processed the data of the cattle farm and calculated the mean achieved productions. Based on the primary data we have established the main technological parameters of milk production: reproductive livestock, number of lactating cows, number of cows in dry period, number of animals in different lactations, mean milk production per each female, daily mean milk production. Meanwhile we have established the milk production evolution at different lactations and the evolution of the total milk production during the whole year. There were also calculated the fat and protein percentage in milk per the whole farm, and also the useful substance index, an important indicator in the milk processing industry, due to its influence in cheese processing. The data were expressed in absolute and relative values from the whole livestock. The data were statistically processed, and the fat and protein percentage were determined by the stipulated standards (Ilie et al., 2011; Petcu, 2006; Tapaloaga, 2008).

RESULTS AND DISCUSSIONS

The evolution of the milk production and main parameters in the studied unit are presented in the following charts. It may remark that from the whole reproductive livestock, which had a low evolution, starting from 392 females in September 2011 and 418 females in May 2011, the number of lactating cows represented 77.14% of the whole livestock in November 2011 and 90.44% in April 2011. The rest of the females, the ones in the dry period represented between 9.56% of the whole livestock in May

2011 and 22.86% in November, the same year, as is seen in chart 1. Comparatively the results quoted in the special literature, the values recorded in S.C.ILYA AGRO S.R.L are superior regarding the percentage of the lactating females, this thing being explained by the fact that this farm is almost new, and the female introduced in the livestock were in lactation.

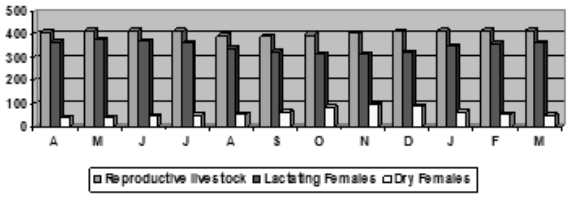

Figure 1. Female distribution during the studied year

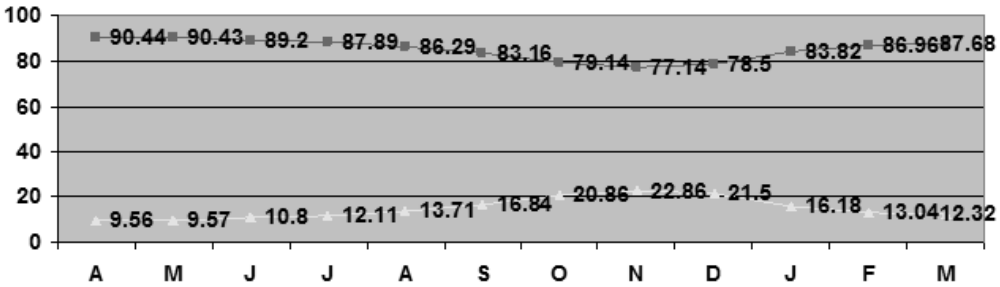

Figure 2. Female distribution at the first lactation

Upon the recorded data, we remarked that the number of the females in the first lactation varied between 105 cows in March 2012 and 212 cows in May 2011.

The average milk production per female oscillated between 18 l per day in July 2012 and 25.1 l per day in March, the same year. On the entire farm, the mean milk production varied between 2438.l in January 2012 and 4388.4 l in May 2011. Chart 3 illustrated the evolution of milk yield per day in the analyzed period.

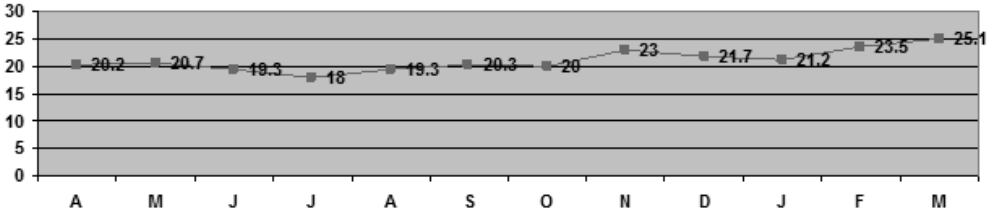

Figure 3. Milk production distribution at the first lactation

In chart number 4, it is presented synthetically the evolution of the milk production in the second lactation. Based upon the primary recorded data, it could notice that the number of the female in the second lactation is inferior the ones at the first lactation, due to the new livestock. The milk mean production at the second lactation oscillated between 19.6 l per day in July 2011 and 31.6 l in March 2012.The mean production recorded in the females at the second lactation had an increasing trend,

starting from 1509.2 l in July 2011 to 3918.4 l in the last studied month.

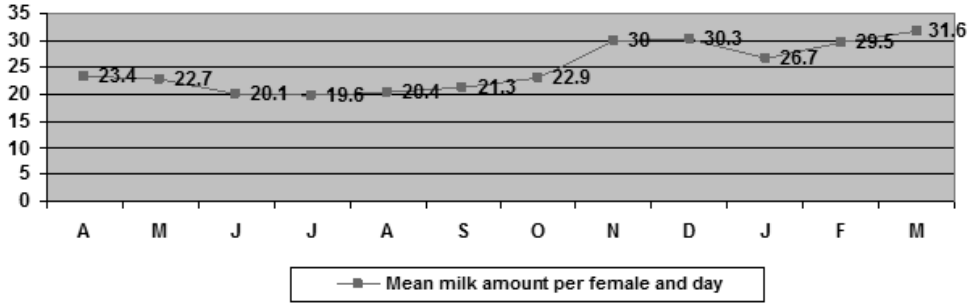

Figure 4. Milk production distribution at the second lactation

Milk production in cows at the third lactation is presented in chart 5. By the recorded date we remarked the increasing trend of the females' number in this category, starting from 88 females in April 2011, to 135 females in February 2012.

The milk amount in this category varied between 19.5 l in July 2011 and 30 l milk in December 2011, these values being inferior to those recorded in the case of the females in the second lactation. Regarding the mean milk production, we noticed that this parameter had superior values besides the ones recorded in females at the second lactation, oscillating between 1821.6 l in June and 3825.8 l in March.

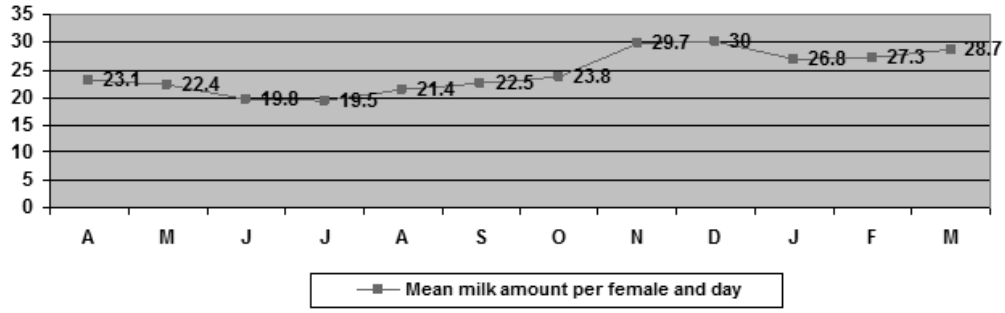

Figure 5. Milk production distribution at the third lactation

By the whole, the milk production dynamics in the three lactations and also daily and monthly is synthetically shown in the following charts. Regarding the total daily amount, it may notice that it exceeded 6801.2 kg l (recorded in July 2011), to almost 10400 kg in the last studied months, these values summing 2945607.7 kg for the whole year.

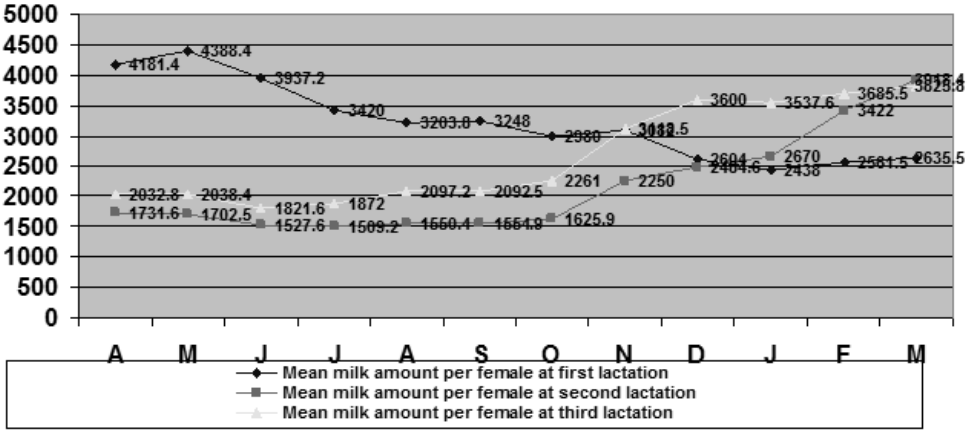

Figure 6. The synthetically dynamics of milk production in the whole livestock, monthly

In chart 7 we may notice the milk production dynamics achieved by the studied females, separately by the three lactation period. It may remark the superiority of the values recorded during the second lactation.

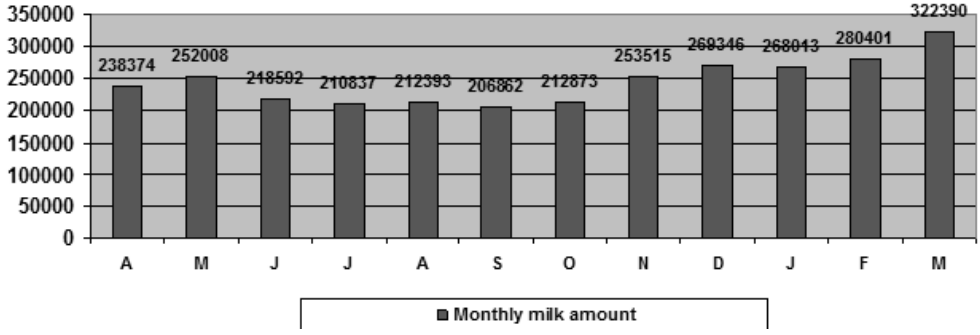

Figure 7. Milk dynamics per whole lactation

In this paper it was followed the quantitative milk amount. Based upon the primary data and after milk samples processing in the laboratory, in charts 8, 9, 10 are presented the evolution of milk amount, fat amount and protein amount in the studied interval, in absolute and also relative values. Beside these values, there are presented too, the values of the useful substance, an important index for animal breeding in our country (Ilie et al., 2010; Savu and Petcu, 2002; Tapaloaga, 2008; Tapaloaga, 2012).

It may notice that in the studied interval, in this farm, the milk fat and protein content varied as the ones in the special literature, framing within 3.90 and for milk fat and 3.31% and 3.40% for milk protein, conformingly these breeds standards. It also could be remarked that the recorded value do not significantly vary depending on season this fact could be justify by the fact that these animals are stock feed by and the seasonal feed does not influence.

The milk fat amount varied from 8046.9 kg in September 2011, to 12863.4 kg in March 2012. The milk protein amount varied from 6971.2 kg in September 2011, to 23728.0 kg in March 2012. Chart 8 presented the evolution of milk fat during the study interval.

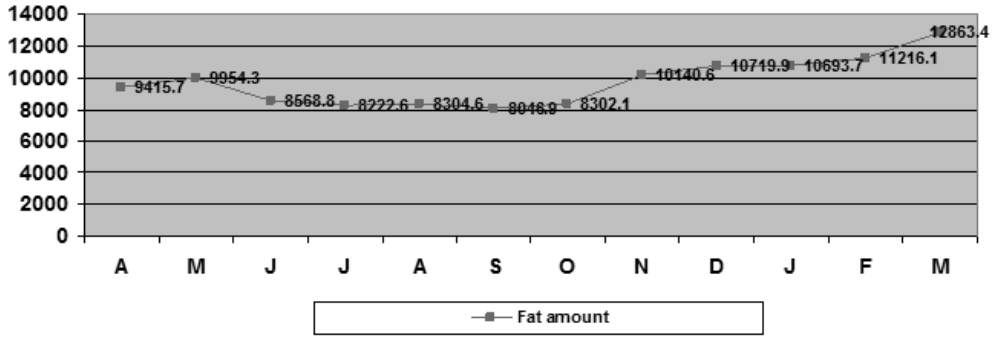

Figure 8. Fat percentage dynamics

Chart 9 presents by short the evolution of milk protein during the studied interval.

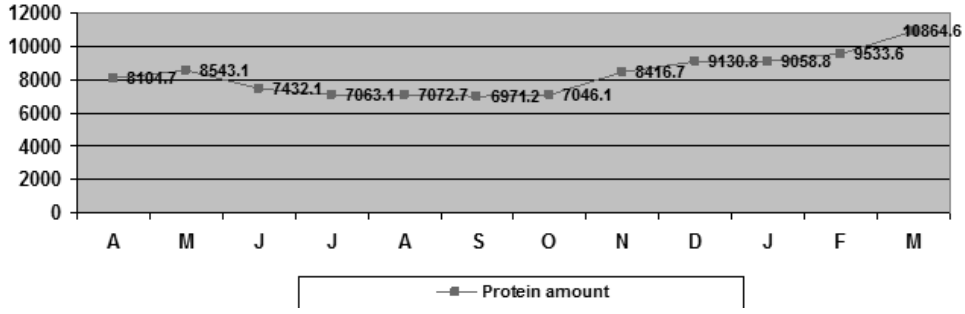

Figure 9. Protein percentage dynamics

As the two indicators, milk protein and fat, the useful substance had almost the same dynamics, emphasizing the special breeding value of the reproductive livestock in this farm, recording a mean value of more than 500 kg useful substance per individuals. The evolution of this index is shown in chart 10.

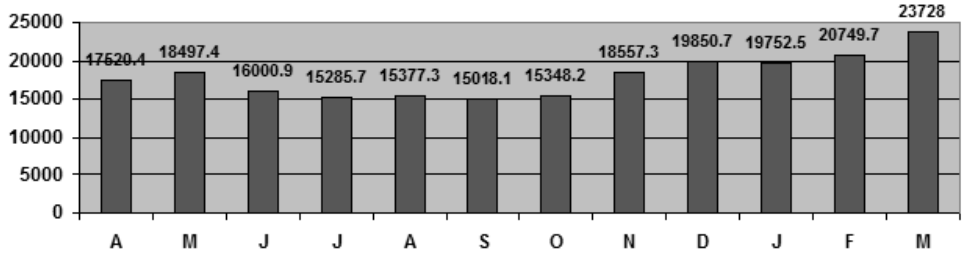

Figure 10. Useful substance dynamics

CONCLUSIONS

Based upon the technologic flow study and the quantitative and qualitative milk amount in S.C. ILYA AGRO S.R.L. we can conclude the following:

The reproductive livestock during the studied interval oscillated between 392 cattle (in

September 2011) and 418 cattle (in May 2011). The lactating females represented 90.44% (in April 2011) and minimum 77.14% (in November 2011), this value being framed within the limits recommended by the special guides to assure a high economic efficiency in dairy cows raising.

Comparing the milk production achieved by each animal category (first lactation cows, second lactation cows and third lactation and more cows) we could notice that during the first part of the studied interval, the percentage of cows at first lactation is superior to the percentage of the other two categories, but in the second part of the interval, this percentage had a decreasing trend to the last month of the study. This fact could be explained by the fact that the first interval of the study is the period of the farm beginning, with a large number of young females, at first lactation, these during the time, passing in the superior categories.

So, based upon the obtained results, this unit is included in the top economic efficient farm in the south of Romania.

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IMPROVING REPEAT BREEDER COWS FERTILITY BYESTRUS SYNCHRONIZATION: COMPARISON OF PRID + PGF_{2ALFA} + GNRH AND GNRH+ PGF_{2ALFA} + GNRH PROTOCOLS

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Abstract

The aim of this study was to compare two protocols for estrus synchronization and pregnancy rates for improving repeat breeder cows fertility over 6 months period. The study consisted of 63 Holstein cows from 3 herds. In each herd, 8 cows were allotted to group I, 8 cows were allotted to group II and 5 cows were allotted to control group. In group I (n: 24), repeat breeder cows received Progesterone Releasing Intravaginal Devices (PRID) on day 0 and PGF_{2alfa} injection on day 8th and Gonadotropin-Releasing Hormone(GnRH) injection on the day 9th. The PRID was removed on day 9th and cows were artificially inseminated after the detection of estrus. In group II (n: 24), repeat breeder cows received GnRH on day 0, PGF_{2alfa} on day 7th and GnRH on day 9th. Cows were artificially inseminated after the detection of estrus. In control group repeat breeder cows did not receive any treatment and repeat breeder cows were artificially inseminated after a behavioral estrus. The 2.00 ± 0.41 and 1.12 ± 0.23 days were found between the end of the treatment and time of behavioral estrus in group I and II, respectively the percentages of estrus detection were 95.8% (23/24) in group I and 91.6% (22/24) in group II. The pregnancy rates after artificial insemination were 56.5% (13/23), 36.3 (8/22) and 33.3% (5/15) in group I, II and control group, respectively. There was not a statistical significant difference for the estrus rates and pregnancy rates between the group I, II and control group. As a result, it is concluded that, both protocol in this study did not improve fertility of repeat breeder cows.

Key words: *Fertility, GnRH, PRID, Repeat breeder.*

INTRODUCTION

Dairy cattle that fail to conceive after several inseminations are a source of frustration and economic loss to the dairy enterprise. These so called 'repeat-breeders' are cows that fail to become pregnant after two or more services but continue to show estrus every 18 to 24 days. There are several potential causes of repeat breeding, including fertilization failure (29 to 41%), embryonic mortality (21 to 35%), defective luteal secretion of progesterone and other hormonal imbalances, errors in heat detection, various defects in sperm or egg function, and nutritional imbalances (Kim et al., 2007).

Ayalon (1978) reported that repeat breeder is a major source of economic loss in dairy herds in North America and its prevalence ranges from 10% to 18% between different states. Maurer and Echternkamp (1985) also reported a higher prevalence of repeat breeder in heifers (15.1%) than in multiparous females (8.3%). Treatment options for repeat breeder in dairy cows have been reviewed by Dawson (1998) and Levine (1999) and except for the administration of GnRH at the time of insemination, as was conducted by Morgan and Lean (1993), responses to other treatment options have been generally poor. Strategies may be used to optimize the time of insemination, including an intravaginal progesterone releasing device through a controlled internal drug release (CIDR) program, as shown by Day et al. (2000) and the Ovsynch protocol described by Pursley et al. (1995). The objective of this study was to evaluate the effect of two estrus synchronization protocols (PRID + PGF_{2alfa} + GnRH and GnRH + PGF_{2alfa} + GnRH) on the fertility efficiency of repeat breeder cows.

MATERIALS AND METHODS

This study was conducted on 63 repeat breeder Holstein cows which aged 3-5 years in three commercial dairy herds, located in Famagusta provinces in North Cyprus. Repeat breeder Holstein cows which were artificially

inseminated 3 or more, controlled by rectal palpation for determining of activities on ovaries and uterus. The cows which were artificially inseminated 3 or more and show estrus every 18 to 24 days without any abnormalities, included in study as repeat breeder cows. 63 repeat breeder cows are divided into three groups. In group I, repeat breeder cows received PRID® (1.55 gr. Progesterone; Sanofi Dogu Ilac, Ankara, Turkey) on Day0 and PGF_{2alfa} (İliren, Farma Intervet) injection on Day8 and GnRH (*Receptal®* inj., 0.0042mg buserelin asetat/ml, Intervet Ltd., Istanbul, Turkey) injection on the Day9. The PRID was removed on Day9 and repeat breeder cows were inseminated after the detection of estrus. In group II (n: 24), repeat breeder cows received GnRH on Day0, PGF_{2alfa}on Day7 and GnRH on Day9. Repeat breeder cows were inseminated after the detection of estrus. In both groups, repeat breeder cows which did not show estrus after the synchronization were not included to the statistical analysis. In control group, repeat breeder cows did not receive any synchronization and were artificially inseminated after behavioral estrus. The uterus of repeat breeder cows that could not be observed in estrus was palpated per rectum 45-50 days after artificial insemination to determine pregnancy status. The differences in estrus rates and pregnancy rates between two protocols and control group were analyzed by using Chi-square Test.

RESULTS AND DISCUSSIONS

Consummately, 63 repeat breeder cows were used in the study. 24 of them were allocated in group I, 24 in group II and 15 in control group. Estrus rate and timing of estrus in group I and II are presented in Table 1.

Table 1. Estrus rate and timing of estrus in group I and group II

	Estrus Rate	**Timing of Estrus (Day)**
Group I (PRID + PGF_{2alfa} + GnRH)	95.8% (23/24)	2.00 ± 0.41
Group II (GnRH + PGF_{2alfa} + GnRH)	91.6% (22/24)	1.12 ± 0.23
P	(p>0.05)	

In group I, 23 repeat breeder cows were detected in estrus and these repeat breeder cows were artificially inseminated. 13 repeat breeder cows were palpated as pregnant by rectal palpation 45-50 days after the artificial insemination in group I. In group II, 22 repeat breeder cows were detected in estrus and these repeat breeder cows also were artificially inseminated and 8 repeat breeder cows were palpated as pregnant by rectal palpation 45-50 days after artificial insemination in group II. In control group, 5 repeat breeder cows were palpated as pregnant (Table 2).

Table 2. Pregnancy Rates at Day 45-50 in group I, II and control group

	Pregnancy Rates at Day 45-50
Group I (PRID + PGF_{2alfa} + GnRH)	56.5% (13/23)
Group II (GnRH + PGF_{2alfa} + GnRH)	36.3% (8/22)
Control Group	33.3% (5/15)
P	p>0.05

The main objective of this study was to compare the effectiveness of the PRID + PGF_{2alfa} + GnRH and GnRH + PGF_{2alfa} + GnRH protocols for improving repeat breeder cows fertility. The treatments of repeat breeder cows have been evaluated for estrus rates, timing of estrus and pregnancy rates and statistically significant difference were not found between pregnancy rates of groups in the study.

In this study, timing of estrus was detected after 2.00 ± 0.41 day in group I and 1.12 ± 0.23 day in group II. Xue et al. (1984) reported 52.0 ± 5.8 hours as a time of estrus after removal PRID and Ozyurtlu et al. (2008) reported 3.22 ± 0.97 days as a time of estrus in their study. In group I, same results were observed after removal of PRID in repeat breeder cows. Aral and Colak (2004) reported 62.6 hours after

PGF_{2alfa} injection as time of estrus in GnRH + PGF_{2alfa} + GnRH protocol. Beside similar results were obtained in group II. Estrus rates are over 90% in two groups because of application of the treatment focus on the cyclic cows. Many studies indicated the conception rate following Ovsych program varied from 27 to 39% (Burke et al. 1996; Pursley et al. 1997; Pursley and Mee 1995). Kasimanickam et al. (2005) reported a 21.0% pregnancy rate following treatment with the Ovysch protocol in repeat breeder cows. In group II, it was reported 36.3% pregnancy rate for Ovsych protocol at 45-50 days and it′s similar to the other results of studies. 56.5% of pregnancy rates reported for the PRID protocols in this study. Hokmabad et al. (2005) reported a significant preganancy rate (28%) in the improvement of fertility by CIDR in repeat breeder cows. In the other studies of PRID, pregnancy rates reported between 14.28% and 73% after first inseminations (Kacar and Aslan 2004; Lopez et al. 2001; Penny et al. 2000; Zonturlu et al. 2005). In the comparison of the pregnancy rates, it′s shown that there was not any significant difference between group I, II and control group in this study.

CONCLUSIONS

In the comparison of the results of two protocols in the improvement of fertility on repeat breeder cows, statistically significant results were not found for PRID + PGF_{2alfa} + GnRH and GnRH + PGF_{2alfa} + GnRH protocols.

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THE EFFECT OF USING PIETRAIN BREED ON IMPROVING CARCASS QUALITY ON PIGS

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Abstract

The paper aimed the formation concerning the improvement of carcasses and pork meat. It includes the results of quality research on meat, carcasses and hybrids obtained by using Pietrain breed (white and white with black spots) – paternal form Yorkshire and Landrace maternal form. For this purpose, it was determined the carcasses length, thickness of fat on superior part, morphological structure of meat in carcasses. It was found significant difference between hybrids.

Key words: *meat, hybrids, breed, carcass.*

INTRODUCTION

The heterosis effect appears differently according to breed, quality of animals, their combination capacity and other conditions. To produce efficient hybrids there is necessary a good choice of breeds and lines with good combination capacities (Kosovac et al., 2009; Bozac et al., 2008).

The effect oh hybridization depends on nutriation and maintenance conditions, the reaction of organism to the environment and on the creation of every premises in order to achieve a prosper activity. Because the degree of heterosis manifestation concerning qualitative and quantitative characteristics varies, there appears the necessity of a good choice of breeds, to obtain qualitative production according to intended purpose. Nowadays there is not sufficient to produce meet without taking into account to consumers and processors needs (Birta, 2009; Cabanov, 2011).

Combination capacity of breeds in concrete amelioration conditions represents a decisive factor in production process of hybrids for the competitive meat.

Hybrids, being a product of hybridization are not a simple result of crossing, but animals with a rich heredity, which has special capacities of food assimilation and that through decrease of costs, produce more qualitative meat (Dragomirescu, 2007; Grosu and Oltenacu, 2005).

MATERIALS AND METHODS

Researches were made in pigs′ fattening enterprise SC″ West-Resurs″ SRL from the Republic of Moldova. Research material were swine hybrids, obtained from crossing Landrace breeds (maternal form) and Yorkshire (maternal form), white Pietrain and Spotted Pietrain. There were formed 3 experimental groups: I-Yorkshire x Landrace, II-Landrace x White Pietrain, III-Landrace x spotted Pietrain. Reproductive qualities of sows were appreciated by their prolificacy, lactation capacity and number of weaned piglets.

For fattening, there were used 48 young heads, each group having 16 heads. Animals were fattened until 100-120 kg, and then 8 individuals were sacrificed, from each category. The capacity of fattening was appreciated by reaching age, of 100 kg and average daily gain.

Carcasses were appreciated by their weight, length and ham development, determining the length of ham, thickness of lard at withers, spine, loin, croup.

RESULTS AND DISCUSSIONS

The action of genetic factors on animal production is determined by genotype influence, which was formed during selection process (breed, line, family etc.), genes interaction after mating and crossing breeds,

but also of genotypes with the environment. (Murugan et al., 2009).

In table 1, there are presented the results of reproductive quality appreciation of Yorkshire and Landrace sows with different paternal swine forms.

Table 1. The influence of combinative capacity of breeds on sows reproductive quality

Indicators	Groups		
	I Y x L	II L x PA	III L x PB
Prolificacy, head	10.6±0.10	9.9±0.20	9.4±0.20
Lactation capacity, kg	57.5± 0.28	52.1±1.03	47.4±1.60
Weight of piglets lot at 2 months, kg	183.8±1.80	170.4±3.4	160.4±4.3
Number of piglets at weaning, head	9.70±0.10	9.30±0.22	9.10±0.26

Data presented in table 1 reveals that a higher prolificacy was obtained in group number I of sows, and a smaller one in group number III, differences being of 1,2 piglets (B> 0.999) and between I group and III group-0,7 piglets (B> 0.99). Differences between I, II, III groups were insignificant, which proves that sows prolificacy staged by Pietrain boars decreased because of the influence of breeds at where paternal capacities are more developed.

The affirmation reflects upon lactation capacity, which was reduced in the experimental group III, with a difference towards group I equal with 10,1 kg. According to piglets lot weight of 2 months, there were noticed the same decreasing tendencies in group II and III. We must underline that Yorkshire and Landrace breeds, were used as maternal form, contribute to the maintenance of a good prolificacy through superior reproductive capacities and this is why, the number of piglets at furrowing varied from 9.49 and 10.6.

Increase results and development of young pigs obtained from the combination of different genotypes are presented in table 2.

Table 2. Growth speed of young hybrid pigs

Group	Breed combinations	The age of reaching 100 kg, days	Average daily gain from birth until finishing, kg
I	Y x L	211±1.8	542±3.6
II	L x PA	201±1.4	569±6.3
III	L x PB	206±1.6	554±4.1

The results of growth rate appreciation confirm that young experimental pigs prove a moderated growth and, the age of reaching 100 kg was over 200 days, in every pigs group. At the same time, a better result was obtained in group II, where crossing was used on Landrace breed (maternal form) and Pietrain (paternal form). Differences on average daily gain were equal with group I and II, with 27 g, and III with 15 g, being insignificant.

The study of formation capacity of meat production proved that pigs genotype influenced the quality of carcasses. There was identified that the carcass with a bigger length had crossing descendants of Yorkshire and Landrace breed, while their descendents obtained through Landrace and spotted Pietrain, characterized a smaller length. Descendants from group II were on intermediary position, but their carcasses were longer, comparatively with experimental group III at 100 kg category. These differences are less pronounced but significant.

Data concerning quality appreciation of carcasses are presented in table 3.

Table 3. Quality of carcasses at young hybrid pigs

Indicators	Groups		
	I (Y x L)	II (L x PA)	II (L x PB)
100 kg			
Carcass weight, kg	74.0±0.01	77.3±1.93	81.0±0.25
Length of carcass, cm	97.5±0.60	93.4±0.15	90.4±0.25
Ham length, cm	51.0±0.67	51.0±0.90	53.8±0.50
Ham perimeter, cm	71.4±0.87	70.2±1.15	74.4±0.72
120 kg			
Carcass weight, kg	88.2±0.13	92.7±0.85	96.2±0.32
Length of carcass, cm	99.7±0.70	93.3±0.25	92.9±0.73
Ham length, cm	53.8±0.54	54.2±0.93	56.8±0.30
Ham perimeter, cm	76.8±0.18	73.3±0.81	79.2±0.30

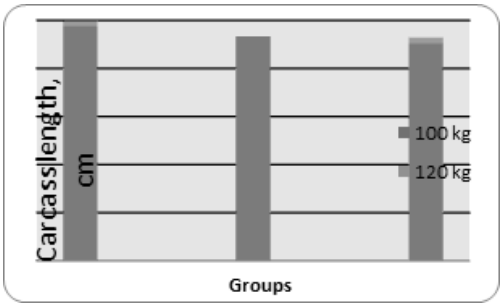

Figure 1. The carcass length in dependence of genotype and body weight

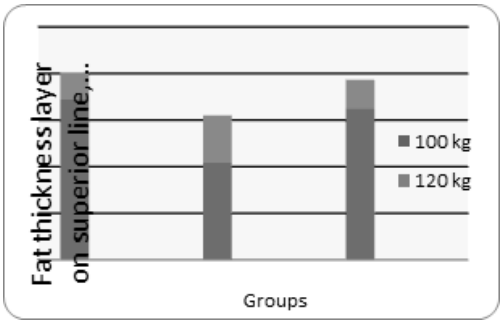

Figure 2. The fat thickness layer on superior line in dependence of genotype and body weight

The quality of carcasses at 100 and 120 kg, depends on pigs genotype and weight at slaughtering (table 3). Carcass mass on young pigs at group III, comparatively with group II at 100 kg was higher with 3.75 kg or 4.6% with 7.9 kg or 8.6% comparatively with group I. Carcasses length varied from experimental groups at 100 kg from 90.4 cm until 97.58 cm, and from 92.9 cm until 99.7 cm at 120 kg. There were registered significant differences between group I and II, I and III which were 4.1 and 7.1 m (B> 0.999).

Meat proportion on carcass is influenced by the level of development on ham, region which provides an important quality of lean meat. Ham length on group III was comparatively higher on group I and II with 2.8 cm at 100 kg and 2.6-3.0 cm at 120 kg. Globular ham were formed on young swine from group III, their perimeter exceeded group I and II with 3-4.2, cm at 100 kg and 1.9-2.4 cm at 120 kg.

The evolution of fat thickness in growth period is presented in table 4.

Table 4. The evolution of fat thickness layer on superior line at carcasses, mm

Carcass region	Groups		
	I (Y x L)	II (L x PA)	III (L x PB)
100 kg			
Wither	28.0±0.95	18.4±1.13	23.3±0.72
Spine	17.2±1.15	10.4±1.06	16.2±0.67
Loin	21.7±1.76	17.9±1.36	21.3±1.06
120 kg			
Wither	30.2±0.80	20.8±1.17	25.1±1.09
Spine	20.1±1.31	15.5±1.13	19.3±0.89
Loin	23.7±1.30	21.3±1.06	22.2±172

Fat layer formation in growth and fattening periods until 100-120 kg went differently, obtaining results which significantly depended on pigs genotypes. In every experimental group, regardless of breed membership or breed combination, there was registered a growth of fat layer thickness together with the increase of weight. The thickness of fat layer at pigs wither from group I equaled with 28.0 mm, being higher than in group III, with 4.8 mm or 17.1% (B> 0.95) and 9.6 mm comparatively with experimental group II (B> 0.999). We must mention that fat layer was thinner than the superior line of carcasses at every genotypes, but a smaller value was noticed at young pigs from group II, obtained by crossing Landrace and white Pietrain breeds. At spine region, fat layer was the thinnest, but differences varied from 6.8 mm (I and II), and 5.8 (II and III) (B> 0,999). In loin region, differences between groups concerning fat thickness were reduced, but it varied from 3.4 and 3.8 mm, between l groups I and II, II and III (B> 0.99).

CONCLUSIONS

1. Reproductive and fattening capacities, but also quality of carcasses is influenced by animal genotype, used at commercial swine hybrid production.
2. Using Pietrain breed boar at crossing, contributed to hybrid production, which formed qualitative carcasses, but this carcasses were longer and with a thinner fat layer, and were obtained from descendants as Landrace x white Pietrain, and globular hams with a bigger length realized Landrace x spotted black Pietrain hybrids.

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BREEDING HOLSTEIN OR NORWEGIAN RED IN ALBANIA?

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Abstract

The study was carried out to assess the economics of small (up to 4 cows) and medium (more than 6 cows) sized dairy farms breeding Holstein and Norwegian Red (NRF) breeds with reference to the Income per Farm including milk and meat sales, Income per Farm only milk sales, and the cost of production in Albania. Data on production, expenses and returns were recorded on a monthly basis for 160 farms (80 for each breed) over a period of 12 months. The "Income per Farm (IpF) " method was used to calculate the farm income. The "IpF" (including milk and meat) for Holstein farms ranged from 1969.9 Euro (small farm) to 8036.6 Euro (medium farm) and for NRF farm from

Key words: *Holstein and NRF breed, economic analysis, income per farm, small and medium sized farms.*

INTRODUCTION

Norwegian Red (NRF) is one of the new cattle breeds introduced in the last 15 years in Albania. Since 1994, NRF breed began to spread out in our country through the importation of semen from Norway. Up to now are imported around 400.000 dose of semen (ADE, 2010; Gjoni 2007). The Norwegian-Albanian association was establish in Elbasan district, supported and financed, at beginning, from a Norwegian project and this is the reason that we find NRF breed mainly in the above mention region. In addition to milk production, farmers likes NRF breed also for good indicator of daily body gain and higher milk fat content compare with Holstein.

During 2010, in Albania were operating 219,952 farms with cattle, out of 350,654 farms in total or 62,7%. The average of cows per farm is 1.5 units. Only 14.5% (3188 unit) of the farms have more than 6 cows and they produce 20% of the total cow milk (MAFCP, 2010).

During the last decade we are witnessed for the emergence of the so-called medium size dairy farms owning 6-20 cows. These farmers have been looking at dairying as an economic activity and they are looking forward to modernize their activities. Another reason to study farms with more than six cows is the Instruments Pre-Accession and Rural Development-Like (IPARD) program which will support such farms.

In Albania, is lacking information on the economics of production on the small and medium size dairy farms, and especially for the farms managing NRF breed. Also farmers are not keeping the financial records for each crop or animal separately. This study was therefore undertaken to collect on farm data pertaining to revenue and expenses on both types of farms and both breeds (Holstein-most spread out breed and NRF breed) and make an economic analysis.

MATERIALS AND METHODS

The research was conducted in the central part of Albania (Elbasan district) where the Norwegian-Albania Association, which imports the NRF semen, has the head-quarter. 80 small size farms (40 per each breed) and 80 medium sizes (40 farms for each breed) were monitored. Data collection lasted from September 2011 till August 2012. Small farms were defined as those having 2 to 4 cows and medium farms those having 6 or more cows.

***Data collection*:** Each farm was visited monthly over a period of 12 months (bi monthly visits). The following data were recorded (according to the questionnaire prepared and tested):

A. Income and expenses: (i) Expenses for the fodder production, like alfalfa, corn, etc., (ii) Expenses for the animal feed bought in the market, (iii) Expenses for veterinary service, including insemination, (iv) Expenses for fuel, electricity, water, trips, lease on land, and land tax, (v) Estimated cost of labor (unpaid labor) needed to take care of the herd per year at Euro 2,150 (Bernet et al., 2000), (vi) Incomes per Farm from sales of milk and meat (IpF milk+meat); (vii) Incomes per Farm from sales of milk (IpF milk); (viii) Incomes per Cow from sales of milk and meat (IpC milk+meat); (ix) Incomes per Cow from sales of milk (IpC milk); (x) Milk yield: the amount of milk produced by each cow during one lactation; (xi) Milk price per liter; (xii) Calves price per kg live bodyweight; (xiii) Quantity of milk sold in the market or to the dairy processor (quantity and price).

B. Technical data, such as: Insemination (artificial or natural mating), milking (milking machine or by hand), type of animal feed used (including microelements or premix), animal health (diseases and parasites), training needs.

***Data analysis*:** A model was developed in Microsoft Excel program for data analysis, and statistical data processing was done with Statgraphics Centurion XVI.

RESULTS AND DISCUSSIONS

Data on number of cattle and cows, milk yield, Incomes per Farm (IpF milk + meat), IpF (milk), milk cost (Bernet et al., 2000; Frank et al., 2001), the ratio milk quantity sold in the market vs. total milk production, prices of milk and meat sold, the manner of milking the cows and method of insemination, are summarized in Table 1, as shown below:

Table 1. Technical data

Number of heads	No of cattle per farm		No of Cows per farm		Milk yield (liter)		IpF (milk+meat) Euro		IpF (milk) Euro		Milk cost (Euro/kg)	
	Holstein	NRF	Hol	NRF	Hol	NRF	Hol	NRF	Hol	NRF	Hol	NRF
2-4 cows	4.25	4.15	3.05	3.08	4525	4480	1969.9	2416.2	784.1	958.8	0.34	0.36
6+ cows	11.92	12.42	9.67	10.17	4838	4780	8036. 6	9235.7	5208. 4	6541.2	0.25	0.28

Table 2. Milk data

Number of heads	Milk sold vs milk produced (%)		Price of milk sold (Euro) By hand (%)		Price of meat sold (Euro)		Milking				Insemination			
							By machine (%)				Natural		Artificial	
	Hol	NRF	Hol	NRF	Hol	NRF	Hol	NRF	Hol	NRF	Hol	NRF	Hol	NRF
2-4 cows	78.4	82.0	55.0	60.0	421	479	100	100	0	0	0	0	100	100
6+ cows	89.0	86.7	49.6	57.2	402	417	35	22.5	65	77,5	0	0	100	100

From table 1, we can see that cows of small and medium farms breeding Holstein produced 45-58 kg milk more milk (1-1.2%) than farms bred NRF. However the farms with NRF breed are taking more incomes compare with Holstein farms (14.9-25.6%). The differences are coming as result of milk and meat price sold which is higher for the farms breeding NRF, because the fat content of milk produced by NRF breed is higher than Holstein and the daily gain also (Gjoni, 2007; Bernet et al., 2000).

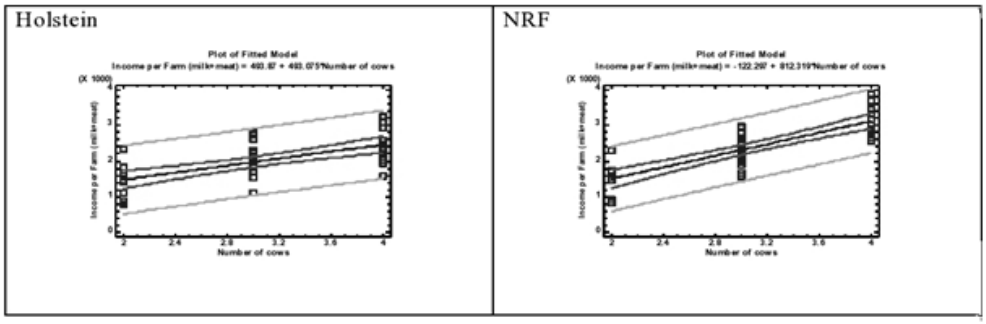

Figure 1. Holstein and NRF small farms IpF (milk+meat) vs Number of cows per year

Holstein: Income per Farm (milk+meat) = 493.87 + 493.075*Number of cows. Since the P-value in the ANOVA table is less than 0.05, there is a statistically significant relationship between Income per Farm (milk+meat) and Number of cows at the 95.0% confidence level.

NRF: Income per Farm (milk+meat) =-122.297 + 812.319*Number of cows. Since the P-value in the ANOVA table is less than 0.05, there is a statistically significant relationship between Income per Farm (milk+meat) and Number of Cows at the 95.0% confidence level.

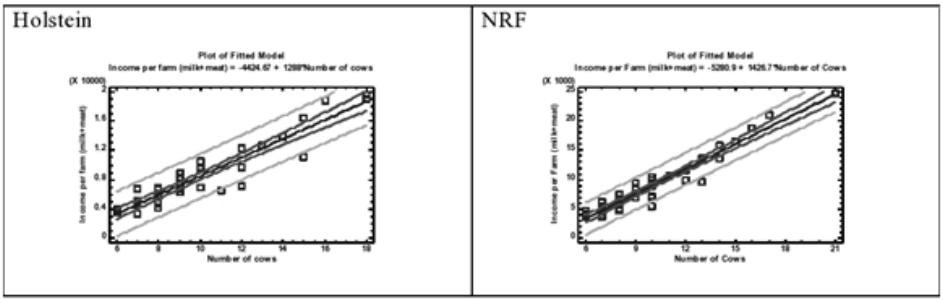

Figure 2. Holstein and NRF medium farms IpF (milk+meat) vs Number of cows per year

Holstein: Income per Farm (milk+meat) = -5280.9 + 1426.7* Number of Cows. Since the P-value in the ANOVA table is less than 0.05, there is a statistically significant relationship between Income per Farm (milk+meat) and Number of Cows at the 95.0% confidence level.

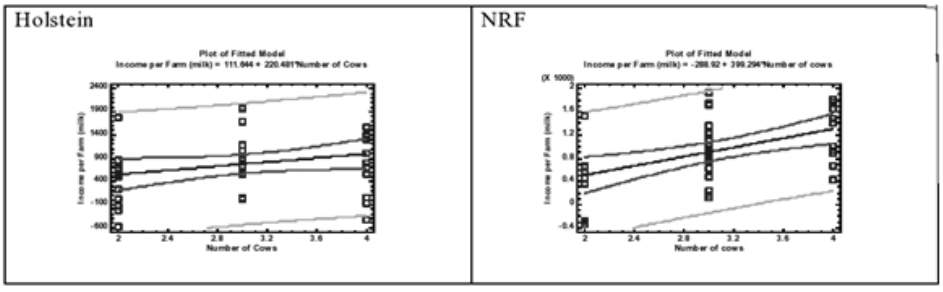

Figure 3. Holstein and NRF small farms IpF (milk) vs Number of cows per year

Holstein: Income per Farm (milk) = 111.644 + 220.481*Number of Cows. Since the P-value in the ANOVA table is greater or equal to 0.05, there is not a statistically significant relationship between Income per Farm (milk) and Number of Cows at the 95.0% or higher confidence level.

NRF: Income per Farm (milk) =-288.92 + 399.294*Number of cows. Since the P-value in the ANOVA table is less than 0.05, there is a statistically significant relationship between Income per Farm (milk) and Number of cows at the 95.0% confidence level.

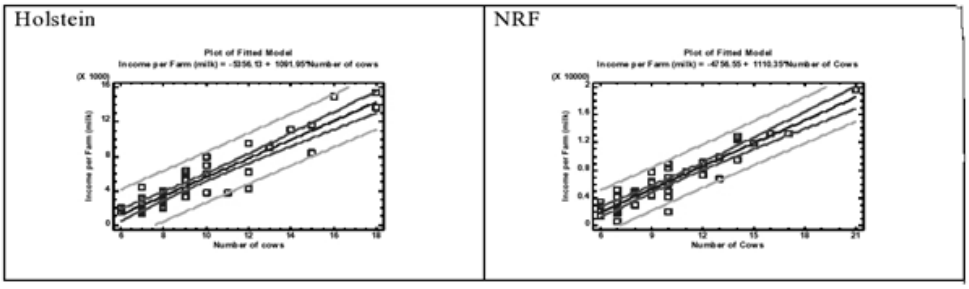

Figure 4. Holstein and NRF medium farms IpF (milk) vs Number of cows per year

Holstein Income per Farm (milk) =-5356.13 + 1091.95*Number of cows. Since the P-value in the ANOVA table is less than 0.05, there is a statistically significant relationship between Income per Farm (milk) and Number of cows at the 95.0% confidence level.

NRF: Income per Farm (milk) =-4756.55 + 1110.35*Number of Cows. Since the P-value in the ANOVA table is less than 0.05, there is a statistically significant relationship between Income per Farm (milk) and Number of Cows at the 95.0% confidence level.

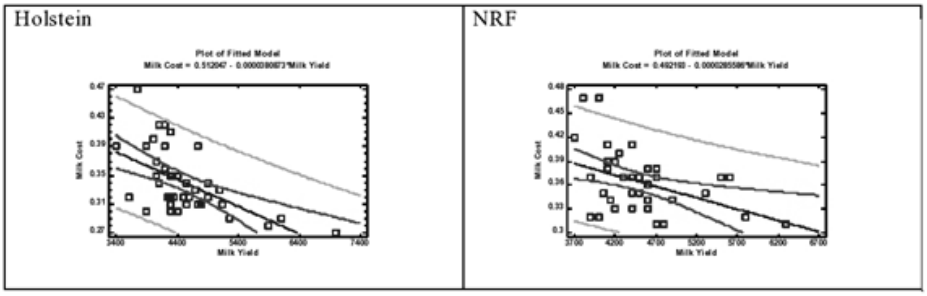

Figure 5. Holstein and NRF small farms Milk yield vs Milk cost

Holstein: Milk Cost = 0.512047- 0.0000380873*Milk Yield. Since the P-value in the ANOVA table is less than 0.05, there is a statistically significant relationship between Milk Cost and Milk Yield at the 95.0% confidence level.

NRF: Milk Cost = 0.492193- 0.0000285586*Milk Yield. Since the P-value in the ANOVA table is less than 0.05, there is a statistically significant relationship between Milk Cost and Milk Yield at the 95.0% confidence level.

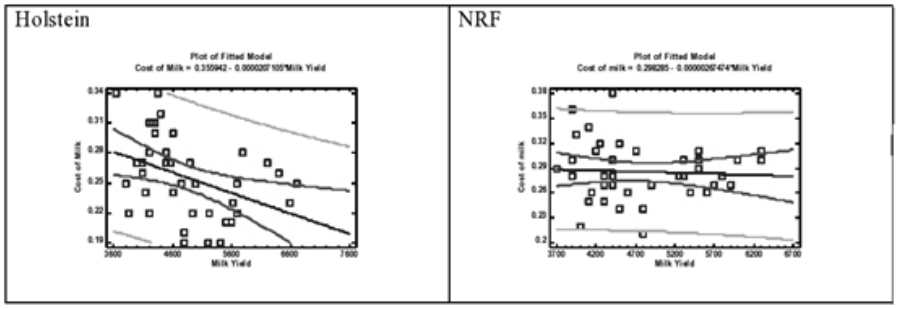

Figure 6. Holstein and NRF medium farms Milk yield vs Milk cost

Holstein: Cost of Milk = 0.355942-0.0000207105*Milk YieldSince the P-value in the ANOVA table is less than 0.05, there is a statistically significant relationship between Cost of Milk and Milk Yield at the 95.0% confidence level.NRF: Cost of milk = 0.298285-0.00000267474*Milk Yield.Since the P-value in the ANOVA table is greater or equal to 0.05, there is not a statistically significant relationship between Cost of milk and Milk Yield at the 95.0% or higher confidence level.The comparison of IpF (milk+meat) data for Holstein and NRF breed are shown in Table 3 and Table 4:

Summary Statistics

	IpF NRF (milk+meat)	*IpF Holstein (milk+meat)*
Count	40	40
Average	2416.2	1995.5
Standard deviation	726.711	600.53
Coeff. of variation	30.0766%	30.0942%
Minimum	811.0	783.0
Maximum	3891.0	3236.0
Range	3080.0	2453.0
Stnd. skewness	-0.51395	-0.314459
Stnd. kurtosis	-0.464041	-0.326298

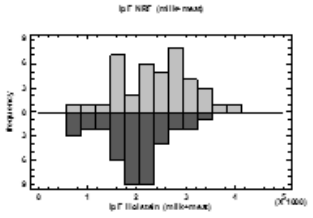

Figure 7. Summary Statistics of small farms IpF (milk+meat) for the two studied breed

This table shows summary statistics for the two samples of data. Values of these statistics outside the range of-2 to +2 indicate significant departures from normality, which would tend to invalidate the tests which compare the standard deviations. In this case, both standardized skewness and standardized kurtosis values are within the range expected.

Summary Statistics

	IpF NRF (milk + meat)	IpF Holstein (milk+meat)
Count	40	40
Average	9235.75	7840.68
Standard deviation	5011.48	4542.65
Coeff. of variation	54.2617%	57.9369%
Minimum	3560.0	0
Maximum	24710.0	19624.0
Range	21150.0	19624.0
Stnd. skewness	3.26985	3.253
Stnd. kurtosis	1.87978	1.5152

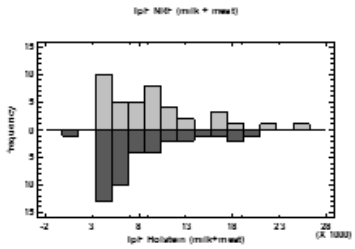

Figure 8. Summary Statistics of medium farms IpF (milk+meat) for the two studied breed

This table shows summary statistics for the two samples of data. In this case, both samples have standardized skewness values outside the normal range while the standardized kurtosis values are within the range expected.

The R-Squared statistic indicates that the model as fitted explains 41.8129% to 88.9456% of the variability in IpF (milk+meat) of Holstein breed and 65.2775% to 93.0524% of the variability in IpF (milk+meat) NRF breed. The correlation coefficient equals 0.646629 to 0.94311 for Holstein breed and, 0.807945, to 0.964637, i for NRF breed indicating a strong relationship between the variables

The average cost of producing a liter of milk for small and medium farms was 0.24 Euro/liter (Holstein) and 0.28 Euro/liter (NRF). Since the P-value in the ANOVA table is less than 0.05, there is a statistically significant relationship between Milk Cost and Milk Yield at the 95.0% confidence level for small and medium sized farms of Holstein and for small farms of NRF but not for the medium farms of NRF.

In addition the farms bred Holstein are selling 78-89% of their milk production and the rest is used for the calves and for the home consumption, while the farms bred NRF only 82-87%, because the medium sized farms are using more milk for raising calves. The small sized farms have higher the price of meat sold because they are slaughtering and selling the meat by themselves while the medium farms are selling live calves in the market. Another difference between the small and medium farms is that the medium ones are using milking machine 65-77,5%) to milk the cows and this is a reason that they have better milk price as the milk quality is higher.

Regarding the trainings the owners of small farms breeding Holstein did not participate in any training while for the medium farms 30% of them participated. 30% of the owners of small and medium farms breeding NRF participated in trainings. In addition all the farmers interviewed are asking for trainings and the main subjects are related with cows feeding and feed ration, animal feed preparation, animal health, and livestock best practices.

From this study we found that farms bred NRF had better results than those bred Holstein breed. In addition, the medium farms (of both breeds) had higher production, better Net Farm Income and lower cost of production compare with small farms.

CONCLUSIONS

Both breeds are performing well in conditions of Albania and specially NRF that is introduced in the last 15 years. However the milk yield of both breeds is low compare with the production in countries of origin (ERDBA, 2010). The state extension service should train the owners of medium sized farms for best management practices, as they lack knowledge of feeding the cows during milk period and dry one. In addition the farmers could get better results

from the calves as the daily gain must be increased with proper feeding.

The 'IpF' increases with an increase in the number of cows kept on the farm. So, the medium sized farms have better financial indicators than the small ones and the Ministry of Agriculture should support the medium farms in the future through IPARD Like program and through the programs financed by Albanian Government.

Farmers in Elbasan region likes NRF breed, as calves has better daily gain and the price of calves is better than Holstein. In addition the milk fat content is higher for NRF breed and as result the milk price is better than Holstein.

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STUDY REGARDING REPRODUCTIVE ISOLATION IN ROMANIAN SPORT HORSE FROM JEGĂLIA STUDFARM

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Abstract

This study is a part of an ample research concerning influences factors in the competitive traits of Romanian Sport horse from Jegălia studfarm. The genetic analysis studies are a part of Animal Genetic Resources Management because just start of them we elaborate the strategies for inbreeding management. This study has as purpose to present one important aspect of genetic analysis: reproductive isolation. This parameters has a capital importance in animal breeding because there has a directly influence in animal population evolution.
The reproductive isolation situation was quantified using the relation elaborated by S. Wright in 1921.

Key words: *sport horse, Jegălia, reproductive isolation.*

INTRODUCTION

This study is a part of an ample research concerning the genetic analysis (history) of Romanian Sport Horse from Jegălia studfarm. The genetic analysis studies are a part of Animal Genetic Resources Management because just start of them we elaborate the strategies for inbreeding management (Popa R., 2005). This study has as purpose to present one important aspect of genetic analysis: reproductive isolation. This parameters has a capital importance in animal breeding because has a directly influence in animal population evolution.

The population acceptance criteria are four: reproductive isolation, morphological and physiological differences, environmental requirements and genetic size (Popescu-Vifor, 1990). The reproductive isolation level is the most important criteria for population acceptance, the other three being in according to them (Drăgănescu C., 1979). This parameter is very important because only reproductive isolated populations have an own evolution, in contrary they are influenced by evolving of immigrants populations.

MATERIALS AND METHODS

The biologic material are represented by 4 sire stallions and 52 mares, Romanian Sport Horse, representing the entire reproductive nucleus from Jegălia stud farm at this time (December 2012).

The reproductive isolation level was quantified using the follow relation [1]:

$$C.I.R. = \frac{AA-(AI+II)}{AA+AI+II},$$

where: AA – number of individuals accepted for reproduction in analyzed interval with both autochthons parents; AI – number of individuals accepted for reproduction in analyzed interval with one autochthon and one immigrant parent; II – number of individuals accepted for reproduction in analyzed interval with both immigrants parents.

RESULTS AND DISCUSSIONS

The results regarding reproductive isolation coefficient (RIC or CIR) are showed in table 1.

The moment of this study is approach to the last imports, and that is the reason who make easy for us the identification of immigrants parents.

Before analyzing dates presented in table 1 we must specificate some things. We can observe, from table 1, big number of fathers who activate in reproductive nucleus by two reasons: first because of the immigrants stallions imported and introduced in

reproductive nucleus, and second because of overlapping generation. All this make possible finding of current sire stallions also at the parents level, especially at the parents of reproductive nucleus level. Such notice a genetic persistence of immigrants in reproductive nucleus, as a following of maintaining of individs with minimum one immigrant parent, to create genetic variability necessary for selection and for changing some characters in the direction of immigrant populations.

Observing the sire stallion ascendence for analyze of R.I.C., we discover one immigrant individual (Condor stallion, recently imported). Regarding to the brood mares, we identify 16 mares with one immigrant parent (great proportion of father who belongs to another horse population: Shagya, Furioso North-Star, Throughbreed).

Dates presented in table 1 relieve the fact that the Romanian Sport Horse livestock from Jegălia studfarm became a population with his own evolutive way (R.I.C.=0.3929). The sire stallion livestock have R.I.C. = 0.5, and the broodmares livestock (R.I.C. = 0. 3846), is dominated by autochthon mares (N = 36) The situation is very different at the parents level (R.I.C. = -0.2069) and at the grandparents level (R.I.C. = -0.439), as we can see in table 1, where we observe a great weight of individuals with one immigrant parent (50% in parents of reproductive nucleus, 39% at the grandparents level) and imported (10.34% in parents of reproductive nucleus, and 32.93% at the grandparents level).

Table 1. The reproductive isolation coefficient values

Specification		No. AA	Immigrants (I)	Parents			R.I.C.
					AI	II	
Reproductive nucleus (RN)	♂	4	1	3	-	1	+0.5
	♀	52	-	20	16	2	+0.3846
	Total	41	1	21	18	2	+0.3929
Parents of RN	♂	18	4	2	6	5	0
	♀	40	2	18	13	2	-0.3
	Total	58	6	20	19	7	-0.2069
Grandparents of RN	♂	33	18	5	7	19	-0.5758
	♀	49	9	26	6	9	-0.3469
	Total	82	27	31	13	28	-0.439

CONCLUSIONS

The Romanian Sport Horse from Jegălia stud farm became a population with his own evolution. The value of reproductive isolation coefficient, are inconstant in generations successions because of crossbreeding. The sire stallion number is too small to allow a good management of inbreeding, if will maintain Romanian Sport Horse as a population with reproductive isolation.

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RESEARCH REGARDING EVOLUTION OF HEAVY WEIGHT OF YOUNG RAMSIN TELEORMAN'S BLACK HEAD SHEEP

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Abstract

Study of average performances in a population have a huge importance because, regarding to a population, the average of phenotypic value is equal with average of genotypic value. So, the studies of the average value of characters offer us an idea about the population genetic level. This study have the principal purpose to analyze heavy weight in young rams because this indicator is used in selection process.
The biological material is represented by a sample of 327 lambs from Teleorman's Black Head Sheep, exploited, at different ages, breed in Braila County.. The average performances of character is presented in the paper. We can observe an important grade of variability with some differences between gain classes. The average performances of the characters are very good and between characteristic limits of the breed.

Key words: *Black Head Sheep, body weight.*

INTRODUCTION

This study is a part of an ample research concerning the opportunity for creating regional rams centers in direction to improve milk production in Teleorman Black Head Sheep. This study have the principal purpose to analyze heavy weight and daily gain of young rams because this character it is used in selection process also for milk production (Taftă V., 1998). The daily gain is associated with milk production, and that is the reason for using this criteria (Taftă V., 2003).

MATERIALS AND METHODS

The biological material is represented by 327 lambs, 157 females and 170 males, breed for reproduction in a single exploitation from Braila county. The individuals were analyzed through daily gain at 7 days old and at 1 month old, through heavy weight, and, most important, through population average performance of daily gain. All measurements was made by us in Braila County, using an electronic scale.

RESULTS AND DISCUSSIONS

The results are showed in table 1. Is very clearly that the males are superior to females regarding heavy weight (figure 1) and daily gains at analyzed moments. The differences between sexes, in relation with all sample, is not so big. The males have 5.3467 kg. average heavy weight at birth (103.06%), 7.337 kg at 7 days (104%), and 14.2 kg at 30 days old (103%). Females presents an average heavy weight of 5.0293 kg. at birth (96.9%), 6.7728 kg. at 7 days (96%), and 13.675 kg. at 30 days old (98.12%). The average performances of sample was 5.188 kg at birth, 7.0549 kg at 7 days, and 13.9375 kg at 30 days old. Regarding the average daily gain, the differences between sexes can be observed in figure2.

Table 1. The reproductive isolation coefficient values

Specifications	Average heavy weight at:			Average daily gain between:	
	Birth	7 days	30 days	0 - 7 days	7 - 30 days
Males	5.346705882	7.336970588	14.2	0.267696429	0.291039698
Females	5.029299363	6.772802548	13.675	0.23738626	0.276888587
All	5.188002623	7.054886568	13.9375	0.252541344	0.283964142

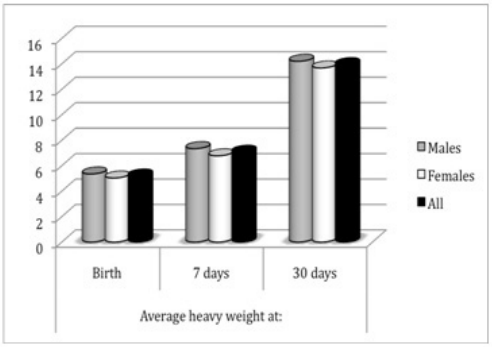

Figure 1. Average heavy weight

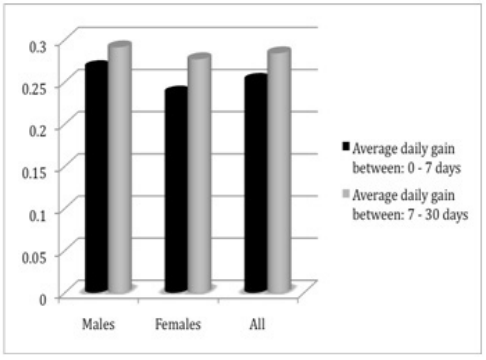

Figure 2. Average daily gain

CONCLUSIONS

The males performances are superior in relation with female performances. The selection criteria body heavy weight, and also daily gain, it is a good way to approximate milk production of the ewes. All this, correlate with selection of rams from twins birth for increasing the birth rate indicator, is a good selection method used in sheep breeding practice for Teleorman′s Black Head Sheep breeders.

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THE UTILIZATION OF FAST FOOD WASTE PRODUCT ON THE PROTEIN EFFICIENCY RATION OF LOCAL MALE DUCK

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Abstract

Day old male local ducks are the hatching duck eggs by product, that could be raised intensively as ducks meat, because very cheap and by fast growing age of eight weeks can be harvested, and slightly fatty. Friend chicken fast food restaurant waste product as bone and meat which rich of high level of protein and utilized for alternative feed local ducks. Waste product contains 50.18 % crude protein, so it can be used as an alternative source of animal protein feed. The experiment using the fast food restaurant waste going as meat and bone meal (MBM) and utilized of its to increase duck meat production. The experiment was held to find out of protein efficiency ratio of male local ducks fed diets containing fast food waste (MBM). One hundred day old local male ducks were raised in cages until eight weeks old. The experiment conducted with Completely Randomized Design, five meat and bone (MBM) meal levels in the ration, namely: 0 % (R0), 5 % (R1) ,10 % (R2), 15 % (R3), and 20 % (R4), repeated five times, where each replication consist of five local male ducks, and continued with Dunnet test, if there were any significantly differences among the treatments. Feed consumption, protein consumption, body weight gain and protein efficiency ratio were parameters observed. The result indicated that fast food restaurant waste (MBM) doesn't give negative effect on feed consumption, protein consumption, body weight gain and protein efficiency ratio. The real conclusion of this experiment that by giving fast food restaurant waste going as meat and bone meal until 20 percent gave the best protein efficiency ratio of local male duck.

Key words: *Fast food restaurant waste product, local male duck, meat and bone meal, product, protein efficiency ratio.*

INTRODUCTION

Male Day old ducks are by-product of the local ducks hatching, Local male ducks feasible for intensively reared meat producers, because it's cheap, fast-growing, and slightly fatty, more efficient in use of ration than female ducks (Bakrie et al 2006; Srigandono, 1996). The optimal ducks growth rate achieved by the age of 6-8 weeks, and generally male ducks harvested at 8 weeks (Hardjosworo and Rukmiasih, 2001). To obtain the maximum yield needs to be balanced with the provision of rations qualities, and rations are balanced and proportionate nutrient content. Ration of quality feed ingredients should be supported by well qualified (Leeson and Summers, 2001). Rations quality usually are relatively expensive, so it would have an effect on the production cost. The solution needs to look for alternative feed ingredients capable in substituting a price relatively cheaper, but still good quality expected to reduce production cost. Fast food restaurant selling dishes from chicken, lots of waste disposal in the form of residual bone with a little meat attached. It is predicted that a fast food restaurant (looks like KFC) was able to spend an average of 125 chickens per day, with a cut into 8 pieces so that the fried chicken products are sold every day, totaling 1000 pieces and approximately 60% (600 pieces) consumed in restaurant. From one piece of chicken waste is expected to generate as much as 10 gram, that of the 600 pieces of waste generated as 6 kilo grams per day. Waste in the form of residual bone and little meat attached, disposed of as waste (Supratman, 2008). In order to optimize the utilization of waste as a source of fast food restaurants feed ingredients, processing needs to be done by processed into meal, called meat and bone meal, and can be used as feeding ducks because of high nutrient content of crude protein content of 63.23%, crude fat 14.53 %, and 10.72% mineral (Poultry Nutrition, Non

Ruminant and Industrial Laboratory, 2007). High protein and mineral content with calcium and phosphor which derived from bone waste was able to be an alternative ingredient mixture rations for male local ducks. Growth is a very complex process that includes body weight gain due to changes in the shape and weight of the tissues except fat tissue (Cherry and Morris, 2008). Feed intake should affect the rate of growth achieved. Consumption and high protein content in the ration will affect protein consumption (Wahyu, 1992). Protein is essential organic substances and essential for growth and production (Leeson and Summers, 2005). To determine the biological evaluation of protein quality is needed to see its effect on livestock. One of the measures of protein quality is the protein efficiency ratio (PER), which is simply the weight gain of animal divided by protein intake (Leeson and Summers, 2005). PER is best that could produce a high number, and it indicates a good quality protein.Until now there is no information about the PER value in using of rations containing meat and bone meal given from a local male ducks. Meanwhile, local male duck is prospective in supply of animal protein of birds, so we need research toward quality protein ration that will give the best weight resulting affect.

MATERIALS AND METHODS

The research used 100 DOD local male ducks, with the average of body weight was 39.87 gram and 8.97% percent of variable coefficient. The ducks kept in flock over 8 weeks, as much as 20 flock, and each flock consisted of 5 ducks. Every flock is equipped by feeder and round waterer, 25 watts of bulb lamp as heater and hanging in the middle of each flock, where a 10 watt of tube lamps as house light.

The ration consisted of yellow corn-meal, fish meal, rice bran meal, soy-bean meal, meat and bone meal, rice polished, salt and premix as additive feed in 22 percent protein and 2900 Kcal/kg of metabolisable energy (Scott and Dean, 1991). The meat and bone meal (MBM) were made from fast food restaurant waste product in the Poultry Nutrition, Non Ruminant and Industrial Laboratory, Faculty of Animal Husbandry, Padjadjaran University West Java.

The ration treatments consisted of:

R_0 = Ration control, without meat and bone (MBM) meal

R_1 = Ration contained 5 percent meat and bone meal

R_2 = Ration contained 10 percent meat and bone meal

R_3 = Ration contained 15 percent meat and bone meal

R_4 = Ration contained 20 percent meat and bone meal

The formula composition of ration is showed in Table 1, and the metabolisable energy and nutrient content in Table 2. Completely Randomized Design was used in this experiment with 5 treatments, and each treatment repeated 5 times. Then the data was analyzed by Random Simple Test, and among treatments with Dunnet Test (Stell and Torrie, 1989). Variable analysis were feed consumption, protein consumption, body weight gain, and protein efficiency ratio.

Table 1. Composition of The Formula Rations (%)

Ingredients			**Ration**		
	R0	**R1**	**R2**	**R3**	**R4**
Yellow corn meal	50.00	50.00	50.00	50.00	50.00
Soy-bean meal	6.65	6.45	6.02	5.70	5.40
Rice bran meal	17.25	17.25	17.24	17.20	17.15
Fish meal	20.00	15.00	10.00	5.00	0.00
Rice polished	5.00	5.30	5.64	6.00	6.35
MBM meal	0.00	5.00	10.00	15.00	20.00
Salt	0.10	0.10	0.10	0.10	0.10
Premix	1.00	1.00	1.00	1.00	1.00
Total	100.00	100.00	100.00	100.00	100.00

Table 2. The Nutrient and Metabolism Energy Content in The Rations

The Nutrients	R0	R1	R2	R3	R4
Crude Protein (%)	22.01	22.01	22.00	22.00	22.00
Crude Fat (%)	5.32	4.85	4.39	3.92	3.45
Crude Fiber (%)	6.25	6.54	6.62	7.11	7.39
Calcium (%)	1.40	1.13	0.85	0.58	0.30
Phosphorus (%)	0.69	0.55	0.41	0.27	0.13
Metabolisable Energy (Kcal/kg)	2.900	2.900	2.900	2.900	2.900

RESULTS AND DISCUSSIONS

Table 3. The Feed consumption, Protein Consumption, Body Weight Gain, and Protein Efficiency Ratio

Variables	R0	R1	R2	R3	R4
Feed Consumption (gram)	3022.45[a]	3052.35[a]	3275.04[b]	3369.11[b]	3473.52[b]
Protein Consumption (gram)	664.94[a]	671.52[a]	720.51[b]	741.20[b]	771.12[b]
Body Weight Gain (gram)	758.25[a]	789.70[a]	848.40[b]	907.05[b]	1019.20[b]
Protein Efficiency Ratio	1.14[a]	1.18[a]	1.18[a]	1.22[b]	1.32 [b]

Note : The similar superscript in the same row show non significant difference (P<0,05)

The feed consumption, protein consumption, body weight gain, and protein efficiency ratio are showed in Table 3.

Feed Consumption

Table 3 shows that feed consumption tends to increase proportional because of meat bone meal increased in the ration.

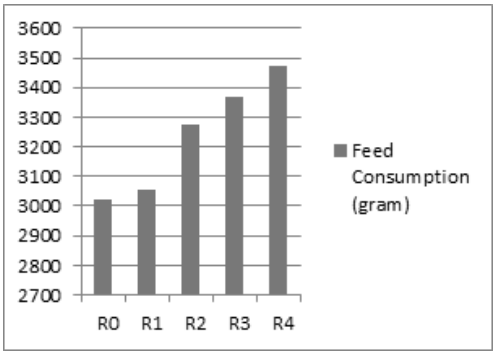

Figure 1. Feed Consumption

The results variance analysis showed, by giving meat and bone meal until 20% in the ration providing significant effect (P <0,05) on ration consumption. Average consumption of rations in the treatment uses 10%, 15% and 20% meat bone meal was significantly higher compared with the control ration consumption. While by using of 5% meat and bone meal showed no significant difference with the control ration. Increased feed consumption on rations containing meat bone meal are palatable because of the higher value. According (North and Bell, 2004) palatability is a major factor affecting consumption and palatability ration depend on texture, smell and taste, although taste not an important role in poultry

Protein Consumption

Protein consumption is obtained by calculating the amount of ration consumed multiplied by the protein content of the ration (Table 2).

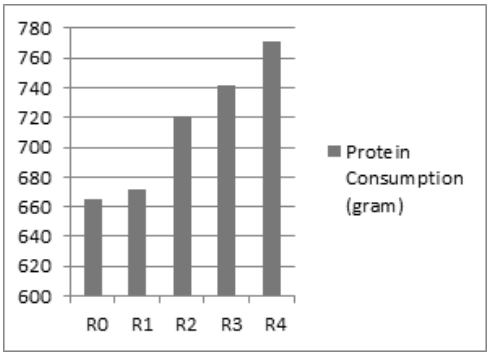

Figure 2. Protein Consumption

Analysis of variance showed that by addition of meat and bone meal in the ration has significant effect (P< 0,05) on protein consumption. From Dunnet Test results showed that an increase in protein consumption in line with the increasing of meat bone meal addition in the ration. This means that rations containing meat and bone meal more palatable, thus by

increasing the consumption of rations will have an impact on increasing of protein intake. The better quality of the ration, the higher consumption of rations, so that more nutrients including protein absorbed by the body which finally result in good growth. These results agree with the opinion of (Cherry and Morris, 2008; Scott and Dean, 1991), where the consumption of protein is affected by the rations consumption and protein content in the ration which will ultimately affect the growth of ducks.

Body Weight Gain

The body weight gain of each treatment is showed in Table 3. The average of body weight gain was 758.25 – 999.20 gram, showing that duck's feeding increase because of there were meat and bone meal composition in rations.

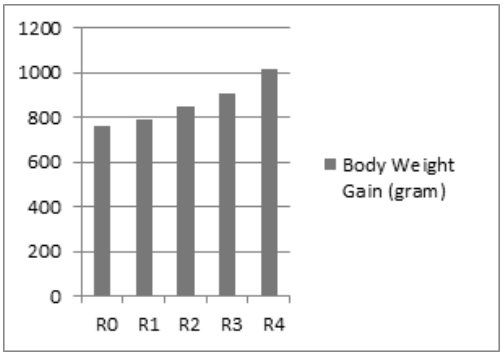

Figure 3. Body weight gain

Analysis of variance showed that by addition of meat and bone meal has significant effect on body weight gain of male local duck. By adding the meat and bone meal until 20 percent in the ration of male local duck still gave a good result. The result was parallel on feed and protein consumption those were also no significant different (P> 0,05) among the treatment 10 %, 15% and 20% meat and bone meal (R2, R3 and R4) in the ration but significant different to R0, (without meat and bone meal) and R1 (2.5% meat and bone meal). Its mean that the meat and bone from 10 percent up until 20 percent in ration did not influence palatability and duck appetite, so the body weight gain was increased. This is because of protein content in meat and bone meal is more better (63.23%) than fish meal protein (54.43%), fat is also higher, at 15.85%, while 8.69% on fish meal. Animal protein from meat and bone meal has a composition similar to the form of the protein inside the duck's body, making it easier for ducks to realignment would be a form of protein to the muscles. Because of that, the body weight gain gave better, than the control treatment without meat bone meal.

Protein Efficiency Ratio

In Table 3 can be seen that highest of protein efficiency ratio on male local duck which receiving 20 percent meat and bone meal in the ration R4 (1.32), and the lowest was R0 ,ration without meat and bone meal (1.14). The results of variance analysis showed that the treatment by using of meat and bone meal gave significantly affected on protein efficiency ratio. This means that the use of meat and bone meal to 20% in the ration produces more better quality than the control ration. This is due to the protein content of meat bone better than fish meal protein, so that the resulting quality of rations is also better. And because the content of amino acids meat and bone meal derived from chicken similar to amino acids in the body of duck, then it is not difficult to change the amino acid feed into meat fibers in duck body.

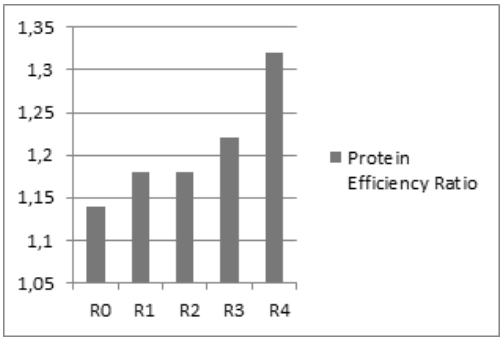

Figure 4. Protein Efficiency Ratio

According to (Leeson and Summers, 2005) a high quality protein will promote more weight gain per unit of protein consumed than will a low quality protein. This is evident from the body weight gain in the treatment of meat bone meal additions higher than the control ration. Scott, and Dean (1991) states that the Protein Efficiency Ratio in the ration directly related to

the biological value of protein ration itself. When seeing from the feed consumption, protein consumption and body weight gain were significantly higher then the resulting Protein Efficiency Ratio also higher. So meat bone meal can be used as an alternative feed ingredients for animal protein supplements of fish meal. Cherry and Morris, (2008) said that protein quality was not only reflected in the amount of protein contained in the feed material or of the amount required but determined by the quality.

CONCLUSIONS

It was concluded that by using the meat and bone meal until 20 percent level in the ration was still able to produce an optimal result on Protein Efficiency Ratio and meat bone meal can be an alternative source of animal protein feed.

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DAIRY SECTOR IN ALBANIA-CHALLENGES AND PERSPECTIVES

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Abstract

Agriculture still remains subsistence-oriented due to a very small average size of farms (1.26 ha per family) and 85,8% are mixed farms. Hence, only 30–40% of crop and livestock products are being sold. Generally, livestock production is seen as a backbone of Albania's agriculture. The objective of this analysis is to give a quantitative and qualitative description of the Albanian dairy sector, its challenges and perspective. About 75% of household incomes are contributed by sales of livestock products. The productivity and the economic efficiency are at low level. Evaluation of performance at dairy farm level is not possible because data are missing. The statistical yearbook of Ministry of Agriculture and Consumer Protection does not include information for each product at farm level. To improve the situation several economic and policy programs are needed to increase the productivity of the dairy sector, the quality of products, to implement the good livestock practices, good hygiene practices and animal welfare standards, as dairy sector provides 21.5% of the value of agricultural production.

Key words: *dairy sector, livestock products, subsistence, productivity, small sized farms.*

INTRODUCTION

Albania continues to be predominantly a rural economy with 20.4% of GDP (World Fact Book) generated by agriculture, while livestock provides 52% of the value of agricultural production (MAFCP, 2012). Presently, 50% of the population continues to live in rural areas and farming constitutes main employment option for people in these areas. Official employment data indicate some 750 thousand people are employed in the private agricultural sector (this accounting for 60% of employment in Albania). Food produced locally fulfills some 70% of the total food requirements of Albania. However value of processed and imported foodstuffs remains still very high.

Agriculture still remains subsistence-oriented due to a very small average size of farms (1.26 ha per family) and 85.8% of them are mixed farms. One farm family as average is managing 2.32 cattle, 31.8 sheep and 31 goats (1.67 cows 24.4 ewes and 23.7 milking goats).

More specifically, dairy activities have a long tradition in Albania due to the favorable natural resources for dairy production. In the plains, cattle production is dominant, while in the hills and mountains, sheep and goat production are more suitable. Traditional dairy products are yogurt, butter, curd and different kinds of cheese from cow, sheep and goat milk.

Livestock occupies a very important place in the Albania′s overall agricultural production. In 2011, it had a share of 52% of the overall agricultural production compared to 42% in 1992 and 35% in the 80ies (MAFCP, 2012).

The dairy sector of Albania is characterized by large number of small milk producers. There are over 210,000 agricultural holdings producing cow milk, the overwhelming part with less than 5 cows. Larger scales of dairy farms are in the western part of the country, where country's milk production is concentrated. 80,000 farms are rearing small ruminants, with 1.93 million milking sheep and goats. The milk production is in total 1,1 million tons and 86,8% of milk production is coming from cows. Most milk producers are semi-subsistence households. Only up to 46% of milk production is delivered to milk processors. The rest is used for self consumption, direct sale to consumer or for feeding of animals (Schröder et al, 2010). 3.370 dairy farms have more than 6 cows per farm and 11.800 farms have more than 50 sheep or goats per farm. The number of farms keeping more than 6 cows and more than 50 sheep or goats started to operate in the last 10 years and

the trend is to be increased. With the support of the Albanian Government funds and IPARD-Like program those farms can achieve further steps to come closer to good hygiene and management practices and standards.

Holstein, Jersey and their crossbred are largely extended as dairy cattle breeds in the farms of the country. The small ruminants are all local breeds. Artificial insemination is covering only 62% of the cows′ population; while natural mating is used for the small ruminants.

The cow′s milk yield is not increased significantly in Albania in the last 20 years (in the year 1990-the yield was 1403 liters milk/cow, whereas in the year 2011-2696 liters milk/cow), as except the problems are related with genetic improvement, and the feeding of animals is unsatisfactory. The farmers mainly use fresh fodder and hay, of a relatively low quality. Concentrated feed are used in insignificant quantities, which result from lack of tradition as well as from rather high price of concentrated feed.

As Albania is in the process of approximation to the European Union (EU), seeks potential to increase competiveness and food standards to improve import/export relation with agriculture and food products. With regard to milk and dairy products Albania is almost self-sufficient (in the level of 99%).

In the last decade there is a tendency of establishment of farms for milk production with a capacity of 10-100 heads of milking cows.

MATERIALS AND METHODS

The aim of this study is to describe and to estimate the state and performance of the dairy sector in Albania, to identify key constraints of the sector and to develop policy interventions to improve the competitive position of the sector. In this study are used agricultural statistical data published by Ministry of Agriculture, Food and Consumer Protection (MAFCP), desk studies, meetings and collection of detailed information of dairy farms managing cows and small ruminants (sheep and goats), as well several meetings with dairy sector specialists of public and private organizations. In order to complement information from the key informed stakeholders, we also posed open-ended questions and had discussions to obtain insights on the relevant issues. Representatives of selected institutions and experts were interviewed and some field visits were conducted to identify the sector problems and get a thorough insight into the structure and performance of the sector.In addition opinions of stakeholders on policy intervention were collected during several focus group meetings.

RESULTS AND DISCUSSIONS

Animal origin products represent a main source of food, and a high share of production still serves subsistence purposes. Dairy activities have a long tradition in Albania due to the favorable natural resource base for dairy production. In the lowland, cattle production is dominant, while in the hills and mountains, sheep and goats′ production are more suitable.

The livestock development in general and milk production in particular, are closely related with several basic factors, which are:

- The Albanians′ tradition, which have historically developed livestock.
- Need for livestock products.
- Daily income provided by milk sales.
- Milk as an improver of the protein diet.

Livestock is totally privatized and it was the first one started before the privatization of the land and finished in 1993.

Due to the significance of livestock and milk production, particularly in rural areas, MAFCP has selected the milk sector as a policy priority (MAFCP, 2007). The Albanian government and MAFCP are inclined to support primary production and one of the declared objectives is to improve the competitiveness of products in order to substitute for import.

a. Overview of milk production and structural features at primary level.

Table 1, illustrate the small-scale structure and the subsistence orientation of dairy farms in Albania. In average are kept 2.32 heads of cattle/farm (only regarding farms that keep cattle at all), which includes calves, heifers and bulls, 31.8 sheep, 31.0 goats. Thus, the number of milking animals is even smaller (1.67 cows per farm in average, and/or 24.4 ewes, and/or 23.7 does).

Table 1. Data on milk farms and production

Description	1990			2000			2011		
	Cattle	Sheep	Goat	Cattle	Sheep	Goat	Cattle	Sheep	Goat
Farms with cattle, sheep or goat (thousands)	*All animals were under state sector (socialist system)*			*315.2*	*109.9*	*51.6*	*212.4*	*5.,3*	*24.4*
Number of farms with more than 5 cows, or 50 sheep, or 50 goats	*All animals were under state sector (socialist system)*			*1011*	*5569*	*2712*	*3372*	*8347*	*3506*
% of farms more than 5 cows, or 50 sheep, or 50 goats	*All animals were under state sector (socialist system)*			*0.3%*	*5.1%*	*5.25%*	*1.6%*	*15.1%*	*14.4%*
Animals (thousands heads)	*632.6*	*1646*	*1145*	*728*	*1939*	*1106*	*492*	*1758*	*759*
Milk production per year (tons)	*421*	*43.8*	*52.6*	*807*	*70*	*71*	*955*	*80*	*66*
Average number of animals (heads/farm)	*All animals were under state sector (socialist system)*			*2.31*	*17.64*	*21.4*	*2,32*	*31,8*	*31.0*
Milking animals (heads)	*300*	*1142*	*776*	*448*	*1448*	*800*	*354*	*1.349*	*580*
Average number of milking animals (heads/milk producing farm)	*All animals were under state sector (socialist system)*			*1.42*	*13.2*	*15.5*	*1,67*	*24.4*	*23.7*
Milk yield per animal (liters/head/year)	*1403*	*38.4*	*67.8*	*1801*	*48.5*	*88.5*	*2696*	*59.2*	*113.4*

Source: Statistical Year Books-2000-2011, MAFCP

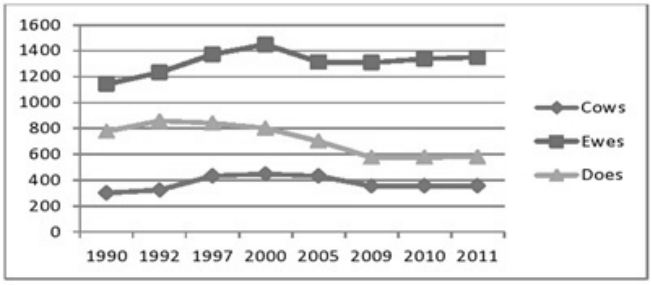

Figure 1: Number of milking animals for the period 1990-2011

Table 2. Amount of milk per cow, ewe and doe for the period 1990-2011 (kg)

Descriptions	**1990**	**1992**	**1997**	**2000**	**2005**	**2009**	**2010**	**2011**
Cows	1403	1500	1636	1800	2163	2581	2631	2696
Ewes	38.4	44.6	49.6	48.5	57.2	56.8	58.7	59.2
Does	67.8	81.7	88.1	88.5	101.3	105.4	110.3	113.4

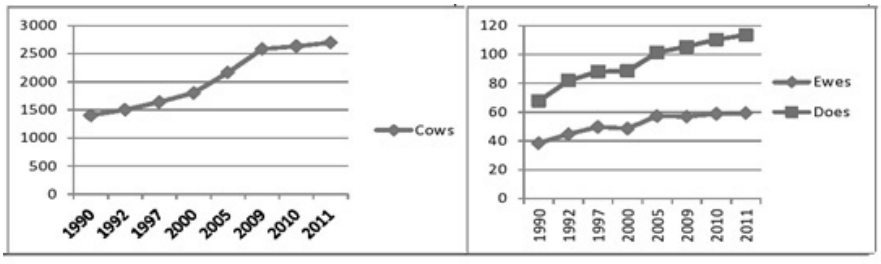

Figure 2: Milk yield per milking animal for the period 1990-2011 (kg)

The privatization of land and livestock started in Albania on August 1991. As farmers in the last 10 years, before the privatization, were not allowed to manage livestock in the first decade

after privatization were eager to increase the number of animals and did not pay too much attention to the productivity. While, after the year 2000 the number of animals is decreased and the yield is increased as farmers are more aware on the productivity of the animals. However the average milk yield per cow/year in Albania is 2,696 liters currently very low in comparison with the average of the EU-27 which is more than 6,000 liters per cow/year. Also sheep and goat milk production is organized based mainly as capital extensive production system (based in natural pasture grazing) and milk yield per ewe with about 59 liters/year and per doe (milking goat) with about 113 liters are also very low.

Even today the number of dairy farms is high (Table 1) and most of cattle/cows farms (63%) are concentrated in the western part of the country (lowlands).

Considering the share of subsistence farms, it is very likely that the primary dairy sector in Albania will face structural changes when the status of "candidate member" will be granted to the country.

Breeding: Another factor that has its negative impact in the milk yield is the genetic improvement and the breeds that are managed in the country. Currently, crossbreeds of 'Black and White' and "Jersey" breeds makeup 80% of the cows' population, and the rest are dual purpose crossbreds (Simmental, Brown Swiss, Norwegian Red, and Tarentaise). While in the small ruminants predominantly are local breeds, because no new breeds have been introduced and the continuation of the natural mating. Few heads of Alpine breed (goat) from France were introduced by the end of the 80ies and continued to be imported during 90ies in southeast of the country as a result of a donation from French Government.

Feeding system and input supply: During summer fresh forage, mainly alfalfa and grasses, and grazing in the plots free of crops, are used to feed the cows. During winter hay and corn or wheat bran are used. Corn or grass silage is used in the ration to feed the animals only by the medium and large dairy farms (10-100 cows). In summer sheep and goats graze in meadows. During winter hay and a limited quantity of wheat bran is provided to animals for the lambing/kidding and lactation period. For feeding the animals are used the permanent pasture that are around 440 000 ha and also about 204 000 ha are planted with forage crops. However the average yield of forage crops is still low in the level of 26-28 Tons/ha (MAFCP, 2012), where are faced problems with the use of high quality seeds, limited amount of fertilizers applied for the forage crops. All inputs are traded by private dealers but a lack of credit on the farmers' side inhibits an expansion of their use. Veterinary services are provided by private veterinarians.

Price of Animal Feed: Animal feed prices are very high and this is an obstacle to the increase of productivity, especially in cows. Thus, a kilogram of compound feed for cows is 50% higher than 1 kg of milk.

Milk quality issues: The system for the control of milk quality is still weak and not functioning very well. A part of the milk continues to be sold on the road or directly to the home door what makes difficult the quality control. The control agencies laboratories are not efficient and the farmers have to pay for the analysis, therefore they neglect to analyze samples which might be dangerous in regard to public health. Closed cooling chains from producer to consumer exist only for the producers that are managing more than 10 cows. EU quality and food safety standards are not yet implemented however through the financial support of EU through IPARD-Like and Albanian Government programs is expected to start the improvement of situation especially for the farms managing more than 6 cows.

Health situation: In general the animal health situation is under control due to the state-run veterinary service available all over the country. However, there are few problems with Brucellosis and Anthrax (in Southern and Northern part of the country) in sheep and goats that render it impossible to export milk, lambs and kids, which in other regions are as organic products.

As a result of all the above mention issues the low capital intensity of production, in all the milking animals, is resulting in low yield, relatively high production costs and low profitability; many dairy farms managing less than five cows have negative balance from milk production however as a farm activity they are profitable as a result of calves' sales.

b. Milk flow and Milk value.

The diary industry in Albania is not vertically integrated. As it mentioned above the majority of milk producer are semi-subsistence farms. The milk market (mainly cow milk) is characterized by the existence of informal (direct selling from farmers to the processors and markets) and formal market channels (collection & distribution by dairies). According to national statistics only up to 46 per cent of the milk production is being delivered for processing. The chain linkage is typically weak in Albania. From the graph below it is clear than a significant share of the milk produced is consumed on the farm, for own consumption (16%) or for animal feed (14%), or processed in the farm (cheese, butter, curd for the family consumption and market). Farmers, still, are selling milk and milk products directly to consumers on street markets.

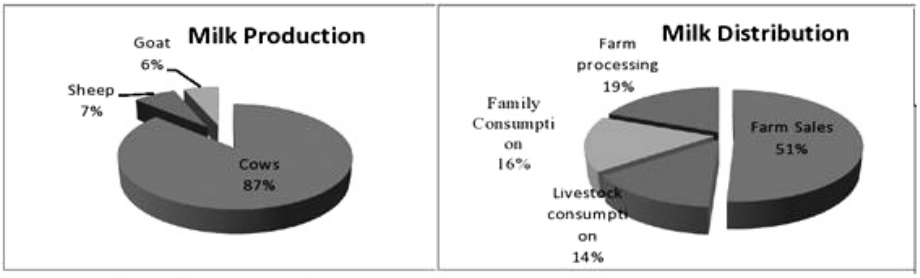

Figure 3. Milk production and milk distribution

The high level of farm usage and direct selling is a consequence of several factors, including the small-scale structure of production, a consequential lack of commercial orientation amongst many producers, an underdeveloped milk collection system, attractive street market prices compared to the price offered by processors, and the unreliability of milk payments made

by some processors (Berkum, 2009). A major challenge for the development of dairy sector in Albania is to increase the supply of good quality raw milk to the processing sector in a cost effective manner. The majority of subsistence farms (less than 5 cows) lack the necessary capital to improve the situation according to EU standards. According to literature review, farm visits and interviews with farmers and field experts only the farms with more than five cows or 50 sheep/goats and a part of the farms managing 3-5 cows will be able to make the necessary investments for operational improvements in order to comply with increasing quality and product safety requirements.

Table 3. Share of milk production in Agriculture and Animal Production Value (Gross Agriculture Output in million EURO-prices of 2006)

Description	Years				
	2000	2005	2009	2010	2011
Agricultural Production	901	1016	1136	1227	1267
Animal production	510	602	627	641	656
Milk production	274	315	315	321	330
Increase rate of milk production value (%)	--	14.96	0	1.9	2.8
Share of milk in aggregate animal production output (%)	53.7	52.3	50.2	50.0	50.3

Source: *MAFCP Statistics Yearbook of Albania for 2000-2011 and authors' calculation*

The value of milk production is increased with 1-2% in the last years and the share of milk in animal production is reduced from 53.7% to 50.36% in the last 10 years.

CONCLUSIONS

The sector needs are related to the development and implementation of specific strategies at national level, the access to updated technology to improve competitiveness of the local businesses, the reinforcing quality standards in

terms of their effective application, the access to financing opportunities especially the EU funds.

According to the SWOT – Analysis, interviews and the discussion with farmers, experts and businessmen s*ome of the factors that limit milk production are:*

- Unfavorable entity structures in production,
- High level of land fragmentation,
- Low competitiveness and efficiency of production,
- Insufficient knowledge on modern production techniques/technologies and standards,
- Difficult access to financial resources for investments,
- Insufficient professional advisory service,
- Insufficient attainment of national and/or EU standards,
- Non appropriate VAT system,
- Insufficient of enforcement of national legislation (e.g. food safety),
- Lack of rural infrastructure, markets, collection points, as well as lack of information on prices, markets, etc,
- Lack of credits for investments in livestock farms and milk processing factories,
- Insufficient managerial skills both in fields of technology and economy of production,
- Weak vertical integration of farmers and processors.

There are no incentives and motivation for the extension services and veterinary and food inspectorates to help farmers in livestock husbandry, animal welfare, milk hygiene, etc.

The future development of the sector will be directly determined by the country's economic and social development. Along with the growth of the economy and the improvements in living standards, the consumer changing habits will generate further opportunities on the food processing market, in terms of both volumes and quality standards. The increase of high income population will refine the demand and generate the drive for a higher diversification of the offer.

The Government and Ministry of Agriculture needs to find the mechanism to raise consumers demand for the high quality products, increasing technology standards, environmental demands, technological innovation, and increasing scale of production and networks of farms and companies.

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GREAT BUSTARD RESTOCKING IN EURASIA

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Abstract

The significant risk of biodiversity loss in Eurasia there is the habitat fragmentation of great bustard (otis tarda). This reduces the flow of individuals between populations. The great bustard is a globally endangered species that has recently suffered dramatic declines due to agriculture intensification as well as other antropical actions. The paper presents a comparative inventory of the population in the sites from Spain up to China. There are presented the threats and the necessary conservation measure for prevention of the extinction of the species, based on the integration of our large data series obtained during the last two decades in the world. The data collection includes informations referring to individual behavior and population dynamics (mating system, dispersal capability, migratory behavior, annual recruitment, mortality, and longevity), habitat availability (carrying capacity, satellite imagery) and genetic structure of the population.
The paper also presents the Romanian approach of the great bustard restocking by the development of national center in National Park Comana, using the site of a former tank polygon Mihai Bravu, Giurgiu County. These research works are conducted in the Program of scientific cooperation with Moldavia Republic, in the theme Eco-economics research regarding compared restoration in Romania and Moldavia Republic.

Key words: *biodiversity conservation, great bustard, Romania.*

INTRODUCTION

The great bustard is on the IUCN Red List of Threatened Species, and European populations have been in long-term decline, only arrested by conservation projects in some areas. The running projects will contribute to conservation of the species in the world.
Great bustards favourite lowlands, river valleys, and undulating open country, avoiding steep or rocky terrain, deserts, wetlands, forests, and savannas or parklands with more than isolated or small clumps of trees. Arable fields bearing crops such as oilseed rape, kale and lucerne now apparently appear to be more attractive than natural steppe, although farmland areas with high agricultural disturbance near human settlements are often avoided.

OTIS TARDA IN EUROPE

In Europe have been managed projects for otis tarada restocking with the following objectives:

- Significantly increase the population of great bustards in Europe
- Detailed monitoring to improve understanding of the interaction between released bustards and their environment
- Development of a long-term strategy to guide future work on great bustard
- Formulation and promotion of agri-environment options to improve the suitability of the 'wider countryside' for great bustards
- Re-establishment of the great bustard as an integral part of Europe avifauna
- Translocation of juvenile great bustards from Russia and Spain each year
- Management of the release area to maximize its value to great bustards year-round
- Secure extensive areas of suitable habitat for great bustard across a wider area through the development and promotion of targeted options for inclusion within agri-environment schemes
- Protection of bustards and their nests and eggs from threats such as disturbance, egg-collecting and predation
- Rigorous monitoring program to improve knowledge of bustard distribution, ecology and behavior

- Communication and dissemination actions undertaken to develop a high profile for the project both in local communities and in key target
- Links developed with projects targeting great bustards elsewhere in the EU to allow the multi-way exchange of experiences and lessons learned.

Some lessons learned in UK and Spain are presented below:

UK

The main objective of the British project is to increase the small population of great bustards on Salisbury Plain-Southern England, in order to develop a self-sustaining population in the country. A five years activity was conducted in a LIFE+ project.

In the UK, the great bustard became nationally extinct when the last bird was shot in 1832. This iconic species of the Wiltshire landscape returned to the UK in 2004 when the Director of the Great Bustard Group, David Waters, established the 10-year trial reintroduction. The project sourced birds rescued from agricultural operations in Russia with a plan to release 20 birds per year onto Salisbury Plain. The project had early success with females laying infertile eggs in year three, males reaching maturity in year five followed by the first chicks to be hatched for over 170 years fledging in the same year.

The great bustard was originally a locally common and widely distributed breeding species in many parts of the UK. It occurred on chalk downland in central southern England and in the open sandy Brecklands of eastern England. In addition, great bustards from continental Europe moved to the UK during the colder months.

Traditionally birds of expansive grass plains (steppe), they have adapted well to modern agricultural landscapes. They are frequently found in semi-cultivated/managed grasslands, arable farmland and traditional lowland hay meadows. (Burnside et all,2012)

With help from Natural England, bustard-friendly habitat options are being developed to be implemented through the ELS and HLS agri-environment scheme. Work with the landowner of the new confidential release site to agree predator control strategy, and deploy electrified fox-proof fencing around the perimeter of the release field, and erect soft-release pens within the field these also having fox-proof electrified fencing surrounding them. Although predator control is practiced by the local farming community, predation of ground-nesting birds by foxes is still the biggest problem.

Spain Habitat fragmentation reduces the flow of individuals between populations, constituting a major risk of biodiversity loss. The great bustard (Figure 1) is a globally endangered species that has recently suffered dramatic declines due to agriculture intensification, and human-induced habitat fragmentation. The Iberian Peninsula represents, with more than half the world total, the species′ last stronghold, but conservation measures are urgently needed to maintain genetic diversity, counteract isolation, and prevent the species′ extinction. This project aims to assess the impact of changing land-use patterns, and other important human-induced sources of mortality, on great bustards in Iberia, and to propose ways to reconcile agricultural and rural development with species survival. This will be achieved through integration of our large data series obtained through radiotracking during the last two decades on individual behavior and population dynamics (mating system, dispersal capability, migratory behavior, annual recruitment, mortality, longevity), habitat availability (carrying capacity, satellite imagery) and genetic structure of the population (mitochondrial and nuclear DNA analyses) in spatially-explicit simulation models. The models will (a) help testing relevant hypotheses of metapopulation theory, (b) provide an analytical framework for assessing how patterns of land use affect the long-term survival of bustards, and (c) indicate ways to minimize human impacts on the conservation of the species and its habitat.

The project aims to assess human impacts on the viability of great bustards in Iberia, the last stronghold of this globally endangered species. We will test the hypothesis that changing land-use patterns, habitat fragmentation, and other human impacts are affecting the species′ survival by reducing the contact between isolated groups, decreasing genetic diversity, and altering fundamental demographic parameters (annual recruitment, sex ratio, mortality).

There are established some research directions as follows:

- To assess the current status, population trends and habitat availability of great bustards in selected Iberian subpopulations by defining the sizes and shapes of suitable habitat patches, and assessing for each patch its carrying capacity and degree of isolation from all others.
- To update and revalidate the current distribution and habitat availability of the species in Iberia during the breeding and non-breeding season.
- To assess the current impact of human-induced negative factors on the population dynamics of selected subpopulations
- To assess the extent and distribution of the species′ composite genetic diversity in the Iberian Peninsula, weigh up the significance of individual populations to the overall genetic diversity, and relate patterns of genetic variability with spatial distribution in habitat quality (landscape genetics).
- To elucidate the historical landscape-level processes or human factors that have shaped the current distribution pattern of the species.
- To identify non-viable populations and propose rational measures for the conservation of the species in the context of sustainable development.

Availability of steppe habitats is not a limiting factor for great bustards in the Spanish region. It has profound implications for the conservation of this globally endangered species. It occurs in open flat or somewhat rolling landscapes, usually with a mixture of crops (cereals, vineyards, fodder plants, in some countries also with steppic grassland [J. C. Alonso *in litt*. 2012).

An important help for the teams of restocking projects was given by the farmers in the areas concerning the biodiversity conservation.

Figure 1. Otis tarda (by courtesy of photo author)

ROMANIAN APPROACH

In Romania has been developed public politics of restocking for some species, by projects funded from state budget as well as European Commission. In this respect it was designed a National Centre for great bustards in Mihai Bravu,Giurgiu county (Figure 2). This decision regarding the site based on soil analysis, pollution assessment, hunting evaluation, remediation of the ground, Natura 2000 sites, national parks, heritage and tradition.

Figure 2. Proposed site for great bustard reserve in Romania – Mihai Bravu, Giurgiu county (by courtesy of FDR, 2012)

The main objectives of the project are the followings:

- development of infrastructure for reproduction and growth in semicaptivity Great Bustard, including establishment of the sanctuary and adult offspring
- developing a multidisciplinary research program
- initiating international collaboration with advanced European centers (Spain, UK) and other countries (Russia, Ukraine, Moldova, Serbia)
- reintroduction of the species Otis tarda in the natural environment
- development of modern methods for public acceptance and support activities restocking tourism project in reserve
- activities of global biodiversity conservation.

A part of activity includes the gathering of the rules concerning the project. There were evaluated European legislation, public politics in different countries, ongoing activities for conservation of the species. Trends of the population development, monitoring schemes as well legal protection status have been studied. (Garlea et all, 2012)

The design of the physical protection system based on the otis tarda vulnerabilities. There are special fences (Figure 3), the last one, inside in the reserve, must be smooth. The site is guarded with rangers and it is provided with electronic surveillance.

Figure 3. Smooth fence to sanctuary
(by courtesy of FDR)

The advanced methods for education and increasing of public acceptance have been studied, the success of the project being significant influenced by the support of people. The National Centre for great bustard developed on the former military site could be an important research area promoting the transborder cooperation in the field.

RESTOCKING POLITICS IN THE WORLD

The Asian subspecies of Great Bustard, found only in Mongolia, China, and Russian South Siberia, is of special concern. Though few surveys have been undertaken in Mongolia, the population has been estimated at just 1500 birds. These bustards are particularly at risk as Mongolia transitions from communism to a free-market economy, replete with road construction, increased natural resource development, and land privatization.

Threats to the Great Bustard are numerous. Bustard nests, simple scrapes in the ground, are destroyed by the activity of agricultural machinery in the fields they inhabit. The insect food base so important to the rapid growth of chicks is eliminated with pesticides. Though they are strong fliers, heavy-bodied bustards are not maneuverable enough to avoid collisions with electrical lines. And though now illegal, hunting by humans also plays a role in these declines. Our team is working to quantify the risks to bustards in Central Asia, and we communicate with local people and conservation agencies to develop conservation plans. (Li Lin et al, 2008)

Great Bustards have a range stretching across Eurasia, from Iberia and Morocco in the west to China in the east, though their distribution is extremely fragmented and numbers are low in many parts of their range. The world Great Bustard population is estimated to be between 43,500 and 51,200 individual birds. The species has undergone a long-term and marked decline, especially since the early 19th century. This decline has been slowed in the past 20 years by major conservation action in many countries. In that period the European population has increased, driven by a rise in the large Iberian population as well as in the world. Over 50% of the world population of Great Bustards is found in Iberia. The only other substantial population is found in western Russia. Several countries have small, threatened populations. Some are in the initial stages of recovery, like those in, Hungary, Austria and Germany, thanks to conservation work, but the continuing existence of others, for example in Morocco or Iran, is less certain.

The East Asian population of the subspecies *Otis tarda dybowskii* is thought to total 3,500 to 4,700 birds. (Kessler, 2012)

Great Bustards are highly gregarious birds that form social units termed 'droves'. Males and females live in separate droves and there is a tendency for birds of the same age to keep together. Large, often loose, flocks form in winter, which may wander in search of food, sometimes joining up with other flocks. Female droves visit groups of displaying males briefly during the breeding season.

Gait is slow and deliberate but bustards are capable of surprisingly fast dashes. Feeding action is a swift pick-up of food from the ground and fast 'snatching' of vegetation. They have a very wary nature. They will often withdraw into tall vegetation when alarmed, but never into bushes or trees, and sometimes they will fly away. Flight is between 30-100 m above ground, with an action noticeably regular and uninterrupted. They never glide, but beat their wings slowly and majestically, making

rapid progress. Wings are long and deeply fingered appearing mostly white. They are generally silent, unless flushed or threatened at very close range, when a nasal bark is sometimes heard. The lack of an opposable hind claw means they cannot perch, so they are a completely ground-dwelling bird. Their notoriously shy and wary behavior makes them very difficult to observe.

Great Bustard is omnivorous, meaning it eats both animal and plant matter. Diet is mainly composed of plants during spring, autumn and winter. Typically they take young shoots, leaves, flowers, ripe and unripe seeds but occasionally also rhizomes, bulbs, berries and fruits. The proportion of animal food varies with season, locality, age and sex of bird, but they are mostly carnivorous in summer.

Females typically become sexually mature from two years of age and males typically from four years. Great Bustards have a mating system termed 'lekking'. Males compete for females with an elaborate visual display. Females appear to visit several males before copulating and appear to be very selective in their choice of mate. Mating success is strongly skewed, with the majority of mating performed by a small proportion of males at a leak site. No pair bonds are formed and pairings may differ from year to year.

Populations are migratory in the east, and dispersive or resident elsewhere. In the former USSR, Great Bustards are often considered truly migratory, except in southern Ukraine where resident. The Ukrainian population is boosted by up to 10,000 birds in the winter, mostly from the Russian Federation. However, recent winter observations of birds wintering in Russia at-30C with deep snow cover suggests that even in hard winters not all migrate. Great Bustards have been reported from Syria and Iraq in winter but whether birds still breed in these countries is unknown.

Young Great Bustards begin developing their adult plumage at about two months, and begin to develop flying skills at the same time. They practice by stretching, running, flapping, and making small hops and jumps to get airborne.

By three months they are able to fly reasonable distances. Juveniles are independent by their first winter, but normally stay with their mother until the next breeding season. The natural mortality of Great Bustards in the wild is over 80% in the first year.

Table 1. Otis tarda population reported in 2012

Countries with current breeding records	**Number of Great Bustards**
Austria	175
Bulgaria	0
China (NW Xinjiang)	2,000 – 3,000
Czech Republic	1 – 6
Germany	110
Hungary	1,353
Iran	89 – 161
Kazakhstan	0 – 50
Moldova	0
Mongolia, NE China, SE Russia	1,500 – 1,700
Morocco	91 – 108
Portugal	1,399
Romania	0 – 4
Russia (European)	8,000 – 11,000
Serbia & Montenegro	35 – 40
Ukraine	500 – 850
Slovakia	8 – 16
Spain	27,500 – 30,000
Turkey	764 – 1,250

Taking into account the previous biological characteristics of great bustard can be evaluate the population and world restocking politics. In the Table 1. are presented data reported by different territories. (Li Lin et al, 2012).

CONCLUSIONS

The actions for biodiversity conservation in Eurasia are extended in the last three years.

The advance research is studying the genetic structure of the population.

The development of the Romanian project in Mihai Bravu National Center depends by European support.

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We are proud to express our thanks to Spanish colleagues who sent to FDR the most important part of their dedicated results as well as to UNESCO office in Mongolia. The authors also collaborated with Lucian Dumitru and V.E. Puscasu in previous works referring to great bustards restocking in Romania.

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THE POLLUTION LEVEL OF TENIOSIS IN SHEPHERDS AND IMPACT OF SOME OF THEM IN APPARITION AND DEVELOPMENT OF CENURIOSIS FOCUS POINTS IN YOUNG SHEEP

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Abstract

The investigations performed in May 2012 on a group made up of 15 shepherd individuals, (9 adults and 6 young from 2 sheepfolds), concerning the incidence and intensity of parasitism in some teniosis, emphasized their high prevalence, of 80.8% in adult dogs from the sheepfold 1 and 75.0% from the sheepfold 2, and 66.6% in young dogs from the sheepfold 2. The share of the oncosphere copro-eliminations in shepherd dogs teniosis from both sheepfolds emphasized average and massive levels in adult dogs (50.0% in sheepfold 1 and 33.3% and 66.6%, respectively, in sheepfold 2), and in young dogs low and massive levels (50.0% sheepfold 2). The incidence of the cerebro-spinal cenuriosis in young sheep during previous year exhibited values of 24% in sheepfolds 1 and 10.2%, in sheepfold 2, and anatomo-clinical picture is dominated by serious locomotory disorders (42.8%) and nervous disorders (28.5%).

Key words: *sheepfold, taenia, parasitism, oncosphere, nervous disorders.*

INTRODUCTION

It is well known that the incidence of the teniosis is increased in dog populations located around animal husbandry farms, slaughtering houses, in accompanying and sheep flocks guardian shepherds dogs, but also in haunting dogs (Şuteu and Cozma 2004; Negrea, 2007). The infested carnivores represent real dangerous pools for both humans and some domestic animals (sheep, cattle, swine) with risk for contacting diseases as echinococosis, cerebro-spinal cenuriosis, etc. In animal husbandry sector, these parasitary diseases (meto-cestadosis) produce major economical prejudices (decrease of the animal productions, weakness, necessity slaughters) (Şuteu and Cozma 2004; Negrea, 2007; Cosma et al., 1998). In the present paper there are evaluated the incidence and intensity of the parasitism of some teniosis in shepherds from two sheepfolds from the county of Cluj, major risk for some of them to harm the sheep young individuals.

MATERIALS AND METHODS

The investigations were carried out in spring of 2012, on 15 Ciobănesc carpatin and Ciobănesc mioritic dogs, belonging to 2 private sheepfolds from a village limitroph to Cluj − Napoca municipality. The dog population was divided as follows:

- sheepfold 1: 8 dogs of which 5 adults and 3 young
- sheepfold 2: 7 dogs of which 4 adults and 3 young.

From these, there were prelevated, individually, coprological samples in polyethylene bags, and for evaluation the incidence and intensity of teniosis parasitism, ovoscopic coprological methods of enriching by flotation (Willis method) were used. The level of parasitism intensity in diagnosed cestadosis was established according to the following protocol:

- reduced infestations: 1-5 oncosphere/ microscopic field
- average infestations: 5-10 oncosphere/ microscopic field
- massive infestations: more than 10 oncosphere /microscopic field

In the mean time, clinical examinations were performed on 116 young sheep stocks, from previous year (67 animals - sheepfold 1 and 49 animals - sheepfold 2), in order to identify cases with behavior alterations (nervous disorders, movement disorders, prolonged decubitus), phenomena characteristic for cerebrospinal cenuriosis developed by one year old young sheep (TOAP). From total cenuriosis suspect cases, 4 animals were hospitalized and then submitted to surgical intervention at the Clinic of Surgical Diseases from the Faculty of Veterinary Medicine Cluj-Napoca. The rest of 17 animals were necessity slaughtered and used for familial consumption.

RESULTS AND DISCUSSIONS

The incidence of teniosis in examined dog group, according to age category and provenience source is presented in table no.1 as follows:

Table 1. The variation of extension of teniosis in shepherds by age categories and provenience

Sheepfold	Adult dogs			Young dogs		
	Examined samples	Of which		Examined samples	Of which	
		positive		%	positive	%
No. 1	5	4	80.0	3	-	-
No. 2	4	3	75.0	3	2	66.6
Total	9	7	77.7	6	2	33.3

Analyzing the values obtained from above presented data, concerning the incidence of the main teniosis in shepherds from 2 studied sheepfolds, we found a high level of incidence, as follows: sheepfold no.1, the prevalence of the parasitism in adult dogs is of 80.0% and75 % in sheepfold no. 2. In the mean time, we note that in young dogs the teniosis parasitism is present only in sheepfold no. 2, in share of 66.6%. It is known that teniosis exhibit an increased incidence in dogs from animal husbandry and slaughter houses surroundings, in shepherds, etc. (Şuteu and Cozma, 2004; Cosma et al., 1998; Daraban, 2006). The carnivores represent dangerous sources for both animal species (cattle, sheep, goats, etc.) with risk of contacting chronic diseases (hydatidosis, cenurosis, cisticercosis) and human collectivities due to the risk of contamination with hydatic cist or cerebrospinal cenuriosis. Because the cestodes eggs are infested in the moment of faeces expulsion the risk of contamination is amplified (Şuteu and Cozma 2004; Losson, 1993).The variation of teniosis extent in shepherds by age categories and provenience is presented in the following graph.

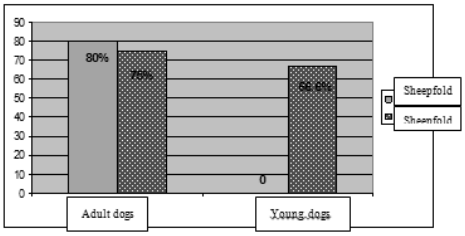

Figure 1. The variation of teniosis incidence in shepherds

The results of the coproparasitological examination concerning the level of the intensity of the parasitism with taenia oncospheres, in shepherds from both sheepfolds are presented in table 2.

Table 2. The level of teniosis coproparasitary pollution in shepherds

Sheepfold	Adult dogs							Young dogs						
	Positive samples	Of which						Positive samples	Of which					
		+		++		+++		+	++			+++		
		+	%	+	%	+		%	+	%	+	%	+	%
1	4	-	-	2	50.0	2	50.0	-	-	-	-	-	-	-
2	3	-	-	1	33.3	2	66.1	2	1	50.0	-	-	1	50.0
Total	7	-	-	3	42.8	4	57.3	2	1	50.0	-	-	1	50.0

The quantum of the oncosphere copro-elimination in carnivore teniosis diagnosed in shepherds from both private sheepfolds is correlated with the level of intensity parasitism produced by different parasite taenia. The obtained data emphasize average and massive levels for parasitism intensity in adult dogs, 50.0% in sheepfold 1 33.3% and 66.6% in

sheepfold 2, respectively, while reduced and massive levels of this parasitism were recorded in young dogs (50.0% in sheepfold 2). We note that the ovoscopical coproscopic examination is relevant for positive cases, but it has group value, while only the morpho-pathological examination confer certitude (Negrea, 2007; Cosma et al., 1998). In following graph (fig.2) is presented the level of intensity of the parasitism with oncospheres of taenia in shepherds from both sheepfolds, by age categories and provenience. The data of investigations performed on an effective of 116 young sheep one year old, concerning the incidence and anatomo – clinical picture in cerebrospinal cenuriosis are presented in table no. 3.

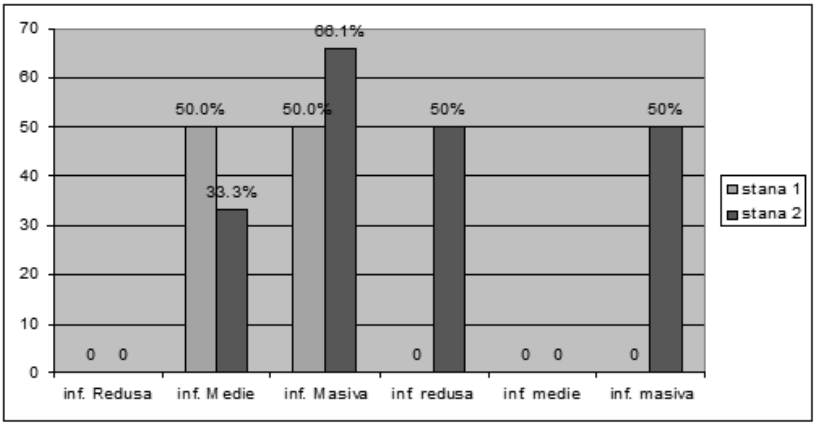

Figure 2. The variation of the oncosphere coproparasitary charge in shepherds

Table 3. The incidence and anatomo – clinical picture in cerebrospinal cenuriosis in TOAP

Sheepfold	TOAP Effective	Of which					
		Positive	%	Anatomo – clinical picture			
				Nervous disorders	Movement disorders	Decubits	Cranial retarding
1	67	16	24.0	4	25.0	5	31.3
2	49	5	10.2	2	40.0	4	80.0
Total	116	21	18.1	6	28.5	9	42.8

From data presented in table no. 3 result an 18.1% incidence of cenuriosis in TOAP, for both sheepfolds, with variations correlated with their provenience. Thus, in sheepfold 1, the incidence of the meta-cestodiosis is of 24.0% compared to sheepfold 2 where incidence is of 10.2%. Generally speaking, cenurosis is a grazing disease, being advantaged by the contamination dog – sheep, sometimes by humans, which ignore or measures of destroying the epidemiological chain (Losson, 1993; Negrea, 2007). The picture of the morpho-clinical alterations emphasized in table 3 is the result of the cerebral migration of the hexacant embryos and consequent development of the cenuric vesicles, this fact being more or less alarming function of the number of the migrating hexacant embryos. This evolves from slight disorders of coordination of movements, sometimes hard to observe, to serious nervous disorders, movement disorders, or contrary, prolonged decubitus (Şuteu and Cozma, 2004; Daraban, 2006; Losson, 1993). The variation of the incidence and morpho-clinical picture of cenuriosis in TOAP are presented in the following graph.

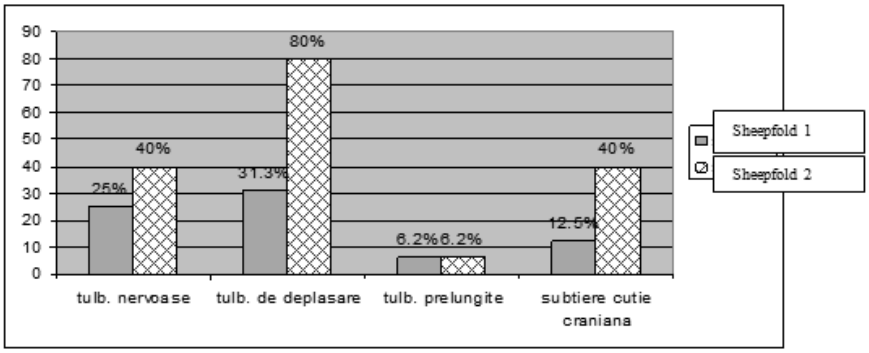

Figure 3. The variation of the incidence and morpho-clinical picture of cenuriosis in TOAP

CONCLUSIONS

The investigations concerning the extension and intensity of parasitism with taenides performed on a group made up of 15 shepherds owned by 2 private sheepfolds from the county of Cluj, as well as the incidence and morpho-clinical picture in cenuriosis in 116 young sheep from the same sheepfolds, reveal the following aspects:

1. The prevalence of the teniosis in shepherds from both sheepfolds, by age categories recorded different values, of 80.0%, 75.0%, respectively in adult dogs, and 66.6% in young sheep from the sheepfold no. 2.
2. The quantum of the oncosphere copro-eliminations in teniosis had average and massive levels in adult dogs (50.0% sheepfold 1, and 33.3%, 66.6% respectively in sheepfold 2) and low and massive levels in young sheep (50.0% in sheepfold no. 2).
3. The incidence of the cenuriosis in young sheep recorded values from 24.0% in sheepfold no. 1 to 10.2% in sheepfold no. 2, and morph-clinical picture is dominated by locomotory disorders (12.8%) and nervous disorders (28.5%).

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SOME ASPECTS CONCERNING THE INCIDENCE OF PODAL DISORDERS IN CATTLE REARED AND MAINTAINED IN FREE SYSTEM

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Abstract

The investigations performed at S.C. Agronova Impex SRL. from the county of Cluj, on a stock of 490 Simmenthal cattle (430 dairy cows and 60 gestant heifers), during December 2011 - March 2012, emphasizes a prevalence of the podal disorders of 26.0% in cows (112 animals with podal disorders) and 15% pregnant heifers (9 animals with podal disorders).The incidence of the podal disorders on cattle nucleus stock in this unit, in correlation with the evolution type, emphasizes some aspects: necrotic pododermatites 9.5% (47 cases); podal ulcer – Rusterholz 5.7% (28 cases); necrosis between fingers 6.5% (32 cases); tiloma 2.8% (14 cases). We must note that frequency of these podal disorders reported to the nucleus stock is with 10.4% biger in posterior trend compared to anterior trend (35 cases anterior trend – 7.1% and 86 cases posterior trend – 17.5%. The used therapeutic patterns aimed a rigorous surgical cleaning and use of different drug formulas function of evolution form, meaning: in pododermatites, ether iodoform + Petlain (3 treatments at 3 days interval); in necrosis between fingers, ether iodoform +Spray (2 treatments at 3 days interval); in podal ulcer Spray with antibiotics + sulfonamides (2 treatments at 4 days interval), and in tiloma Spray with antibiotics (2 treatments at 3 days interval)

Key words: *prevalence, pododermatites, ulcer, onglon.*

INTRODUCTION

In the mean time with development of cattle farms in intensive systems with free stabulation, the podopaties in dairy cows are continuously increasing. Majority of specialists recognizes that the podal disorders are the result of the conjugated actions of determinant and advantaging factors on the basis of major deficiencies of zoo-hygien, feeding and motility (Susan et al., 2004; Constantin, 2009; Negrea, 2007). According to performed investigations there was found that recorded loosing, consequent to pododermatitis, in Simmental cows, aimed both dairy production and reproduction process (Constantin, 2009; Telezhenko and Bergten, 2005). Due to these considerations, in order to reduce the harmful economic effects, among others, it is imposed the adaptation of a prophylactic programme consisting in surgical cleaning of onglons within entire nucleus stock at 6 months interval.

MATERIALS AND METHODS

The investigation concerning the incidence of podal disorders in dairy cows reared and maintained in free system were performed in an animal husbandry farm from the county of Cluj, on a nucleus stock of 490 Simmental cattle (430 cows and 60 heifers in gestation). The studied cattle stock was sheltered in 2 modern shelters, built with Sapard European none reimbursing founds. The shelters are supplied with 6 rows of boxes for resting, separated by metallic balustrades, and between them are provided 4 roads destined for manure collection and hygienization. The manure is then directed to a collection channel covered by a lamellar. The animal feeding consists in administration of unique forage, prepared in farm forage kitchen. The study of the frequency of the podal disorders was performed using a serious anatomo-clinical examination of the entire nucleus stock, studying:

- the incidence of the podal disorders by the total studied stock, function of evolution form and age category, respectively;
- their prevalence according to localization (anterior and posterior members).
- putting into practice therapeutic patterns and their value.

RESULTS AND DISCUSSIONS

The data resulted from the anatomo-pathological examinations, performed on a nucleus stock of 490 Simmental cattle, concerning the incidence of podal disorders are presented in table 1.

Table 1. The incidence of podal disorders in cattle

No. crt	Podal disorder	Total stocks: 490 animals, of which:	
		positive	percent, %
1	Pododermatite	47	9.5
2	Interdicital necrosis	32	6.5
3	Podal ulcer – Rusterholz	28	5.7
4	Tiloma	14	2.8
5	Total	121	24.6

From data presented in above mentioned table is emphasized an increased incidence of the anatomo-clinic forms of pododermatites (47 cases – 9.5%) and interdigital necrosis (32 cases - 6.5%), compared to Rusterholz podal ulcer (28 cases – 5.7%), and tiloma (14 cases - 2.8%) (Negrea, 2007; Lischen and Ossent, 2001; Oană and Timen, 2005). The increased incidence of these podal disorders in studied stock emphasize the presence of some major deficiencies in application of the technology of exploitation, as: the excessive humidity at acropoidal level, floor with many irregularities, prolongued stabulation, onglons excessively glowed (favorizing factors), but also presence of an unspecific shelter microbial charge consequent to presence of a big number of cows with endometritis and mammites (determinant factors) (Constantin, 2009; Negrea, 2007).The prevalence of the podal disorders in cattle nucleus stock is graphically presented in figure no. 1.

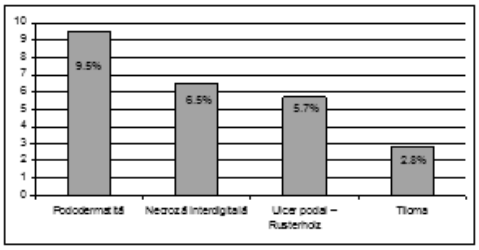

Figure 1. The incidence of the podal disorders in cattle, by evolution forms

The incidence of the podopaties in cattle function of age category and evolution form is presented in table no. 2.

Table 2. The incidence of the podal disorders function of evolution form and age category

Category	Total stocks	Of which							
		Pododermatites		Necrosis between fingers		Podal ulcer		Tiloma	
		No. cases	%	No. cases	%	No. cases	%	No. cases	%
Dairy cows	430	40	9.3	30	6.9	28	5.7	14	2.8
Pregnant heifers	60	7	11.6	2	3.3	-	-	-	-
Total	490	47	9.5	32	6.5	28	5.7	14	2.8

From presented data, we note an increased incidence off the infectious pododermatites, in dairy cows (9.3%) and pregnant heifers (11.6%), but the rest of recorded evolution forms also have big enough values, in dairy cows, especially: interdigital necrosis (6.9%), Rusterholz podal ulcer (5.7%) and tiloma (2.8%). The big frequency of these podal disorders in studied nucleus stock, reveals major deficiencies in exploitation technology applied to dairy cows, especially excessive humidity, micro- and macro-traumas at acropoidal level, excessively grow onglons, metritis and mammitis, thus facilitating the opening of gates for the unspecific poly-microbial microflora (Constantin, 2009; Oană and Timen, 2005; Mates, 2009). The graphic representation of the incidence of the podal disorders in cattle, function of age category and evolution form is presented in figure no. 2. Concerning the prevalence of the podal disorders in cattle function of location; recorded data are presented in table no. 3.

Table 3. The prevalence of the podal disorders in cattle function of location

Localization	Total cases		Of which							
			Pododermatite		Necrosis between fingers		Podal ulcer		Tiloma	
	No.	%	No.	%	No.	%	No.	%	No.	%
Anterior members	35	7.1	17	3.4	8	1.6	2	0.4	8	1.6
Posterior members	86	17.5	30	6.1	24	4.9	26	5.3	6	1.2
Total	121	24.6	47	9.5	32	6.5	28	5.7	14	2.8

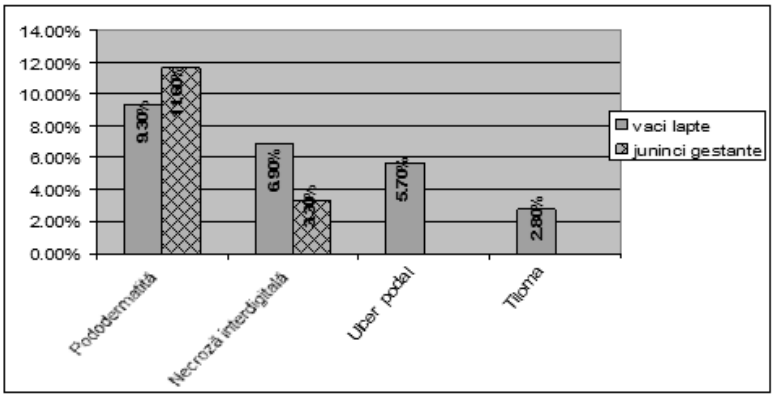

Figure 2. The incidence of the podal dermatitis in cattle function of age category and evolution form

The data we obtained emphasize an increased incidence of podal disorders in posterior members (17.5%) compared to anterior members (7.1%). This is possible, mainly, because of the resting boxes of ″short bed type″, which constraint the animals to permanently expose their posterior members to mechanical and traumatic risks. In the following graph we emphasize the prevalence of the podal disorders in cattle, according to localization (fig.3)

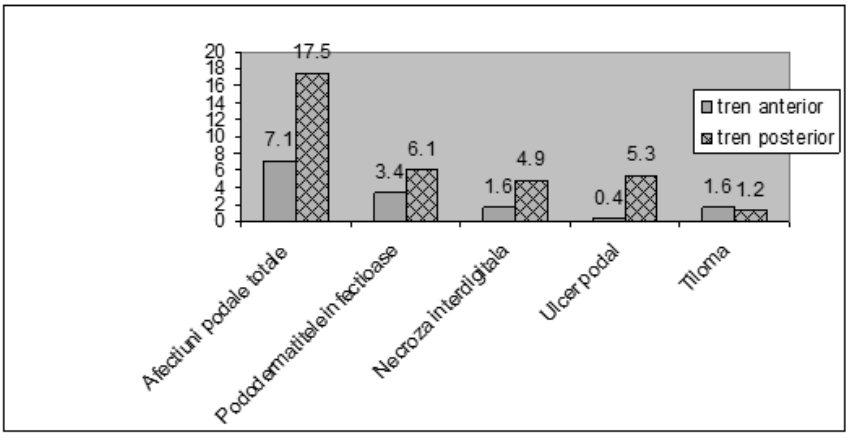

Figure 3. The prevalence of the podal disorders in cattle, function of localization

CONCLUSIONS

The investigations carried out within an animal husbandry unit located in the county of Cluj, în in the Ist trimester of the year 2012, on a nucleus stock consisting of Simmental 490 animals (430 cows and 60 pregnant heifers),

concerning the incidence of the podal disorders, emphasize the following aspects:

1. The prevalence of the podal disorders is 26.0% in cows (112 cases) and 15.0% in pregnant heifers (9 cases), of the total studied stock.
2. The incidence of the padopaties, in correlation with the evolution form exhibits differentiate values, meaning: infectious padodermatite 9.5% (45 cases), Rusterholz podal ulcer 5.7 % (28 cases) interdigital necrosis 6.5 % (32 cases) and tiloma 2.8 % (14 cases). The dairy cows are more exposed to the risk of podal disorders appearance (26.0%) compared to pregnant heifers (15%)
3. The extension of podopaties, according to localization, emphasizes their increased values in posterior members (17.5%) compared to anterior members (7.1%).

The used therapeutic patterns aimed to a serious surgical cleaning and putting into practice of some drug formula correlated to the evolution forms.

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THE UMBILICAL RING OF RUMINANTS, NATURAL GATE FOR TRANSPONDER IMPLANT INTO THE PREPERITONEAL SPACE

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Abstract

The ruminant identification is an important tool aimed to establish safely animal ownership, for animal health monitoring and control, food safety control, and finally for genetic selection.
For ruminants the technologies developed during the last years do not allow to ensure that a certain set of basic requirements are met: absence of migration of transponder, fast recovery of transponder in slaughterhouse, possible application in the second part of the meat cutting chain. Therefore, a safe and reliable identification system is needed to ensure live animals and products traceability throughout the food production chain. Implanting of an electronic device into the preperitoneal space by patent of umbilical cord and ring, for performing electronic identification of ruminants may represent a positive answer for the above requirements.
This procedure relates to an electronic system for identification and monitoring of small and big ruminant animals, from birth until cutting and utilization of meat cuts.
Experiments were carried out in experimental farm conditions of National Research Development Institute for Animal Biology and Nutrition. Our preliminary investigations about methodology, were made with the intent, to explore if is possible to be used, in real farm conditions.

Key words: *Animal individualization, transponder, umbilical cord, umbilical ring.*

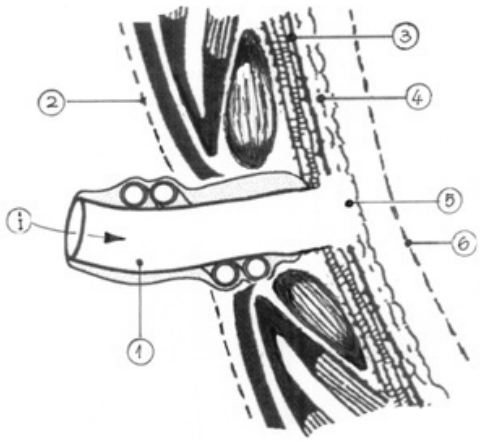
INTRODUCTION

The ruminant identification is an important tool aimed to safely establish animal ownership, for animal health monitoring and control, food safety control, and finally for genetic selection. Therefore, a safe and reliable identification system is needed to ensure live animals and products traceability throughout the food production chain.
According to recent EU Council Directives, concerning identification and registration of small and big ruminants, must be marked as soon as possible with double identification: an ear-tag and as second tag, an electronic device.
Frequently as second electronic tag, is used the ruminal bolus. Is a transponder housed in a specific gravity container (e.g. ceramic) which is orally administered to a ruminant and that remains (due to its weight, shape and size) permanently in the reticulo-rumen (forestomach). Because the electronic device is deposited in reticulum, there is some limitation to the minimum age - 3 weeks and older - at which a bolus can be introduced. The application of boluses requires appropriate training.
On this background of good results, but in the same time, sufficient space for new solutions, we have had idea to use what the nature gave us, namely the temporal opening of umbilical ring, as a good passageway for implanting of an electronic device into the preperitoneal space.

MATERIALS AND METHODS

Animal test group. The study involved a sample size of 54 newborn animals (lambs and calves), in experimental farm of National Research Development Institute for Animal Biology and Nutrition. Technical and clinical investigations concerning the present breeding process were carried out. In particular, the preliminary investigations concerned the husbandry environment during the farrowing phase (cleaning and disinfection procedure, ventilation system) and animal condition (vaccination, health condition).
Electronic identifiers. A cylindrical, glass encapsulated transponder, has been used to identified electronically the newborns.

Brief Description of the Implantation Technique. The implant procedure has been designed for ruminants. The neonate should be restraining in dorsal recumbent position, in a resistless position; only in this position the muscles of abdominal wall will pass into relax, offering no resistance.



I. Direction of transponder implant;
1. Umbilical cord;
2. Skin plus fatty layers;
3. Transversalis fascia posterior lamina;
4. Preperitoneal fat and space of Bogros;
5. Properitoneal space, appropriate for transponder′s implant by patent of umbilical cord;
6. Parietal peritoneum.

The first steps of cleansing the umbilical stump, and holding the umbilicus upright, it are followed by the introduction the tip of the transponder into the lumen as deeply as possible; once the transponder is in good position into the cord, continue to advance with gentle motion; when the tip of transponder is arrived at the umbilical ring level, a normal resistance is encountered. After this moment it is sufficient, to bent at 50 - 60 degree and by a simple gentle pushing toward to xiphoid, midline direction, to implant the transponder into properitoneal tissue (Figure 1). By palpation, it is possible to fell, the size, firmness, location of transponder.

RESULTS AND DISCUSSIONS

In contrast to the vital role played by the umbilicus in utero, it has minimal physiologic importance after birth. Umbilical ring is a natural defect, an opening in the abdominal wall through which the umbilical cord passes and meets the fetus. Shortly after birth, the opening in the abdominal wall, begin to close.

These two aspects, the presence of a flexible cord and temporary opening of umbilical ring, are key elements in our methodology used for individualization of ruminants.

The flexibility of umbilical cord plus the presence of Wharton's Jelly lead the electronic device in direction of umbilical ring, and opening of umbilical ring, the implant the transponder in region of preperitoneal. Preperitoneal (properitoneal) space there is between the peritoneum and posterior lamina of the transversalis fascia.

Because, shortly after birth, the reduction in temperature starts a physiological process which causes the Wharton's Jelly from umbilical cord to swell, the implant of transponder is made early, in the first 1-15 postnatal hours, also due to concern about the closure of the umbilical ring. Functional closure of the umbilical ring usually occurs within minutes of birth and structural closure occurs within three to seven days of birth in term neonates.

Due the fact that the tendinous aponeurotic fibers what surround the umbilical ring, evolve the closing process, in a way similar to the shutter mechanism used in optical instruments, process that occurs, from the inside out, the implant of transponder, stops at level of transverse fascia. Therefore we have had use this properitoneal space, as a suitable, appropriate place for transponder's implant, by patent of umbilical cord (Figure 2).

The readability of the transponders before and after the implant was verified using a portable reader. With the second reading performed after the tagging as 'control reading' to verify the transponder readability, was possible to issue the 'previous list' (i.e. the list of tagged lambs expected to be read during the following control readings). The comparison between reading sessions performed at predefined dates allowed to record the retention

rate as well as the proper functioning of transponders.
Up to the date of this communication, was not possible the slaughtering of an animal from experimental lot, for assess if in total, the set of basic requirements are met: absence of migration of transponder, fast recovery of transponders in slaughterhouse, possible application in the second part of the meat cutting chain.

But, according to our latest experiments, in normal conditions, the reaction of the host connective fibrous fascia provides a good encapsulation for the transponder. That because deep implanted transponder behaves like any common inert foreign objects, causing a benign reaction in host tissues. Capsule formation around the transponders is able to maintain them in situ.

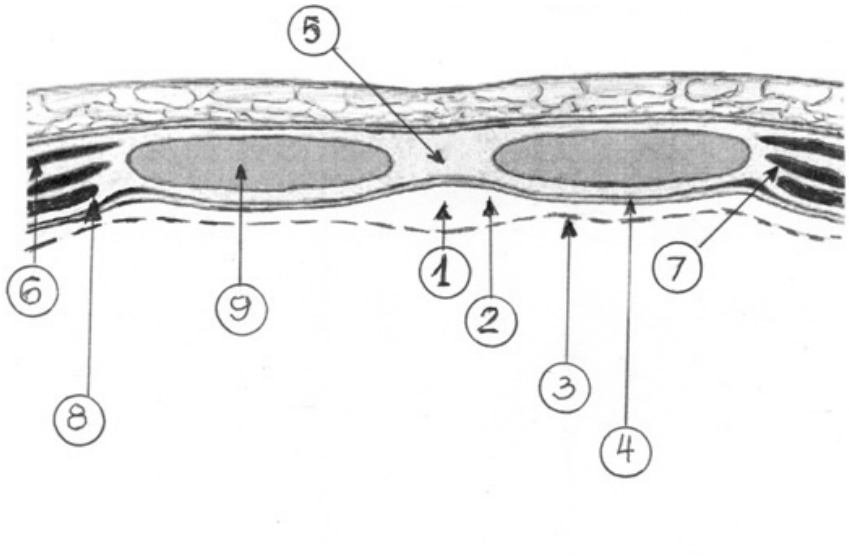


Figure 1. Representation of the layers of the abdominal wall and the properitoneal zone of the implant

1. Properitoneal space, appropriate for transponder′s implant by patent of umbilical cord;
2, Properitoneal fat (tela subserosa);
3. Parietal peritoneum;
4. Transversalis fascia;
5. Linea alba;
6. External oblique muscle;
7. Internal oblique muscle;
8. Transversus abdominis muscle;
9. Rectus abdominis muscle

The fact that more than ten reading sessions had been performed with success, it is a clear confirmation of presence of the electronic device in respectively area.
But, according to our latest experiments, in normal conditions, the reaction of the host connective fibrous fascia provides a good encapsulation for the transponder. That because deep implanted transponder behaves like any common inert foreign objects, causing a benign reaction in host tissues. Capsule formation around the transponders is able to maintain them in situ. The fact that more than ten reading sessions had been performed with success, it is a clear confirmation of presence of the electronic device in respectively area.

For moment, in base of this aspect we can only suppose that, the electronic device can be easy localized in the umbilical zone of carcasses, supposition what follow to be clarified by slaughtering of some animals from experimental lot.

According to our knowledge, the application of a transponder in the properitoneal zone, by patent of umbilical cord has never been explored.

CONCLUSIONS

This methodology may represent a positive answer to the above requirements and can also ensure an easy and efficient mean to trace-back the animal(s) and the product(s) to the farm of origin (due to the fact that the animal will be individually identified). In addition, it allows keeping the ruminant identification throughout the slaughtering process (where it could play the role of carcass identification) and the meat cutting process.

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STUDY ABOUT INFLUENCE OF FEED PARAMETERS ON SLAUGHTERING PERFORMANCES IN CERTIFICATE- TYPE BROILERS

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Abstract

In feed milling nutrient levels there should be established both chicks need and financial circumstances and any variation of nutrient composition should be followed by a prompt answer in slaughtering performances leading to a competitive feed cost and so to a competitive final product cost with no change in chemical meat composition.
Some values about carcass output and percentage of body parts in carcass were determined for this purpose using feed combination with variable parameters.
Experiments were performed at S.D.E. Avicola Moara Domnească during 56 days with 150 birds (Ross 308) divided in three treatments based of nutritive value of combined feed given (CM, C1, C2). Data that was obtained during the experiment was processed and so it was noticed that the best commercial output was obtained in the group with constant nutritive parameters (CM − 81.4 ± 0.53%) and the best breast percentage from carcass was noticed in the group with constant nutritive parameters (CM − 25.9 ± 0.94%) and the best in leg percentage was noticed in the group with variable protein (C1 − 27.7 ± 0.54%).

Key words: *Certificate chickens, carcass, output, performance.*

INTRODUCTION

Quality standard of products is one of the base criteria in choices made by consumers of animal products. Technically and economically carcass quality is being a result o carcass size (weight, output, parts shares) and carcass conformation (muscles profile, fat layer, etc) (Tudorache et.al., 2010). Data about production performances, slaughtering output, cut shares and main internal organs from both poultry live weight and poultry carcass were registered and processed to establish the quality of produced carcasses (Tudorache et al., 2009; Waller, 2007).

MATERIALS AND METHODS

Experiment was performed at S.D.E. Avicola Moara Domneasca which is the research farm of the University of Agricultural Science and Veterinary Medicine Bucharest on broilers of type Bio supplied in three experimental variants with uniformity of body weight and sex percentage and the experimental plan in blocks was used. Plymouth Rock bared was used for this experiment and birds were raised according to standard technology for this broiler and in the same management, feeding and watering conditions (Tudorache et al., 2012; Van et al., 2003).

Three treatments were performed for each experimental flock to determine quantitative and qualitative features and experiments were preformed in the same time interval and on the same biological material and in the same unit.

Working schedule realized for broilers of type Bio was as following:

· treatment I (M): constant energy level and constant protein level;

· treatment II (El): variable protein level and constant energy level;

· treatment III (E2): constant protein level and variable energy level.

5 groups by treatment with 10 heads each were used in all three experiments (table 1).

Groups were formed at one day of age with chicks from the same hatchery. Chicks came from parents of same age to diminish genetically influence on final results. Experimental interval had 84 days. Two phases feeding technology was used. Feed combination used in experiments was produces at

I.B.N.A. - Baloteşti according to feeding requirements of the broiler used and based on the experimental schedule.
The following performance traits: live weight, feed consumption and livability were established for each treatment and they were followed weekly during the experiment.

Table 1. Work schedule for Certificat type broilers

Specification	U.M.	Phase		
		Rising		
		T_1	T_2	T_3
Time	days	28	28	28
Flock	birds	50	50	50
Pens	no.	5	5	5
ME	MJ/kg	100	100	93.96
Protein	%	100	95.36	100

Specification	U.M.	Phase		
		Finishing		
		T_1	T_2	T_3
Time	days	28	28	28
Flock	birds	50	50	50
Pens	no.	5	5	5
ME	MJ/kg	100	100	93.06
Protein	%	100	95.30	100

RESULTS AND DISCUSSIONS

Broilers weight gain is highly variable based on genetically potential of the hybrid, birds' sex and housing conditions during production. Phenotypical manifestation of the genetic potential depends mainly on feed quality and especially on feed protein and essential amino acids content. A good understanding of nutritional limits of commonly used feedstuffs is a key for both improvement of feed milling and feeding procedures and for better solutions to many problems with feed composition, consumption and usage and with carcass quality (Tudorache et al., 2009).
Production performances were established and based on them data about economical output and cut shares and main internal organs from both carcass and live weight cat and din carcass were registered and processed to establish the quality of products based on the structure of diets used (Tudorache et al., 2010).
Table 2 (a, b, c) and figure 1 (a, b, c, d) are showing final production performances of the Certificat broiler. Their analyze has showing that average body weight at 8 weeks of age is varying between 2384.86 g in CM and 2224.2 g in Cl but the treatment applied did not influence significantly the results obtained.

Table 2. Final production performances of the Certificat broiler

Specification	**M.U.**	**Group**	
		CM	
		X	Sx
Live wieight	g	2384.86	56.09
Student Test	-	CM-Cl =1.266	
Average daily gain	g	41.98	0.91
Student Test	-	CM-Cl=1.280	
Feed intake	kg	2.54	0.16
Student Test	-	CM-Cl=25.031	
Cumulative mortality	%	8.00	1.23
Student Test	-	CM-Cl=0,283	

Specification	**M.U.**	**Group**	
		C1	
		X	Sx
Live wieight	g	2224.20	69.88
Student Test	-	CI-C2=0.336	
Average daily gain	g	39.11	1.24
Student Test	-	CI-C2=0.045	
Feed intake	kg	2.65	0.01
Student Test	-	CI-C2=6.299	
Cumulative mortality	%	10.40	1.56
Student Test	-	CI-C2=0.024	

Specification	M.U.	Group	
		C2	
		X	Sx
Live wieight	g	2269.60	64.57
Student Test	-	C2-CM=0.952	
Average daily gain	g	39.92 I	1.15
Student Test	-	C2-CM= 1.282	
Feed intake	kg	2.70	0.01
Student Test	-	C2-CM=22.161	
Cumulative mortality	%	10.20	1.11
Student Test	-	C2-CM=0.311	

Daily average gain is between 41.98 g in CM and 39.11 g in Cl with no statistically assured differences. Feed intake is minim in CM (2.54) and maximum in C2 (2.70) and all differences between groups are statistically assured. Cumulative mortality is high enough but insignificantly smaller in CM with 8% compared to C1 and compared to about 10% in the other groups. Difference C2 - CM is not statistically assured. So best results of 'certificat' broilers are also obtained in CM group with constant protein and energy level and with a significantly better feedstuff capitalization.

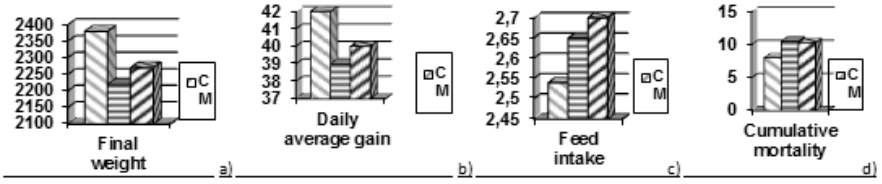

Figure 1. Final production performances of the Certificat broiler (a – final weight, b – daily average gain, c – feed intake, d – cumulative mortality)

Table 3 (a, b, c) is showing the slaughtering performances which is influencing the economical value if meat and the economical value if meat is given by quantity and quality of marketed meat. Analyze of data reveals that performances are different for each experimental group and each parameter (indicator) taken into account as following: commercial output is between 76.80± 0.37% in group C1 and 81.40± 0.53% in group CM; breast share from carcass is 21.50± 1.35% in group C1 and 25.90± 0.94% in group CM; legs share from carcass is between 27.30 ± 0.56% in group CM and 27.70 ± 0.54% in group Cl; wings share from carcass is between 9.60± 0.38% in group CM and 9.90± 0.37% in group C1.

Table 3. Slaughtering performances of the Certificat broiler

Specification		Group CM	
		X	S_X
Output (%)		81.4	0.53
Brest Share (%)	Live weight	21.1	0.67
	carcass	25.9	0.94
Legs share (%)	Live weight	22.2	0.47
	carcass	27.3	0.56
Wings share (%)	Live weight	7.8	0.35
	carcass	9.6	0.38
Head share (%)	Live weight	3.4	0.11
	carcass	4.2	0.11
Gizzard share (%)	Live weight	1.4	0.04
	carcass	1.7	0.05
Back share (%)	Live weight	19.5	0.48
	carcass	24.0	0.57
Hart share (%)	Live weight	0.5	0.02
	carcass	0.6	0.02
Liver share (%)	Live weight	2.0	0.11
	carcass	2.5	0.14
Drumsticks share (%)	Live weight	3.4	0.26
	carcass	4.2	0.30

Specification		Group C1	
		X	S_X
Output (%)		76.8	0.37
Brest Share (%)	Live weight	16.5	0.98
	carcass	21.5	1.35
Legs share (%)	Live weight	21.2	0.51
	carcass	27.7	0.54
Wings share (%)	Live weight	7.6	0.26
	carcass	9.9	0.37
Head share (%)	Live weight	3.6	0.14
	carcass	4.7	0.20
Gizzard share (%)	Live weight	1.8	0.11
	carcass	2.4	0.14
Back share (%)	Live weight	19.4	0.64
	carcass	25.3	0.74
Hart share (%)	Live weight	0.4	0.04
	carcass	0.6	0.05
Liver share (%)	Live weight	2.4	0.11
	carcass	3.1	0.15
Drumsticks share (%)	Live weight	3.8	0.34
	carcass	5.0	0.43

Specification		Group C2	
		X	S_X
Output (%)		78.9	0.39
Brest Share (%)	Live weight	18.1	1.07
	carcass	22.9	1.43
Legs share (%)	Live weight	21.6	0.57
	carcass	27.4	0.70
Wings share (%)	Live weight	7.7	0.28
	carcass	9.8	0.39
Head share (%)	Live weight	3.8	0.18
	carcass	4.8	0.22
Gizzard share (%)	Live weight	1.8	0.13
	carcass	2.2	0.15
Back share (%)	Live weight	19.2	1.06
	carcass	24.3	1.24
Hart share (%)	Live weight	0.6	0.04
	carcass	0.7	0.05
Liver share (%)	Live weight	2.4	0.12
	carcass	3.1	0.16
Drumsticks share (%)	Live weight	3.8	0.40
	carcass	4.8	0.51

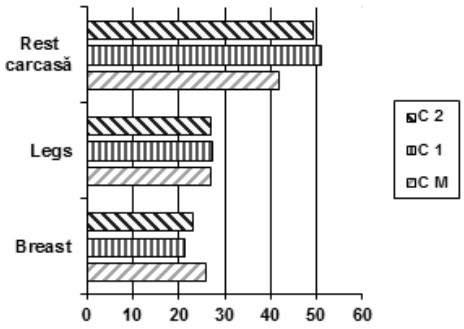

Figure 2. Shares of different carcass parts in Certificat broiler

In conclusion the group CM (which received a diet with constant protein and energy) offered best results about carcass quality for the Certificat broiler thanks to both economical output and percentage of valuable parts followed by the group with variable energy level of diet (C2).

CONCLUSIONS

- Live weight altered between 2224.20 ±69.88 g in Cl group and 2384.86± 56.09 g in CM group; .
- highest average daily gain was registered in CM (41.98± 0.91 g) and lowest average daily gain was registered in Cl (39.11 ± 1.24);
- feed intake altered between 2.54 ±0.16 in CM group and 2.70 ± 0.01 in C2 group;
- cumulative mortality altered between 8.00± 1.23% in CM group and 10.40± 1.56 % in C1 group;
- commercial out put was between 76,80 ± 0,37 % la Cl and 81,40 ±0,53, la Group CM;
- highest breast percentage was registered in CM group (25.90 ± 0.94 % from whole carcass) and highest legs percentage was registered in C1 group (27.70± 0.54 %);
- variation of nutrient percentages in broilers diet offered weaker slaughtering performances compared to situation when nutrient percentage are constant.

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RESEARCH ON INFLUENCE OF NUTRITIONAL PARAMETERS ON UNIT COSTS IN BIO TYPE BROILER CARCASSES

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Abstract

A production cost means all costs for inputs consumption performed by the enterprise for goods and services produced and offered. Production costs are very meaningful about business quality and it is a decision-making condition for every producer; lowest cost level is the standard in choosing the right option.
These researches were intended to give an overview about these problems. Objectives were first to find unit costs for feeds and kg carcass to broilers type Bio and second to reduce unit costs by changing energy and protein content of feeds for these broilers.
Experiments were performed at S.D.E. Avicola Moara Domnească with 150 broilers type Bio of Plymouth Rock race divided in three treatments based of nutritive value of combined feed given (ME, E1, E2). Experimental period had 84 days same as in production technology of ecological chicks. Data that was obtained during the experiment was processed and so it was noticed that best slaughtering results were obtained in group E2 (receiving a combined feed with diminished energy level). Finally unit costs were analyzed per kg processed feed (1,366 E2– 1,362 EM lei) and per kg carcass (15,044 E1 – 15,223 E2 lei).

Key words: *Bio chickens, carcass, unit cost, feed.*

INTRODUCTION

You should assess effects on profitability before cutting feed coasts. Increasing the levels of nutritional parameters means higher feed costs. Improving poultry performances are offering higher incomes. This will lead to higher profits compared to the increase of feed costs. Obviously maximum profit is being obtained when incomes are higher above costs not as a result of cutting feed costs (Tudorache et al., 2009; Tudorache et al., 2010). It is very important to understand the difference between decreasing feed cost by bird and decreasing feed cost by kg live weight or carcass parts (Tudorache et al., 2012). Decreasing nutritional parameters in diets are offering little reduction of feed cost by bird. Performances will be decreased and results reported to live weight will trigger an increase of production costs (Van et al., 2003; Waller, 2007).

MATERIALS AND METHODS

Experiment was performed at S.D.E. Avicola Moara Domneasca which is the research farm of the University of Agricultural Science and Veterinary Medicine Bucharest on broilers of type Bio supplied in three experimental variants with uniformity of body weight and sex percentage and the experimental plan in blocks was used. Plymouth Rock bared was used for this experiment and birds were raised according to standard technology for this broiler and in the same management, feeding and watering conditions. Three treatments were performed for each experimental flock to determine quantitative and qualitative features and experiments were preformed in the same time interval and on the same biological material and in the same unit.
Working schedule realized for broilers of type Bio was as following:
- treatment I (M): constant energy level and constant protein level;
- treatment II (El): variable protein level and constant energy level;
- treatment III (E2): constant protein level and variable energy level.

5 groups by treatment with 10 heads each were used in all three experiments (table 1).

Groups were formed at one day of age with chicks from the same hatchery. Chicks came from parents of same age to diminish genetically influence on final results. Experimental interval had 84 days. Two phases feeding technology was used. Feed combination used in experiments was produces at I.B.N.A. – Baloteşti according to feeding requirements of the broiler used and based on the experimental schedule.

The following performance traits: live weight, feed consumption and livability were established for each treatment and they were followed weekly during the experiment.

Table 1. Work schedule for Bio type broilers

Specification	U.M.	Phase Rising		
		T_1	T_2	T_3
Time	days	28	28	28
Flock	birds	50	50	50
Pens	no.	5	5	5
ME	MJ/kg	100	100	93.46
Protein	%	100	95.00	100
Specification	U.M.	Phase Finishing		
		T_1	T_2	T_3
Time	days	56	56	56
Flock	birds	50	50	50
Pens	no.	5	5	5
ME	MJ/kg	100	100	92.90
Protein	%	100	94.51	100

To find slaughtering performances 25% of birds were slaughtered for control at 84 days of age. Chicks were weighted before slaughtering and chicks representing the average weight of the flock were chosen.

After slaughtering by cervical dislocation chicks were de-feathered, weighted and cut and weights of carcass, breast, legs, wings, internal organs and rest of carcass were found.

Obtained data were registered and statistically processed and costs by product unit of analyzed broiler type for every experimental group were calculated based on obtained results.

RESULTS AND DISCUSSIONS

Minimizing production costs is crucial for maximizing profit and so the relationship between production cost and a competitive price is emphasized and this is allowing a good capitalization of productive factors available 4 . Which has also consequences for external trade concerning the competitively of products and the efficiency of international trade. When we are facing an increase of feedstuffs costs and so any increase of feeding costs first reaction is usually to find a solution against the financial impact for your own business which usually means a reduction of nutrient levels recommended in diet to reduce feed cost by ton. For this reason these trials aimed finding both unit cost of product at the Bio broiler and the possible reduction of production cost by diminishing feed cost by unit 1 , 6 .In this way, unit costs for carcass were found by taking into account composition and cost of used diets and consumption and cost of other resources and final production performances and also slaughtering performances obtained with broilers type Bio by experimental groups. Average feed price by unit for each experimental group (table 2 and figure 1) were calculated based on combined feeds consumption by production phase and based on production cost for every feed combination.

Table 2. Average unit cost of diet used for broilers type Bio

Specification		Time (days)	Processed feed consumption (grams)	Production cost (lei/kg)	Average cost (lei/kg)
EM	Starter	0-28	1243.99	1.44	1.398
	Finisher	29 - 84	6959.05	1.39	
E1	Starter	0-28	1232.74	1.41	1.376
	Finisher	29 - 84	7304.05	1.37	
E2	Starter	0-28	1291.13	1.40	1.366
	Finisher	29 - 84	7297.10	1.36	

Presented data are showing that production costs for the combined feed of type of Bio broilers differ is different by experimental group and average unit cost is varying between 1.366 lei/kg at El group and 1.398 lei/kg at EM group.

Table 3 and figure 2 are showing final production performances of broilers type Bio The Bio broilers have average weights between 2496.14 g in El group and 2439.84 g in E2 group at 12 weeks of age. Variations of protein and energy levels have no influence on results. There are no statistically assured differences between results. Best feed intake is at EM

group with constant energy and protein levels and the least favorable feed intake is at E2 group with variable energy level between 3.34 – 3.52. All differences between groups are statistically assured.

Table 3. Final production performances of the Bio broiler

Specification	MU	Group		
		EM	El	E2
Live weight	g	2456.00	2496.14	2439.84.
Feed intake	kg	3.34	3.42	3.52
Variability	%	93.80	91.80	89.60
Slaughtering output	%	79.70	79.90	81.20
Carcass weight	g	1957.43	1994.41	1981.15

Chicks livability is also better in EM (mortality 6.2 %) and weaker in E2 (mortality 10.4 %) but differences between groups are not statistically assured. So best production results of Bio broilers are those of EM group with feed intake very significantly lower compared the other two groups and slaughtering performances are showing that best output of Bio broiler (81.20%) is obtained in E2 group with variable energy.

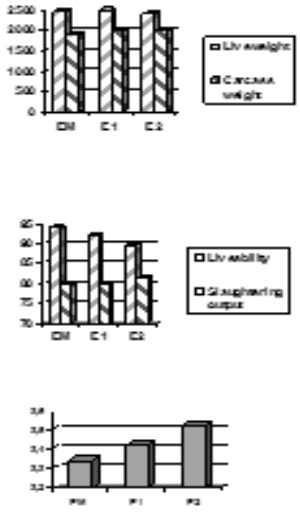

Figure 1. Final production performances of the Bio broiler

Day old acquisition price plus expenses for transport and slaughtering have been taken into account to find unit cost in carcass. Slaughtering output of every experimental group has been also taken into account for finding raw materials cost. This output is between 66.30 and 67.30 % in Bio broiler for processing without head and feet after recovering internal organs. Table 4 and figure 3 are showing the results about the unit cost of meat in carcass for every type of chickens and for every experimental group. After the calculation of these costs acquisition price was of 9113.83 lei/ton in EM group and higher in the other two groups (9157.79 lei/ton in E1 and 9291.47 lei/ton in E2). In control group total cost by unit was intermediate (15130.13 lei/to) compared to E1 (15044.22 lei/ton) and E2 (15223.23 lei/ton).

Table 4. Unit cost of meat in carcass in Bio chicken

Specification	**M.U.**	**Group**		
		EM	E1	E2
Transport cost	lei	9113.83	9157.79	9291.47
Catering cost	lei	151.20	151.20	151.20
Organs harvest	lei	9265.03	9308.99	9442.67
Total meat cost	lei	455.00	437.10	437.1
Slaughtering output	lei	8810.03	8871.89	9005.57
Raw materials cost	%	66..30	67.20	67.30
Slaughtering cost	lei	13288.13	13202.22	13381.23
Total costs	lei	1842.00	1842.00	1842.00
Transport cost	lei	15130.13	15044.22	15223.23

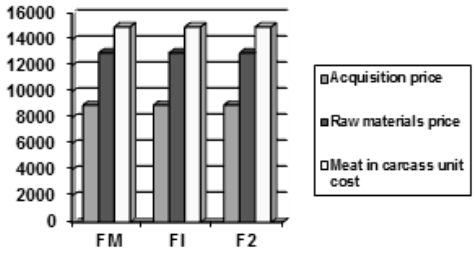

Figure 2. Acquisition price, raw materials cost and unit cost of meat in carcass at the Bio broiler

CONCLUSIONS

Researches described in this paper are pointing to the following conclusions:

- production performances (average daily gain, Feed intake, Variability) are different by experimental group and they are usually better in control group EM excepting body weight in El group and slaughtering output in El and E2 groups;

- average unit cost of diet is different by experimental group and is between 1.3661ei/kg in El group and 1.398 lei/kg in EM group;
- unit cost of product 'live meat production' is between 9113.83 lei/ton in EM group and 9291.47 lei in E2 group and for the product meat in carcass costs are varying between 15044.22 lei/ton in El group and 15223.23 lei/ton in E2 group;
- by reducing nutrient parameters of diet feed cost by unit will be diminished bat production performances will be also diminished
- these effects are emphasizes that when we are dealing with an increase of feed costs we could reduce nutrient levels in diet but the financial impact on whole activity has to be evaluated before taking such a decision.

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RESEARCHES CONCERNING TOTAL WATER CONTENT OF BROILER

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Abstract

In the new vision of the EU, quality and quality control require monitoring food products from the stage of obtaining the raw materials to the final consumer. According to the own research undertaken in this work which had aimed the study of quality indicators of poultry meat, absolutely required at EU level, as a result of the existence of a free trade of food products within the European Union. Thus, in assessing the quality of carcasses from the point of view expressed above, the specific parameters were taken and analyzed for two genetic types, in two consecutive years and different seasons.

The water content of the carcass has different values at the two genetic types analyzed. In season 1, the year I was determined a value of 64.5316 ± 0.4106 percent at ROSS 308 and 65.1820 ± 0.3724 percent at COBB 500, the differences observed between the two hybrids are statistically nonsignificant. In season 2, it was determined a value of 64.4656 ± 0.3989 percent at ROSS 308 and 65.5412 ± 0.3442 percent at COBB 500, the differences are significant from the statistical point of view. In season 1 of the second year of the experiment, at the ROSS 308 hybrids of the average water content was 64.4264 ± 0.3807 percent, while COBB 500 66.3212 ± 0.3051 percent, statistically significant. Season 2 reveals the existence of close values of the average content of water in the two seasons, those two hybrids being taken into question.

Key words: *water, protein, carcasses, broiler.*

INTRODUCTION

Food quality is a very complex notion; It is increasingly obvious tendency approach to quality in terms of consumer safety, became the competitive element for these products. Moreover, the consumer is widely recognized as the most important element in carrying out economic activities whose essence lies precisely in the satisfaction to an extent as possible the needs, desires, preferences and requirements (Ciobanu, 1999; Georgescu et al., 2000; Popa, 2012). In poultry production, quality and quality control require a good management on the entire production chain, aimed, on the one hand, to improve performance and increase profitability, and so on the other hand, the development of appropriate standard products (Vacaru-Opris et al., 2000; Vacaru-Opris et al., 2004). Having regard to the own research undertaken in this work had aimed at the study of quality indicators of poultry meat, absolutely required at EU level, as a result of the existence of a free trade of food products within the European Union (www.thepoultrysh).

MATERIALS AND METHODS

Own research were held over the course of two years. The material studied was represented by groups belonging of two genetic types: ROSS 308 and COBB 500. In accordance with the purpose, were made three series of experiences:

- The A series of experiences who fallow the influence the genetic type (hybrid) on quality indicators track the technology of slaughtering and processing of poultry in accordance with the standards of the European Union;
- The B series of experiences watching the effect of season on quality indicators track the technology of slaughtering and processing of poultry meat in accordance with European Union standards.
- C series of experiences that sought to establish the extent to which quality indicators are maintained in the production unit. Thus, for repeatability testing of results, has resumed the whole experiment and in the following year.

For the *A series* of experiences, research has been carried out on the basis of the results obtained from 100 individuals, commercial hybrids, belonging to the two genetic types mentioned (50-50) for a period of 1 year.
Given the multitude of factors that may influence the quality of the meat and the carcass, raising chickens has been carried out in uniform, on permanent bedding (large captivity), in accordance with standard technologies of the two hybrids, food and water provided 'ad libitum' chickens are slaughtered at the age of 42 days. Also, it was desirable to ensure uniformity in terms of body weight and gender composition.
The birds feed taken into study was represented by the combined feed that have been carried out in accordance with the requirements of the nutritional research hybrids. These have included several types of raw material: cereals, vegetable protein, animal protein, minerals, premixtures and feedingstuffs.
After slaughter, have been appreciated the quality indicators and the differences observed between the two genetic types have been tested as of significance statistically.
In *B series*, the experiment had aims to establish the influence of season on quality indicators. Thus, research has been carried out on the basis of the results obtained from 100 individuals, belonging to the two genetic types. The conditions under which it was made are similar to those of the A series. After the slaughter, the results obtained as a result of quality indicators analyzed were compared between the two seasons (season 1-hot: April-September, and season 2-cold: October-March), the differences observed between averages being tested as statistically significance.
Obviously, the two experimental series have been made together, the separation of the two making it only for the iconography reasons.
In the *C series* of experiments it study the extent to which the quality indicators are maintained in the production unit, in the next year. Thus, in order to ensure repeatability of the results, the entire experiment (series A series B) was resumed in the next year, while maintaining the same environmental conditions as in the previous year. The results obtained as a result of the quality indicators have been analyzed comparatively, between genetic types and between seasons.

RESULTS AND DISCUSSIONS

Protecting consumers′ interest is an open concern of the European Union. For this reason binding to established limits about water composition and water/protein proportion is now mandatory in poultry industry and poultry product trade. However meat freshness indicators are important in this question and sticking to them is a guarantee for the delivery of most trustworthy products. We are emphasizing the average values of each analyzed character and the statistical significance of differences noticed between means in the two studied groups (the two hybrids) to emphasize any influence of genetic type of slaughtered chickens on water share of carcasses. Table 1 and figure 1 are showing values for "carcass moisture" for the two groups in the experiment.

Table 1. Influence of genetic type on carcass moisture, first year, first season

Genetic type	n	(%)	s	c.v.%
ROSS 308	25	64.5316 ± 0.4106	2.0531	3.1816
COBB 500	25	65.1820 ± 0.3724	1.8623	2.8570
Differences significance	$t = 1.1732^{NS}$ $t_{48;0,05} = 2.01$; $t_{48;0,01} = 2.68$; $t_{48;0,001} = 3.51$			

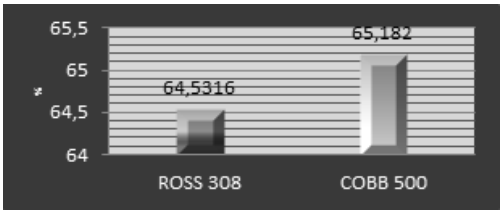

Figure 1. Average carcass moisture at both hybrids, first year, first season

Average carcass moisture was 64.5316 % ± 0.4106 and 65.1820 % ± 0.3724 or almost 1% higher in hybrids ROSS 308 and COBB 500 respectively. Superiority of hybrids COBB 500 has been noticed but it is not statistically significant as Student Test value ($t = 1,1732^{NS}$) has been emphasizing. So differences between the two hybrids would be most probable a consequence of sampling errors. The two

genetic types are actually having an equal carcass moisture and registered are being regarded as very good in analyzed groups.
Table 2 and figure 2 are showing values for average moisture of carcasses of chickens of both genetic types slaughtered during the experiment in first year and second season.

Table 2. Influence of genetic type on carcass moisture, first year, second season

Genetic type	n	(%)	s	c.v.%
ROSS 308	25	64.4656 ± 0.3989	1.9943	3.0936
COBB 500	25	65.5412 ± 0.3442	1.7212	2.6261
Differences significance	$t = 2.0414^{*}$ $t_{48;0,05} = 2.01$; $t_{48;0,01} = 2.68$; $t_{48;0,001} = 3.51$			

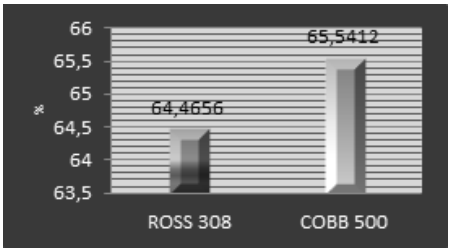

Figure 2. Average carcass moisture at both hybrids, first year, second season

Data presented in table 2 are revealing values close to average carcass moisture of analyzed carcasses from both hybrids taken into account. Like in season 1 hybrid COBB 500 (65.5412 ± 0.3442 %) has carcass moisture 1.64% higher than ROSS 308 (64.4656 ± 0.3989 %). Calculated value of Student Test (t = 2.0414) has been higher than in tables which support some significant differences between the two hybrids most probable due to sampling errors.
Data presented in table 3 are showing that in first season of the second year of the experiment registered average moisture of controlled carcasses was 64.4264 ± 0.3807 % and 66.3212 ± 0.3051 % or 2.86 higher in hybrids cobb 500 and respectively ross 308. Variability of character between the two analyzed groups was small which are suggesting that feeding and housing conditions are identical. In second year we noticed a statistically significant superiority in favor of hybrid Cobb 500 as the value of the student test ($t = 3.8838^{***}$) is emphasizing.

Table 3. Influence of genetic type on carcass moisture, second year, first season

Genetic type	n	(%)	s	c.v.%
ROSS 308	25	64.4264 ± 0.3807	1.9035	2.9546
COBB 500	25	66.3212 ± 0.3051	1.5255	2.3001
Differences significance	$t = 3.8838^{***}$ $t_{48;0,05} = 2.01$; $t_{48;0,01} = 2.68$; $t_{48;0,001} = 3.51$			

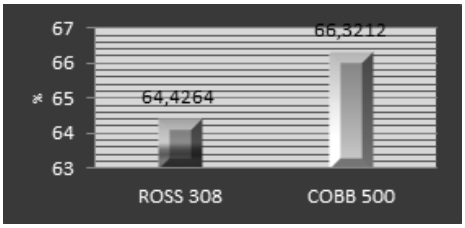

Figure 3. Average carcass moisture at both hybrids, second year, first season

Table 4 and figure 4 are showing values of average carcass moisture of chickens from both genetic types slaughtered during the experiment and second year and second season.

Table 4. Influence of genetic type on carcass moisture, second year, second season

Genetic type	n	(%)	s	c.v.%
ROSS 308	25	64,4768 ± 0,3787	1,8935	2,9368
COBB 500	25	66,7896 ± 0,3072	1,5361	2,2999
Differences significance	$t = 4,7427^{***}$ $t_{48;0,05} = 2,01$; $t_{48;0,01} = 2,68$; $t_{48;0,001} = 3,51$			

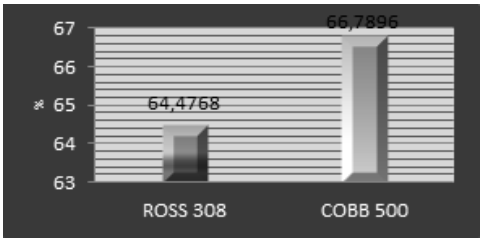

Figure 4. Average carcass moisture at both hybrids, second year, second season

Data presented in table 4 are revealing values close to average moisture of analyzed carcasses from the two hybrids taken into account in both seasons. Similar to first season se hybrid COBB 500 (66.7896 ± 0.3072 %) is 3.46% higher than hybrid ROSS 308 (64.4768 ±

0.3787 %). Calculated value of Student Test (t = 4.7427**) has been higher than in tables which support some significant differences between the two hybrids.

The two hybrids might be considered as appropriate from the point of view of the analyzed character as average carcass moisture is a very important character in the assessment of meat quality and how appropriate for processing the meat is.

CONCLUSIONS

Researches described in this paper are leading to following conclusions:

- Compliance with quality standards has no connection with the hybrid. The differences that arise between the genetic types examined in this paper, are caused by genetically controlled aspects (weight, water physiological linked, protein content, etc.).
- The season has an influence on the quality of the meat, as it emerged from the study in this paper. Both the research cited in the study, and on the basis of the results obtained in own research showed that, in most situations, the hot season has had a negative influence on the quality of the meat.

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CATTLE AND SMALL RUMINANT BREEDING ACTIVITIES IN TURKEY

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Abstract

In recent years, due to the increasing global warming, food security is one of the world's biggest problems. Each country is willing to take under guarantee the safety of their food in order to feed the population. In order to realize this strategy, rather than increasing the number of animals in production to increase yield obtained per animal. The most effective and accurate way to increase productivity per animal is breeding. Breeding success depends on agricultural policies applied, in addition to the objectives to be accomplished.
Breeding activities started with the republic in Turkey, but could not achieve the desired success. Herd book records belongs to species are collected by the cattle, sheep and goat breeders associations. Type of pedigree cattle records history dates back to 1995. Projects carried out jointly by Turkey, Germany and Italy began pedigree studies resulted in the establishment of Cattle Breeders' Association. By means of this associations, breeding activities have gained momentum and National Breeding Program was started in 1999, Cattle Breeders' Association of Turkey, in partnership with the Ministry of Food, Agriculture and Livestock. In this program, pre-herdbook and herdbook records are kept by the Association and, Progeny Testing System are applied in conditions of the country.
Organizational model in small ruminants are applied similar to cattle, however keeping herdbook records and breeding activities are implemented at the beginning stage yet.
In this study, animal breeding applications in Turkey are evaluated generally.

Key words: *Breeding, Cattle, Small Ruminant, Turkey.*

The presence of animals in Turkey over the years (Table 1) except the cattle for the last twenty years appears to be a significant reduction. The presence of cattle increased by 9% in terms of by the year in 1990, 38% in the presence of sheep and goat decreased by 34%. During this period the most dramatic reduction occurred in number of buffalo presence by 74%.

Table 1. The presence of animals in Turkey over the years, (1000 head)

Years	Cattle	Sheep	Goat	Buffalo
1990	11377	40553	10977	371
1995	11789	33791	9111	255
2000	10761	28492	7201	146
2005	10526	25304	6517	105
2009	10724	21750	5128	87
2010	11370	23090	6293	85
2011	12386	25032	7278	98

Milk and red meat production in Turkey over the years on the basis of species presented in Table 2. Milk production has increased by 56% in the last twenty years. Share of cattle milk production increased up to 92%. Increase in milk production in dairy cattle in favor of the public sector can be said that the effect of its policies.

Table 2. Red meat and milk production in Turkey over the years

Years	MILK (1000 ton)					RED MEAT (TON)				
	Cattle	Sheep	Goat	Buffalo	Total	Cattle	Sheep	Goat	Other	Total
1990	7961	1145	338	174	9617	329	144	23	12	507
1995	9275	934	277	115	10602	292	102	14	7	415
2000	8732	774	220	67	9794	355	111	21	4	491
2005	10026	790	254	38	11108	322	74	12	2	409
2009	11583	734	192	32	12542	325	75	12	1	413
2010	12419	817	273	35	13544	619	136	23	3	781
2011	13802	893	321	40	15056	645	107	23	2	777

The red meat production tend to decrease until 2010, while increased last two years due to the imports of animals for slaughtering. However, short-term solutions for the production of red meat can not be considered ensured continuity. In Turkey, the vast majority of red meat are provided by dairy herds. Therefore, the problems experienced in the production of milk and milk price fluctuations affect the red meat production in same period. In recent years, although there have been imported the breeding beef-breeds, it needs long-term solution by the Ministry.

Breeding studies in Turkey are seen based on initially appears to be generally short-term solutions. However, adequate and balanced feed of the people of the country are required to meet and milk, the only way of satisfying the growing demand in the future, consistent with the applicable government policy, and a recording system that can be integrated multidisciplinary studies of genetic and environmental breeding. In recent years, breeding studies has focused on this awareness.

Stages of a breeding program consists of phases identification of purpose, collection and recording of data, estimating of population parameters, breeding value estimation, selection of high-yielding animals and mating. Each stage is very important for the success of animal breeding studies. International Committee for Animal Recording in the international arena (ICAR) has established a set of standards relating to determination of yields and evaluation of the data obtained and the recording of animals all over the world. Cattle Breeders′ Association is only a member of this organization in Turkey.

Cattle Breeding in Turkey

The first years of the republic in Turkey, on the one hand, the culture breeds imported from abroad and the existing native breeds was crossbred, on the other hand imported culture breeds were grown as pure-breeds. These activities continued for many years, but could not achieve the desired success in producing animal breeding and breeding. One of the most important reasons is the lack of a consistent policy of livestock and keep away the breeders who are responsible for the production. However, The Breeders' Associations and recording systems carried out by these organization play an important role in breeding studies when the breeding background took place in developed countries are examined.

The first step in this regard in the field of dairy cattle issued by the Ministry of Agriculture in 1966 'Instruction on Registration of Holstein Friesian cow' and although the first serious studies began to projects carried out jointly by Italy and Germany by the years in 1989 and 1990. Both projects aim to create a registration system in Turkey in the field of cattle breeders′ associations and the establishment of this system is to carry out.

In fact, with the changing in the current law has given the opportunity to breeders to establish The Breeding Associations for breeding purposes in 1995. Thus, Provincial Cattle Breeders' Association began to be established since 1995, and Cattle Breeders' Association of Turkey was established. 1998. Then the studies gained momentum after the establishment of the Association and Ministry of Agriculture has implemented the National Breeding Program. The aim of the program Holstein breed a lactation (305 days) 7000

liters of milk with 4% fat that can mature at the age 145 cm rump height, reaching 750 kg live weight is solid and healthy, the body is expected to provide a high yield capacity, provide the ability to move freely foot and nail structure, convenient and easy to be milked milking machine, with a capacity of teat to achieve genotype and the population spread is defined as. In this context, Herdbook, Pre-herdbook and Progeny Testing Project is currently being carried out jointly with the Ministry. Established across the province have completed the organization of these units across the country today, operate in 81 cities. Duties of these associations are as follows;

- Tagging new-born calves,
- Identification of new farms
- Monthly milk yield records and milk metering audits,
- Milk sample collection and analysis,
- Records relating to calving, insemination and herd movement
- Classification according to conformation traits
- Breeding and nutrition counseling,
- Input supply (semen, earrings, milk meter bucket, insurance, equipment, etc.),
- Animal health and artificial insemination services
- Procurement and sale of breeding animals,
- Breeding cow show,
- Extension and Training services,

The number of farms joined to Cattle Breeders′ Association which have completed the organization reach approximately 140 thousand in 81 provinces union across the country in cooperation with the National Breeding Program. Number of female cattle registered in herdbook is 3.3 million head and 4.2 million total number of cattle. In addition, the number of registered female cattle is 3.7 million head in 900.000 farms, the total number of cattle has reached 4.5 million head as well in pre-herdbook. The number of registered cattle is 8.735.000 head in more than one million farms.

It is important of selection both cows and bulls in breeding studies, however, selection of males is placed on mostly due to their physiological properties. For this purpose, the progeny testing is applied all over the world. The semens are produced by bulls known daughters′ performance through the country as a result of Progeny Testing Project implemented in Turkey. While the quantity of milk can be measured only at the beginning of the project, in addition fat, protein, somatic cell count, lactose, etc. can be determined currently. Properties of milk yield as well as conformation traits features are identified and this information is published in the bull catalogs. In addition, breeding activities implemented only Holstein Friesian (Holstein Black and White) breed started for Simmental and Brown Swiss cattle in 2011. The milk yield per cows is 3000 kg in Turkey, while this value is 6000 kg in farms recorded herd book.

Sheep Breeding in Turkey

In the traditional sense of the importance of sheep farming in Turkey, as well as the geographical conditions can not be ignored because of its compliance. In recent years, there has been a serious drop in the number of sheep, it has not been compensated for by an increase in productivity per animal and this situation has affected the production of meat and milk. The most important factor in this regard is the public policy concentrate on the cattle.

In Turkey, an important sheep breeding studies were conducted in the past. Initially, the main objective of improving the quality of wool called Merino activities were emphasized, however, desired success was not obtained due to insufficient environmental conditions affecting productivity.

In general, sheep breeding efforts are focused on the cross-breeding and breeding of native breeds in cooperation with the Ministry and the universities to work toward the creation of new types of sheep are held. But these studies were not applied in breeders level.

In recent years, it is aimed the organization like cattle breeders′ associations for sheep breeding activities. Breeding Sheep and Goat Breeders' Association organized in 80 provinces in Turkey and 191.676 farms were registered.

Determination of Association′s breeders to be involved "National Animal Breeding Project" applications in farming condition, selection and registration of herds, breeding programs carried out in a timely and taking all necessary records and data, regulating the relations between the Union and the Ministry, the Ministry deems appropriate qualifications the principles and

procedures for the acquisition of technical services of the Ministry 'published by the National Project Implementation Principles of Animal Breeding at village level were determined.

Goat Breeding in Turkey

The Angora goat is named for Ankara and historically known as Angora producing the lustrous fiber known as mohair is differ from other goat breeds and should be evaluated separately in Turkey. Studies were carried out in order to increase the quantity and quality of the mohair in Ankara goat in some state owned Farms, however have not been successful in the field. As a result of reduced demand for mohair and prices of mohair falls rapidly decrease in the number of goats. So that at the beginning of the 1990′s the number of Ankara goats over 1 million head in 2011 decreased to 151 thousand head.

Hair goats are grown mainly in forested and mountainous area. The rhetorical thoughts about the goats relating to damaging effects on the forest has the negative affect for goat farming, in particular the high nutritional value of goat milk also increased the importance of goats in recent years,

Legal regulations for sheep is current for goat and with two kinds of operations are carried out under the umbrella of the same union. National Animal Breeding Project in farming condition carried out in 31 provinces and 9 breeds.

CONCLUSIONS

In Turkey, breeding facilities are only performed based on the cattle, sheep and goats breeding. However, studies mainly focused on cattle. Cattle breeding program is conducted in partnership with the Public and Breeders Association and herdbook records to be used in breeding activities are kept regularly.

Recording are just new in sheep and goat species. For this reason, an established registration systems to fallow up the records are not available at the moment.

The Purebred Sheep and Goat Breeding Legislation″ has inured in February of 2013. According to the legislation published the herdbook registration conditions, the necessary information for registration, breed-specific performance evaluation methods and criteria of these animals. The semen, ova, recording and documenting of every stage of the embryos rules belonging to breeding sheep and goats was determined. This legislation is expected to accelerate activities to be performed on small ruminants.

In addition to conventional breeding approaches as well as all over the world in the use of biotechnology based applications would be useful.

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STUDY ON DENSITY IN SHELTER AND THE EFFECT OF A VITAMIN-MINERAL SELENIUM PREMIX IN YOUNG QUAILS OF THE BALOTEŞTI POPULATION

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Abstract

In order to establish an optimal density per unit area, and the effect of administration of a selenium vitamin-mineral supplement in the diet of young quails during 0-14 days of life, an experiment was organized on an initial number of 1200 chickens one day old of Baloteşti population between the ages of 1 and 42 days. For the experiment chicks were divided into four equal groups, namely a control group (300 chicks) and 3 experimental groups (each 300 chicks per group).

Following research is recommended to use a density per unit area that decreases faster during the growth, of 300 chickens / square meter during 0-3 days of growth, 200 chickens / sq. m during 4-7 days of life, 150 chicken / sq. m during days 8-21 and of 100 chickens / sq. m during 22-42 days. This leads to superior results with over 10% in young quails mass growth, with a reduction in death rate of 20%, without significant influence over combined feed consumption and feed utilization. Small differences of only 2.47% in live weight, 2.34% in average daily gain, 1.23% in combined feed consumption, by 2.72% in specific consumption and considerable effect of 3% in the death rate of the group with the same low experimental density, recommends the administration of a vitamin-mineral product with selenium in young quails growing, while used density above. Administration of selenium had a greater influence on death rate, which was reduced by 13.7% in the group with high density, equal to that of the control group.

Key words: *density, quail, youth, selenium.*

INTRODUCTION

Quail is the smallest bird species raised for meat and eggs (Panda and Singh, 1990). Japanese quail growth witnessed great development in recent decades due to the biological characteristics of this bird, which causes high production levels and economic efficiency and because of the market requirements for eggs and quail meat with recognized quality (high biological and nutritional value, special taste) and recommended by natural medicine for their therapeutic effect. Among the main productive characteristics of quail stands out: rapid rate of growth (reach adult weight at 5-6 weeks after hatching), early sexual maturity, short interval between generations, high rate of laying, low feed consumption and reduced space accommodation (Adeogun and Adeoye, 2004). To determine the quail productive parameters in Romania were conducted and continue to conduct research on biological material existing in one of the largest quail farm in Romania (Popescu-Micloșanu et al., 2008).

MATERIALS AND METHODS

The aim of the experiment was to determine, on one hand the optimal density per unit area in young quail of Baloteşti population between 0-42 days of life and secondly to determine the effect of administering a vitamin-mineral supplement on the basis of selenium, 'Selevit', in young quails during 0-14 days of life.

The research was conducted within the quail farm of Gheorghița common, Ungureni village, Lucian T. Ioniță individual society enterprise Bucharest.

Density used in the control group was of 300 chicken / m^2 during 0-7 days, 250 chicken / m^2 during 7-14 days of growth, 200 chicken / m^2 from 15 to 28 days of growth and 100 chicken / m^2 between 29-42 days of growth.

In experimental group I was used the same density as the control group and chicks have been given the vitamin-mineral supplement with selenium Selevit the first 14 days of life.

In the experimental group II, the following density per unit area was used: 300 chicken / m^2 during 0-3 days of growth, 200 chicken / m^2 during 4-7 days of life, 150 chicken / m^2 during 8-21 days and 100 chicken / m^2 during 22-42 days of growth. In the experimental group II, it was not used vitamin-mineral supplement with selenium.

In the experimental group III the same density as in the experimental group II was used, but their chicken were administered for 14 days the vitamin-mineral supplement with selenium in the drinking water.

Note that during days 0-28 chickens were maintained at ground, on permanent litter consisting of sterilized shavings in all analyzed groups and from the age of 28 days chicks were raised in battery cages with density of 100 chicken / cage sq. m A cage capacity is 50 heads.

Vitamin-mineral product 'Selevit' contains a concentration of 20000000 IU per ml A vitamin, 2,500 mg of vitamin B_2, 7.5 mg vitamin B_{12}, of 100000 IU of vitamin D3, 2,000 mg of vitamin K3, 1,250 mg of vitamin B_1, B_6 vitamin 1,750 mg, 2000 mg of vitamin C, vitamin E 5,500 mg, 6500 mg calcium pantothenate, folic acid 400 mg, 18,000 mg nicotiamide, of 4,000 mg methionine, 600 mg tryptophan, 4,000 mg lysine and 33 mg selenium / ml. The product was administered in drinking water at a dose of 5 ml product/10 l water. Were used 1000 g of vitamin-mineral product 'Selevit' in the two groups in the analyzed period.

The determinations refers to living weight and combined feed consumption ages at 1 day, 7, 14, 21, 28, 35 and 42 days. They also watched the actual stock losses. Measurements on live weight and combined feed consumption were performed by simple random sampling, individually, during 0-28 days period and on cage during 29-42 days of growth. Based on measurements made, weekly and daily gain, specific combined feed consumption, loss ratio in the analyzed groups were calculated. To test the significance of the differences was applied Fisher's test, followed by Turkey-test.

RESULTS AND DISCUSSIONS

1. Evolution of the average living weight of quail chicks in the 3 experimental groups compared with control group

At the age of 1 day was an average weight of 8.36 ± 0.23 g / capita in the control group, of 8.95 g / head ± 0.34 in the experimental group I, 8.74 ± 0.55 g /capita in experimental group II and 8.67 g / head ± 0.56 in the experimental group III, the differences between the 4 groups were insignificant.

Table 1. Live average weight in quail chickens from the 3 experimental batches compared to the control

Age	Control lot	Experimental lot I I	Experimental lot II	Experimental lot III
	$X \pm s_X$			
Day 1	8.36 ± 0.23 *ans bns cns*	8.95 ± 0.34 *ans dns ens*	8.74 ± 0.55 *bns dns fns*	8.67 ± 0.56 *cns ens fns*
Day 7	19.23 ± 2.35 *a bb ccc*	22.34 ± 2.55 *a dns ee*	24.54 ± 2.77 *bb dns f*	29.33 ± 2.67 *ccc ee f*
Day 14	52.00 ± 2.76 *aa bbb ccc*	58.88 ± 2.95 *aa dns e*	59.55 ± 2.65 *bbb dns f*	63.22 ± 2.78 *ccc e f*
Day 21	100.23 ± 3.56 *aa bb cc*	110.45 ± 4.33 *aa dns ee*	112.34 ± 4.23 *bb dns f*	120.33 ± 3.87 *cc ee f*
Day 28	134.45 ± 3.23 *aa bbb ccc*	145.45 ± 4.02 *aa dd eee*	155.34 ± 3.90 *bbb dd f*	160.45 ± 3.46 *ccc eee f*
Day 35	154.45 ± 4.45 *aa bbb ccc*	165.34 ± 3.68 *aa dd eee*	176.87 ± 4.03 *bbb dd f*	180.67 ± 3.96 *ccc eee f*
Day 42	178.65 ± 3.78 *aa bbb ccc*	188.95 ± 4.34 *aa dd eee*	198.74 ± 3.45 *bbb dd f*	203.78 ± 3.78 *ccc eee f*

At the age of 7 days was an average weight of 19.23 ± 2.35 g / head in control group, of 22.34 ± 2.55 g / capita in experimental group I, 24.54 ± 2.77 g / capita in experimental group II and 29.33 ± 2.67 g / head in the experimental group III. Distinct significant differences were between the control group and experimental group I, distinctly significant between the control group and experimental group II and very significant between control and experimental group III. Between experimental group I and experimental group II there were insignificant differences, between experimental

group I and group III distinct significant and between the experimental group II and III were only significant differences.

At the age of 14 days there was an average weight of 52.00 ± 2.76 g / head in control group, of 58.88 ± 2.95 g / capita in experimental group I, 59.55 ± 2.65 g / capita in experimental group II and 63.22 ± 2.78 g / head in the experimental group III. There were signifycant differences between the control and experimental group I, distinctly significant between the control and experimental group II and highly significant between the control and experimental group III. Between experimental group I and experimental group II differences were not significant, between the experimental group II and III were distinctly significant differences. Between the experimental group II and III were only significant differences.

At the age of 21 days there was an average weight of 100.23 ± 3.56 g / capita in the control group, 110.45 ± 4.33 g / head in experimental group I, 112.34 ± 4.23 g / capita in experimental group II and 8.67 g / head ± 0.56 in the experimental group III. Distinct significant differrences were between the control group and experimental group I, experimental group II and III. Between experimental group I and experimental group II there were not significant differences, between the experimental group I and III they were distinctly significant, between the experimental group II and III differences were only significant.

At the age of 28 days there was an average weight of 134.45 ± 3.23 g / capita in the control group, 145.45 ± 4.02 for g / head in experimental group I, 155.34 ± 3.90 g / capita in experimental group II and 160.45 ± 3.46 g / head in the experimental group III. Distinct significant differences were between the control and experimental group I and very significant differences between the control group and experimental groups II and experimental III. Between experimental batch I and experimental group II there were distinctly significant differences, between experimental group I and experimental group III there were very significant, while between experimental group II and experimental group III differences were only significant.

At the age of 35 days there was an average weight of 154.45 ± 4.45 g / capita in the control group, 165.34 ± 3.68 for g / head in experimental group I, 176.87 ± 4.03 g / head in group II and 180.67 ± 3.96 g / head for the group III. Distinct significant differences were between the control and experimental group I and very significant differences between the control and groups II and III. Between experimental group I and group II there were distinctly significant differences, between the experimental group II and III they were very significant, between the group II and III differences were only significant.

At the age of 42 days there was an average weight of 178.65 ± 3.78 g / capita in the control group, 188.95 ± 4.34 g / head in group I, 198.74 ± 3.45 g / capita in group II and 203.78 ± 3.78 g / head in the experimental group III. Distinct significant differences were between the control group and experimental group I and very significant differences between the control group and experimental groups II and III. Between group I and II were distinctly significant differences, between group I and III there were very significant, while between experimental group II and III differences were only significant.

During the period when chickens were maintained on the ground, average live weight per unit area at 1 day in control group was 2508 kg / sq. m, 4807 kg / sq. m at the age of 7 days, 13,000 kg / sq. m at the age of 14 days, 20,046 kg / sq. m at the age of 21 days, 26,890 kg / sq. m at 28 days. Average live weight per unit area of the cage at the age of 35 days was 15,445 kg / sq. m, while at the age of 42 days it was 17,865 kg / sq. m.

In experimental group I when the chickens were maintained on ground, average live weight per unit area at 1 day was of 2685 kg / sq. m, 6702 kg / sq. m at the age of 7 days, 14,700 kg / sq. m at the age of 14 days, 22,090 kg / sq. m at the age of 21 days, 29,090 kg / sq. m at 28 days. Average live weight per unit area of the cage at the age of 42 days was 18,895 kg / sq. m, 5.5% higher than for the control lot.

During the period when chickens were maintained on the ground, average live weight per unit area at 1 day in experimental group II was 2622 kg / sq. m, 4908 kg / sq. m at the age of 7 days, 8933 kg / sq. m at the age of 14 days,

16,851 kg / sq. m at the age of 21 days, 15,534 kg / sq. m at 28 days. Average live weight per unit area of cage at the age of 35 days was 17,687 kg / sq. m, while at the age of 42 days it was 19,874 kg / m² (10.1% greater than the control).

In the experimental group III, in the period when chickens were maintained on the ground, average live weight per unit area at 1 day was 2601 kg / sq. m, 5866 kg / sq. m at the age of 7 days, 9483 kg / sq. m at the age of 14 days, 18,050 kg / sq. m at the age of 21 days, 16,045 kg / sq. m at 28 days. Average live weight per unit area of cage at the age of 42 days was of 20,378 kg / m², 12.3% higher than the control.

2. Evolution of average weekly gain in quail chicks of the 3 experimental groups compared to the control

In the first week of life, there was a daily average gain of 10.87 g / head in control group, of 13.39 g / capita in experimental group I, 15.8 g / capita in experimental group II and 20.66 g / head ± 0.56 in experimental group III, the differences between the 4 groups were not statistically assured.

In the second week of life, there was a daily average gain of 32.77 ± 1.23 g / head in control group, of 36.54 ± 1.47 g / capita in experimental group I, 35.01 ± 1.35 g / capita in experimental group II and of 33.89 ± 1.55 g / head for the experimental group III, the differences between the 4 groups were not statistically assured.

In the third week of life, there was an average daily gain of 48.23 ± 1.45 g / head in control group, of 51.57 ± 1.83 g / capita in experimental group I, 52.79 ± 1.74 g / capita in experimental group II and of 57.11 ± 2.05g/capita for the group III, the differences between the 4 groups were not statistically assured.

In the fourth week of life, there was an average daily gain of 34.22 ± 2.37 g / head in control group, of 35.00 ± 2.15 g / capita in experimental group I, 43.00 ± 2.56 g / capita in group II and of 40.12 ± 2.10 g / head for the experimental group III, the differences between the 4 groups were not statistically assured.

In the fifth week of life, there was a daily average gain of 26.23 ± 2.45 g / head in control group, of 24.88 ± 2.02 g / capita in experimental group I, 23.21 ± 2.90 g / capita in group II and 25.98 ± 2.32 g / head for the group III, the differences between the 4 groups were statistically assured as follows: not significant between the control group and experimental group I, significant differences between control group and group II, not significant between control group and experimental group III. Between group I and II differences were significant, as in the group I and III. Between the experimental group II and experimental group III there were significant differences.

Table 2. Average weekly gain in quail chickens from the 3 experimental lots compared to the control lot

Age period	Control lot	Experimental lot I	Experimental lot II	Experimental lot III
	$X \pm s_X$			
Week I	10.87 ± 0.75	13.39 ± 0.88	15.80 ± 0.45	20.66 ± 1.34
Week II	32.77 ± 1.23	36.54 ± 1.47	35.01 ± 1.35	33.89 ± 1.55
Week III	48.23 ± 1.45	51.57 ± 1.83	52.79 ± 1.74	57.11 ± 2.05
Week IV	34.22 ± 2.37	35.00 ± 2.15	43.00 ± 2.56	40.12 ± 2.10
Week V	26.23 ± 2.45 *ans b cns*	24.88 ± 2.02 *ans d e*	23.21 ± 2.90 *b d f*	25.98 ± 2.32 *cns e f*
Week VI	17.97 ± 1.34 *ans b cns*	18.62 ± 1.68 *ans d e*	20.19 ± 1.03 *b d f*	17.35 ± 1.97 *cns e f*
Total I-VI week	170.29	180.00	190.00	195.11
Average I - VI week	28.38 ± 5.37	30.00 ± 5.67	31.67 ± 5.89	32.52 ± 5.99

In the sixth week of life, there was an average gain of 17.97 ± 1.34 g / head in control group of 18.62 ± 1.68 g / capita in experimental group I, 20.19 ± 1.03 g / capita in experimental group II and 17.35 ± 1.97 g / head for the experimental group III, the differences between the 4 groups were significant among the experimental group II and control groups and experimental I. The differences between the 4 groups were statistically assured as follows: not significant between the control and experimental group I, significant differences between control and experimental group II, not significant between control group and experimental group III.

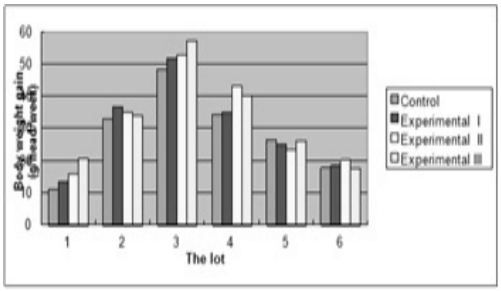

Figure 1. The evolution of average weekly gain in quails chickens for the 4 analyzed lots in the growth period of 1-6 weeks

Between experimental group I and II differences were significant, as in the experimental group I and III. Between the experimental group II and III there were significant differences.

3. Average weekly combined feed consumption trends in the quail chicks of the 3 experimental groups compared with controls from I to VI weeks of growth

Table 3. Average consumption of combined fodder for the 3 experimental lots of quails chicks compared to the control lot in the period of growth I - VI

Specification	Control lot	Experimental lot I	Experimental lot II	Experimental lot III
		$X \pm s_X$		
Week I	30.50 ± 1.68	31.65 ± 1.87	32.76 ± 1.55	33.23 ± 2.24
Week II	69.65 ± 2.33	71.33 ± 2.43	72.45 ± 2.89	73.35 ± 2.85
Week III	110.87 ± 2.76	115.67 ± 2.99	119.45 ± 3.05	123.56 ± 2.38
Week IV	134.56 ± 1.98	137.45 ± 3.15	137.77 ± 2.65	139.15 ± 3.56
Week V	169.55 ± 2.65 *ans* *dns* *cns*	171.24 ± 2.13 *ans* *dns* *ens*	173.12 ± 1.95 *bns* *dns* *fns*	175.35 ± 3.16 *cns* *ens* *fns*
Week VI	182.15 ± 2.45 *ans* *bns* *cns*	185.24 ± 2.18 *ans* *dns* *ens*	186.34 ± 2.14 *bns* *dns* *fns*	188.67 ± 3.96 *cns* *ens* *fns*
Total I-VI weeks	697.28	712.58	721.89	733.31
Average I-VI weeks	182.15 ± 2.45 *ans* *bns* *cns*	185.24 ± 2.18 *ans* *dns* *ens*	186.34 ± 2.14 *bns* *dns* *fns*	188.67 ± 3.96 *cns* *ens* *fns*

In the first week of life, there was an average of 30.50 ± 1.68 g combined feed consumption / head in control group, of 31.65 ± 1.87 g / capita in experimental group I, 32.65 ± 1.55 g / capita in experimental group II and of 32.76 ± 2.24 g / head for the experimental group III; the differences between the 4 groups were not statistically assured.

In the second week of life, there was an average consumption of 69.65 ± 2.33 g combined feed g / head in control group, of 71.33 ± 2.43 g / capita in experimental group I, 72.45 ± 2.89g/capita in the experimental group II and of 73.35 ± 2.85 g / head for the experimental group III; the differences between the 4 groups were not statistically assured.

In the third week of life, there was an average of 110.87 ± 2.76 g combined feed / capita in the control group, 115.67 ± 2.99 g / head in experimental group I, 119.45 ± 3.05 g / capita in group II and 123.56 ± 2.38 g / head for the group III; the differences between the 4 groups were not statistically assured.

In the fourth week of life, there was an average of 134.56 ± 1.98 g combined feed / head in the control group, 137.45 ± 3.15 g / head in experimental group I, 137.77 ± 2.65 g / capita in group II and 139.15 ± 3.56 g / head for the group III; the differences between the 4 groups were not statistically assured.

In the fifth week of life, there was an average of 169.55 ± 2.65 g combined feed / head in the control group, 171.24 ± 2.13 g / head in experimental group I, 173.12 ± 1.95 g / cap in group II and of 175.35 ± 3.16 g / head for the group III; the differences between the 4 groups were not significant.

In the sixth week of life, there was an average of 182.15 ± 2.45 g combined feed / capita in the control group, 185.24 ± 2.18 g / head in experimental group I, 186.34 ± 2.14 g / capita in group II and 188.67 ± 3.96 g / head for the group III; the differences between the 4 groups were not significant.

4. Specific consumption evolution in quail chicks of the 3 experimental groups compared with controls from I to VI growth weeks

In the first week of life, there was a specific consumption of 2.81 ± 0.23 g compound

feed / g weight gain in the control group, 2.36 ± 0.34 in the group I, 2.06 ± 0.17 in the group II and 1.61 ± 0.25 in the group III; the differences between the 4 groups were not statistically assured.

In the second week of life, there was a specific consumption of 2.13 ± 0.14 g compound feed / g weight in the control group, of 1.95 ± 0.55 in the group I, 2.07 ± 0.88 in the group II and 2.16 ± 0.67 in the group III; the differences between the 4 groups were not statistically assured.

Table 4. Specific consumption evolution in quail chicks of the 3 experimental groups compared with controls from I to VI growth weeks

Growth period	Control lot	Experimental lot I	Experimental lot II	Experimental lot III
X ± s				
Week I	2.81 ± 0.23	2.36 ± 0.34	2.06 ± 0.17	1.61 ± 0.25
Week II	2.13 ± 0.14	1.95 ± 0.55	2.07 ± 0.88	2.16 ± 0.67
Week III	2.30 ± 1.13	2.24 ± 0.23	2.26 ± 0.35	2.16 ± 0.75
Week IV	3.93 ± 0.36	3.92 ± 1.35	3.20 ± 1.35	3.47 ± 1.68
Week V	6.46 ± 1.35 *ans* *b* *cns*	6.88 ± 2.76 *ans* *dns* *ens*	7.46 ± 2.37 *b* *dns* *fns*	6.75 ± 2.78 *cns* *ens* *fns*
Week VI	10.14 ± 2.65 *ans* *bns* *cns*	9.95 ± 2.75 *ans* *dns* *fns*	9.23 ± 1.93 *bns* *dns* *fns*	10.87 ± 2.55 *cns* *ens* *fns*
Average I-VI weeks	4.62 ± 1.28 *ans* *bns* *cns*	4.55 ± 1.34 *ans* *bns* *cns*	4.38 ± 1.28 *ans* *bns* *cns*	4.50 ± 1.48 *ans* *bns* *cns*

In the third week of life, there was a specific consumption of 2.30 ± 1.13 g combined feed / g weight gain in the control group, 2.24 ± 0.23 for the group I, in experimental group II of 2.65 2.26 ± 0.35 and 2.26 ± 0.35 for the batch III; the differences between the 4 groups were not statistically assured.

In the fourth week of life, there was a specific consumption of 3.93 ± 0.36 in the control group, 3.92 ± 1.35 for the group I, 3.20 ± 1.35 in the group II and 3.47 ± 1.68 for the group III; differences between the 4 groups were not statistically assured.

In the fifth week, there was a specific consumption of 6.46 ± 1.35 g combined feed / weight gain in the control group, 6.88 ± 2.76 for the group I, 7.46 ± 2.37 in the group II and 6.75 ± 2.78 for the group III; the differences between the 4 groups were statistically assured as follows: significant differences between control and experimental group II and not significant otherwise.

In the sixth week, there was a specific consumption of 6.46 ± 1.35 g combined feed / g weight gain in the control group, of 6.88 ± 2.76 in group I, 7.46 ± 2.37 in group II and 6.75 ± 2.78 for the group III; the differences between the 4 groups were not significant.

5. Evolution of death rate in quail chicks of the 3 experimental groups compared with controls from I to VI growth weeks

In the first week of life, was an average death rate of 8.33% in the control group, of 3.33% in the group I, in the group II of 2.33% and 1% for the group III; the differences between the 4 groups were statistical assured in favor of experimental groups.

Table 5. Evolution of death rate for quails chicks from the 3 experimental lots compared to the control lot for the period of growth I to VI weeks In

Growth period	Control lot		Experimental lot I		Experimental lot II		Experimental lot III	
	head	%	head	%	head	%	head	%
Week I	25	8.33	10	3.33	7	2.33	3	1
Week II	14	4.67	8	2.67	4	1.33	1	0.33
Week III	12	4	8	2.67	2	0.67	1	0.33
Week IV	9	3	7	2.33	2	0.67	1	0.33
Week V	8	2.67	2	0.67	0	0	0	0
Week VI	8	2.67	0	0	0	0	0	0
Total I-VI weeks	76	25.33	35	11.67	15	5.00	6	2.00
Average/ week I-VI	12.67	4.22 ± 0.88	5.83	1.94 ± 0.53	2.5	0.83 ± 0.36	1	0.33 ± 0.14

In the second week of life, there was an average death rate of 4.67% in the control group, of 2.67% in the group I, in group II of 1.33% and 0.33% for the group III, the differences between the 4 lots being statistically assured.

In the third week, there was an average death rate of 4% in the control group, 2.67% in the group I, in group II of 0.67% and 0.33% for the group III, the differences between the 4 lots being statistically assured.

In the fourth week, there was a 3% average death rate in the control group of 2.33% in the group I, in group II of 0.67% and 0.33% for the group III, the differences between the 4 lots being statistically.
In the fifth week of life, there was an average death rate of 2.67% in the control group and 0.67% in the experimental group I, the differences between the 4 groups being statistically assured. In the other groups were not quails lost.
In the sixth week of life, there was an average death rate of 2.67% in the control group; the other groups did not registered death rate.
In a study by Ragab S. et al. (2002) an average weight at age 42 days similar to that determined for the population Baloteşti quail (199.89 g / head) was found, a somewhat lower weight gain (167.67 g / head / period) and a specific consumption of 6.93 g mixed fodder / g weight gain was determined.
In another study by Khalil, H. (2009) the following parameters of growth in young quail are mentioned: live weight at the age of 42 days of 246.98 g / head, weight gain of 238.04 g / head / period, feed consumption of 902.76 g / head / period, specific consumption of 4.06 g / g weight gain).

CONCLUSIONS

1. **The weight between 1-6 weeks in quail chicks from the 4 groups analyzed**

If at the age of one day the live weight of chickens in the 4 groups was approximately the same in all 4 groups analyzed, the differences among them being not significant, from the age of 7 days the differences were significant between control group (with density used normally in the farm and without vitamin-mineral supplementation) and experimental group I (who applied density control group, but taking vitamin-mineral supplement with selenium), experimental groups II (which was applied experimental density and not receiving vitamin-mineral supplement) and experimental III (which was applied experimental density and taking vitamin-mineral supplement). Between experimental group I and II the difference was not significant and between experimental I and III distinctly significant and between experimental group II and III only significant. The differences occurred at ages of 14, 21, 28, 35 and 42 days were alike the same.

Highest weight at age 42 days was recorded for the experimental group III (203.78 ± 3.78 g / head), while the lowest live weight was recorded for the control group (178.65 ± 3.78 g / head) that did not apply any treatment. Live weight at 42 days of age was 5.45% higher in experimental group I, 10.11% in experimental group II and 12.33% higher in experimental group III compared to the control group. Between the control group and group II, with lower density, the difference was very significant, of 10.11%, in favor of group II. Between groups with supplementation of selenium and without supplement, but with the same density, the differences were quite small. Thus, between group II and group III there was a difference of only 2.5%. These results recommend using experimental density in parallel to the use of vitamin-mineral selenium product.

2. Average weekly weight gain in quail chicks of analyzed groups in period I - VI growth weeks

Average weekly weight gain per total studied period showed a similar trend with live weight, respectively the average weekly gain was 5.39% higher in experimental group I, 10.37% higher in experimental group II and 12.72% in the experimental group III compared with controls. Weekly weight gain increased steadily until the third week, when there was the highest in all 4 analyzed groups (between 48.23 ± 1.45 g / capita in the control group and 57.11 ± 2.05 g / capita in group III) and then gradually decreased until the sixth week of growth. Between groups with supplementation of selenium and without supplement, but with the same density, the differences were quite small. Thus, the difference between controls and group I was only 5.4%, and between group II and III of 2.62%.

3.The evolution of the average weekly mixed feed consumption and specific consumption in the analyzed quail chicks groups in the period I - VI weeks of growth

Between 1-VI weeks of growth in the control group the total combined feed consumption was of 697.28 g / capita, with 15.2% less than in the experimental group I, 3.40% less than the

group II and 4.91% less than the group III (who had a total consumption of 733.31 ± 5.99 combined feed g / head), the differences being not statistically assured.

Also, during 1-6 weeks of growth, in the control group was obtained the highest average specific consumption of 4.62 ± 1.28 g mixed feed / g weight gain, with 1.63% more than in the experimental group I, 5.60% less than the experimental group II and 2.72% less than the experimental group III; differences between groups were not significant.

4. Evolution of the average death rate in the analyzed quail chicks groups in period I - VI weeks of growth

The highest death rate between 1-6 weeks of growth was recorded in the control group, of 25.33%, with 13.66% more than the experimental group I, 20.33% more than group II and 23.33% more than group III. Differences were evident for lower density lots and those with selenium supplementation.

As a general recommendation following investigations, the use of a density per unit area to decrease faster during the growth, of 300 chicken / sq. m during 0-3 days of growth, 200 chicken / sq. m. during 4-7 days of life, 150 chicken /sq. m during 8-21 days of growth and 100 chicken / sq. m during 22-42 days of growth brings superior results in raising young quails. Recommendation is justified by obtaining a body weight and weight gain higher with more than 10% and a reduction in death rate of 20.33%, without affecting feed and specific consumption.

Whereas differences between, on one hand, control and experimental groups I (with the same density and without, respectively with selenium) and on the other hand, experimental II (where experimental density was applied, but not received vitamin-mineral product with selenium) and experimental group III (which was applied experimental density, but taking vitamin-mineral product) are small, of only 2.47% in live weight, of 2.34% in average daily gain, of 1.23% in mixed feed consumption, of 2.72% in specific consumption and 3% in death rate (between group II and III), results that taking selenium vitamin-mineral product tends to achieve superior performances in raising young quails while obvious improving viability by using density in experimental II.

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RESEARCH ON BEE KEEPING DEVELOPMENT IN THE SOUTHERN ROMANIA – A QUESTIONAIRE BASED SURVEY

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Abstract

The paper aimed to identify beekeepers' opinion on the development of apiculture in the Southern Romania based on a structured questionnaire based survey. In this purpose, a sample of 60 beekeepers from Calarasi, Ialomitza and Prahova counties answered 34 questions logically listed. The answers were statistically processed according to the specific marketing research methods and the following results were obtained: average apiary size was 67.4 bee families, a number of 4,044 bee families are kept in the 60 apiaries considered in the study and their honey production accounted for 105,161 kg, meaning 26 kg per bee family in 2011. About 55% beekeepers own between 50 and 100 bee families per apiary, 38 % less than 50 and 7 % over 100 bee families. Honey production depends on the number of bee families but also on bee feeding, pickings opportunities, climate conditions, maintenance during winter season. In 2011, the 60 beekeepers earned Lei 20,142,760, meaning Lei 298.75 per bee family from honey sold on the domestic market. As a conclusion, despite that apiary size and honey yield are still very small specific to subsistence farms compared to other countries, beekeeping is continuously developing in Romania being a profitable sector of agriculture. The unbalanced demand/offer ratio on the Western European market is a chance to increase honey production, to intensify export and to improve beekeepers' income.

Key words: *beekeeping, honey, production, questionnaire based survey, Southern Romania.*

INTRODUCTION

Beekeeping is among the priorities of the EU agricultural policy taking into account the increased honey demand in the common market and mainly in the Western countries (Popescu, 2010).

Romania has a long tradition and an exceptional meliferous potential and based on its honey production it is situated among the most important producing and exporting countries (Popescu, 2010)

Beekeeping is suitable to hilly areas but also it is practiced in the plain regions in apiaries of various size, whose average is ranking between 20 and 30 bee families at country level, being specific to subsistence farms (Bodescu et al., 2009). Beekepers are independent producers, most of them operating as physical authorized person with a poor bookeeping regarding expenditure and income, but also as members of beekeepers' association (Popescu, 2012).

According to the EU regulation, important subsidies and funds are allotted per bee family stimulated beekeepers to increase the number of bee families and produce more and higher quality honey (Vural et al., 2006).

In this context, the paper purpose was to analyze the status of apiculture development in the South part of Romania using an opinion test among beekeepers who own subsistence apiaries whose number is predominant in the country.

MATERIALS AND METHODS

The research was carried out in the period April-November 2012, using a sample of 60 beekeepers from three counties situated in the South part of Romania: Calarasi, Ialomitza and Teleorman. Their opinion on various aspects regarding the development of apiculture in the area was collected using a questionnaire based survey, an usual method for such a marketing research (Tull et al, 1976).

The beekeepers answered 34 questions concerning the activity carried out in the year 2011 and their responds were statistically processed and interpreted.

The main indicators used in this study were the following ones: socio-professional characteristics of the individuals selected in the sample (number of beekeepers by county, age, gender, experience in the field, training level), number of bee families, extracted and marketed honey, apiary structure in terms of the number of bee families and extracted honey production, honey yield, honey marketing, clients, income and beekeepers structure by category of income resulted from sold honey, income distribution by apiary size, income per beekeeper, bee family and kg honey, and major problems beekeepers are facing.

RESULTS AND DISCUSSIONS

Beekeepers distribution by county of origin. A number of 20 beekeepers, representing 33.3 % of the sample were selected from each of the three counties: Calarasi (CL), Ialomitza (IL) and Prahova (PH) involved in this study.

Beekeepers' distribution by age category: 3.34 % interviewed persons were younger than 30 years, 16.66 % were of 31-40 years old, 31.66 % of them belonged to the category 41-50 years, 35 % belonged to the category 51-60 years and 13.34 % were over 60 years old.

Beekeepers' gender structure showed that 83.34 % of the respondents were men and 16.66 % women, reflecting that apiculture is mainly practiced by men.

Beekeepers' training level. Most of the interviewed persons, 75 %, were high school leavers and 25 % graduated a higher education institution.

Beekeepers' experience in the field. About 20 % respondents practiced apiculture only during the last 5 years, 40 % have 6-10 years experience, 25 % have 11-15 years experience, 8.83 % have 16-20 years practice and 6.67 % are very experienced with over 20 years practice in apiculture.

All the 60 interviewed beekeepers are members of Local Beekeepers Associations and also are authorized physical persons.

Beekeepers' number of bee families and apiary average size. The 60 respondents were keeping a number of 4,044 bee families, meaning 67.4 bee families per apiary. Of the total number of bee families, 35.75 % are owned by the beekeepers from Calarasi County, 35.68 % are kept in Ialomitza County and 28-57 % in Prahova County. The highest average number of bee families was noticed in Calarasi and Ialomitza counties and it was almost equal to 72 bee families while in Prahova county the average apiary size was smaller, more exactly 57.25 bee families (Table 1).

Table 1. Distribution of bee families and average apiary size by beekeepers' county of origin

	CL	IL	PH	Total
No. of bee families	1,446	1,443	1,155	4,044
%	35.75	35.68	28.57	
No. of apiaries	20	20	20	60
%	33.3	33.3	33.4	100.00
No. of bee families per apiary	72.30	72.15	57.75	67.40

Beekeepers' distribution by apiary size pointed out that most of respondents, 54.98 %, own apiaries whose size varies between 50 and 100 bee families, 38.32 % respondents own apiaries smaller than 50 bee families and 6.70 % have more than 100 bee families (Table 2).

Distribution of bee families by apiary size varied between 0.49 % for the apiaries with 10-20 bee families and 27.77 % for the ones with 91-100 bee families. The highest share, 65.67 %, belonged to the apiaries with 50-100 bee families, 12.17 % for the ones keeping over 100 bee families and 22.16 % to the apiaries with less than 50 bee families (Table 3).

Apiaries' distribution by honey yield reflected that most of the apiaries, more exactly 61.66 %, produced less than 25 kg honey per bee family, 21.67 % apiaries achieved 26-40 kg honey per bee family and 16.67 % apiaries produced over 40 kg honey/ bee family (Tabel 4).

Table 2. Distribution of beekeepers by apiary size

	10-20 bee fam.	21-30 bee fam.	31-40 bee fam.	41-50 bee fam.	51-60 bee fam.	61-70 bee fam.	71-80 bee fam.	81-90 bee fam.	91-100 bee fam.	Over 100 bee fam.	Total
No. of beekeepers	1	2	6	14	3	10	6	7	7	4	60
%	1.66	3.33	10.00	23.33	5.00	16.66	10.00	11.66	11.66	6.70	100.00

Table 3. Distribution of bee families by apiary size

	10-20 bee fam.	21-30 bee fam.	31-40 bee fam.	41-50 bee fam.	51-60 bee fam.	61-70 bee fam.	71-80 bee fam.	81-90 bee fam.	91-100 bee fam.	Over 100 bee fam.	Total
No. of beekeepers	20	55	228	594	177	677	465	619	719	490	40.44
%	0.49	1.36	5.63	14.68	4.37	16.74	11.49	15.30	17.77	12.17	100.00

Table 4. Distribution of apiaries by honey yield

	Less than 10 kg	11-15 kg	16-20 kg	21-25 kg	26-30 kg	31-35 kg	36-40 kg	Over 40 kg	Total
No. of apiaries	9	9	12	7	8	4	1	10	60
%	15	15	20	11.66	13.33	6.67	1.67	16.67	100.00

Extracted honey production by apiary size. The highest honey production, 66.53 % was achieved in the apiaries where 51-100 bee families were kept, 19.35 % production was carried out in the apiaries with less than 50 bee families and 14.12 % in the largest apiaries where over 100 bee families were kept (Table 5).

Table 5. Extracted honey production by apiary size

	10-20 bee fam.	21-30 bee fam.	31-40 bee fam.	41-50 bee fam.	51-60 bee fam.	61-70 bee fam.	71-80 bee fam.	81-90 bee fam.	91-100 bee fam.	Over 100 bee fam.	Total
Extracted honey kg	200	1.200	4.515	14.442	5.730	17.210	11.860	15.845	19.359	14.800	105.161
%	0.19	1.14	4.29	13.73	5.44	16.36	11.27	15.06	18.40	14.12	100.00

Distribution of extracted honey by honey yield. A large variation from 4.89 % in the apiaries where average honey production per bee family was less than 10 kg and 37.03 % in the apiaries where more than 40 kg honey per bee family was noticed. The highest honey production was carried out in the apiaries where the number of bee families varied between 50 and 100 (Table 6).

Honey yield varied between 9.71 kg per bee family for the apiary category less than 10 kg and 48.58 kg per bee family for the apiary category over 40 kg. Average honey production for all the 4,044 bee families kept by the 60 interviewed beekeepers accounted for 26 kg per bee family in 2011.This is a small production performance compared to other countries reflecting important problems regarding pickings and bee family maintenance (Table 7).

Honey yield distribution depending on apiary size. In this respect, it was noticed an increasing honey yield in the larger apiaries. This indicator varied between 10 kg per bee family and 32.37 kg honey per bee family in the apiaries with 51-60 bee families and 30.20 kg honey per bee family in the largest apiaries (over 100 bee families) (Table 8).

Marketed honey was equal to obtained honey and accounted for 105,161 kg. Honey was sold in bulk to Beekeepers Local Associations and also in jars or cans to the direct clients. Honey quality was characteristic for conventional honey, just two beekeepers (3.34 %) obtained and sold organic honey having certified apiaries.

Table 6. Distribution of extracted honey by honey yield

	Less than 10 kg	11-15 kg	16-20 kg	21-25 kg	26-30 kg	31-35 kg	36-40 kg	Over 40 kg
Extracted honey (kg)	5.150	7.170	17.480	11.775	11.067	9.415	4.200	38.904
%	4.89	6.81	16.62	11.19	10.52	8.95	3.99	37.03

Table 7. Honey yield by apiary category depending on honey yield

	Less 10 kg	11-15 kg	16-20 kg	21-25 kg	26-30 kg	31-35 kg	36-40 kg	Over 40 kg	Total
Honey yield (kg/bee family)	9.71	12.96	19.20	24.37	28.82	33.98	40.00	48.58	26.00

Table 8. Honey yield by apiary size

	10-20 bee fam.	21-30 bee fam.	31-40 bee fam.	41-50 bee fam.	51-60 bee fam.	61-70 bee fam.	71-80 bee fam.	81-90 bee fam.	91-100 bee fam.	Over 100 bee fam.	Total
Honey yield kg/bee familiy	10	21.81	19.80	24.31	32.37	25.42	25.50	25.59	26.92	30.20	26.00

Average honey price varied between Lei 8.30/kg to Beekeepers′ Association and Lei 16 lei/kg to direct clients. The lowest honey price, Lei 7/kg, was registered in Calarasi County and the highest one, Lei 22/kg, was noticed in Ialomitza County. Honey price varied according to honey type between Lei 13/kg for acacia honey and Lei 7.66 /kg for rape honey (Table 9).

Table 9. Honey price by honey sort (Lei/kg)

Acacia	Linden	Rape	Sun flower	Poliflora
13.00	10.00	7.66	9.70	9.50

Beekeepers′ structure depending on the other apicultural products delivered on the market. From this point of view, 96.68 % respondents produced and sold wax, in general for exchange with combs, 20 % commercialized polen, 18.33 % propolis and 10 % bee families (Table 10).

Table 10. Beekeepers′ structure according to other apicultural products sold on the market

	Polen	Propolis	Wax	Bee families	Total
No of beekeepers	12	11	58	60	60
%	20.00	18.33	96.68	10.00	100.00

Income from marketed honey for the 60 interviewed beekeepers accounted for Lei 1,208,566 in 2011, of which 23.36 % was carried out in Calarasi County, 39.52 % in Ialomitza County and the remaining 37.22 % in Prahova County.

Average income per beekeeper was Lei 20,142.76 at sample level and by county the situation was the following one: Lei 14,061.50 in Calarasi County, the lowest income, Lei 23,883.40 lei in Ialomitza County, the highest income per beekeeper and Lei 22,483.40 in Prahova County. Over 77 % income was due to marketed honey at the best price to direct clients (Table 11).

Table 11. Distribution of income from sold honey by county

	CL	IL	PH	Total
Income-Lei	281,230	477,668	449,668	1,208.566
%	23.26	39.52	37.22	100.00

Beekeepers′ distribution by income from marketed honey. About 65 % interviewed beekeepers achieved less Lei 20,000 income in 2011, as follows: 33.33 % earned between Lei 10,001-20,000 and 31.66 % less than Lei 10,000 lei. About 3.35 % beekeepers earned over Lei 50,000 income. It was expected as the highest income to be earned by the beekeepers whose apiaries recorded the highest honey production, but the average sale price advantaged the ones who registered a smaller production. This aspect reflects that there are problems with honey marketing. Beekeepers who achieve higher honey production have difficulties to deliver it at a higher price (Table 12).

Table 12. Beekeepers' structure by income from marketed honey (Lei/year)

	Less than Lei 10,000	Lei 10,001-20,000	Lei 20,001-30,000	Lei 31,000-40,000	Lei 40,001-50,000	Over Lei 50,000	Total
No. of beekeepers	19	20	6	8	5	2	60
%	31.66	33.33	10.00	13.33	8.33	3.35	100.00
Income-Lei	128,576	283,770	138,400	280,720	222,000	155,100	1,208,566
%	10.63	23.47	11.48	23.22	18.36	12.84	100.00

Beekeepers' income by apiary size. In general, it was noticed an increased income in the larger apiaries. So, the respondents who kept 91-100 bee families earned Lei 348,340 from marketed honey, the highest income representing 20.54 % of total income at sample level, Lei 1,208,566. Also, an important income accounting for Lei 242,915 was achieved by the beekeepers who kept 61-70 bee families (Table 13).

Table 13. Beekeepers' income by apiary size

	10-20 bee fam.	21-30 bee fam.	31-40 bee fam.	41-50 bee fam.	51-60 bee fam.	61-70 bee fam.	71-80 bee fam.	81-90 bee fam.	91-100 bee fam.	Over 100 bee fam.	Total
No. of beekeepers	1	2	6	14	3	10	6	7	7	4	60
%	1.66	3.33	10.00	23.33	5.00	16.66	10.00	11.66	11.66	6.70	100.00
Inocme-Lei	1,825	13,028	48,023	160,990	62,460	242,915	122,800	157,995	248,340	150,150	1,208,566
%	0.15	1.07	3.97	13.32	5.16	20.09	10.16	13.07	20.54	12.47	100.00

Average income per beekeeper, bee family and kg honey. Income per bee family was in average Lei 298.85 in 2011, ranking between Lei 194.48 in Calarasi County and Lei 389.32 in Prahova County. In average per honey kilogram, an apiculturist obtained Lei 11.49 at sample level, with variations between Lei 10.80/kg in Ialomitza County and Lei 12.23/kg in Prahova County.

At sample level, an apiculturist earned in average Lei 20,142.76 in 2011, the highest income being recorded in Ialomitza County and the lowest one in Calarasi County. Income level depended directly on marketed honey and sale price.

Beekeepers' structure according to the means used to promote honey and other bee products. All the interviewed beekeepers mentioned that they are accustomed to promote honey and other bee products in the moment of sale during the dialogue run with each client. About 30 % respondents took part to various honey fairs, 26.66 % are accustomed to label their products and on the label they write information about their apiary, honey sorts, contact address; 25 % beekeepers answered that they were recommended by their clients to other clients, 11.66 % proceed to make advertising but this is very costing, 8.33 % are accustomed to give a visiting card to their clients, and 5 % beekeepers have a web page with all the needed information for any client (Table 15).

Inputs bought by beekeepers in 2011. Business development or maintenance involves some expenditures as shown in Table 16. Most of the beekeepers bought combs and frames, usually at exchange with wax, also feeders, bee hives and bee families, biostimulators, medicines for treatment of bee diseases, honey extractor and other specific inventory for an apiary.

Table 14. Average income per beekeeper, bee family and kg honey by county

	Income per bee family (Lei/Bee family)	Income per kg honey (Lei/kg)	Income per beekeeper (Lei/beekeeper)
CL	194.48	11.63	14,061.50
IL	331.02	10.80	23,883.40
PH	389.32	12.23	22,483.40
Total sample	298.85	11.49	20,147.76

Table 15. Distribution of beekeepers by promotion form of their bee products

	Orally at the sale place	Announcements	Web page on internet	Visiting card	From a client to another	Label on each product	Participation at fairs	Sheets	Total
No. of beekeepers	60	7	3	5	15	16	18	9	60
%	100.00	11.66	5.00	8.33	25.00	26.66	30.00	15.00	100.00

Table 16. Distribution of beekeepers by input bought in 2011

Input	No. of beekeepers	%	Input	No. of beekeepers	%
Frames and combs	58	96.66	Polen collector	4	6.66
Feeders	18	30.00	Carpenter for bee hives making	4	6.66
Bee hives	15	25.00	Bee smoker	3	5.00
Bee families	15	25.00	Beekeeper suit	3	5.00
Medicins	5	8.33	Selected bee queens	3	5.00
Tray for honey extraction	4	6.66	Sugar	2	3.33
Fork	4	6.66	Auto small trailer	2	3.33
Wire	4	6.66	Total	60	100.00

Major problems beekeepers are facing are the following ones: low honey purchasing price, expensive fuels, high tariff for rent a mean of conveyance in pastoral, high price for apiary inputs, crop spraying, bee diseases, low subsidy, self polynating crop hybrids which do not allow bees to collect nectar, climate variations with a negative impact on pickings (Tabel 17).

Beekeepers' financial resources are mainly represented by their own resources but also by subsidy received from Government and E.U. per bee family under the condition to be a member of Beekeepers Association and deliver a specific amount of honey to an authorized processor.

Beekeepers' opinion on the ways for increasing honey production. Most of the interviewed beekeepers considered that the increase of the number of bee families is the most important way to growth honey production.Also, on the 2nd position they consider that a corresponding feeding for bee families is very important for strengthen their power and enable them to collect more nectar and fill combs with honey.

Table 17. Distribution of beekeepers according to the major problems they are facing

Problem	No. of beekeepers	%
Low honey purchase price	21	35.00
Expensive Diesel	8	13.33
High tariff/km at rent of a mean of transportation	8	13.33
High input price	8	13.33
Crop spraying	7	11.66
Bee diseases	7	11.66
Low subsidy	6	10.00
Self polynating hibrids for sun flower crop	6	10.00
Climate variation with a negative impact on pickings	5	8.33
Low quality medicines	3	5.00
Lack of transportation means	2	3.33
Steelers in pastoral	1	1.66
Bureaucracy	1	1.66
Total	60	100.00

Bee hives transportation in pastoral and the use of biostimulators as well as treatments in case of disease are also extremely important for increasing honey production. The use of selected bee queens should not be neglected too because they contribute to the development of the bee family (Table 18).

Table 18. Distribution of beekeepers according to their opinion on the ways how to increase honey production

	Increased number of bee families	Use of selected bee queens	Maintenance of bee family	Application of treatments in case of diseases	Better pickings	Use of biostimulators	Additional feeding	Total
No. of beekeepers	16	5	58	48	28	13	26	60
%	26.66	8.33	96.66	80.00	46.66	21.66	43.33	100.00

Beekeepers' opinion regarding the modalities to obtain a higher price at honey deliver was the following one: about 30 % interviewed beekeepers opinated that production diversification based on a better use of acacia, linden, sun lower, rape etc pickings; 40 % interviewees answered that they have to incorporate more value added into sold product in order to sell it at a higher price; for example, they use to add nuts, almonds, sea buckthorn, comb parts, polen etc; 65 % respondents considered that more honey has to be sold to direct clients and a minim amount of honey to be delivered to beekeepers association or processors or intermediaries; 13.33 % beekeepers proposed to sell honey abroad because on the EU market honey price is higher than on the domestic market, but there is a huge bureaucracy and high taxes to get the export authorization; 6.66 % considered that organic honey could bring a better price by 20-30 % higher than the actual one for conventional honey; other 6.66 % respondents considered that they need to improve their negotiation abilities in order to get a higher price from beekeepers' association and intermediaries; 13.33 % considered that honey price should be differentiated from client to client; 25 % respondents considered that embattled honey could bring an additional price compared to honey delivered in bulk; 6.66 % considered that they need to establish their own brand as a recognition and guarantee of product quality; 13.33 % respondents considered that subsidies are not enough to cover the increased input price; 13.33 % considered that beekeepers association has to be more involved in protecting its members' interests; 10 % respondents proposed the reduction of honey import from China which has a smaller price and lower quality, the dumping price being more attractive for consumers compared to Romanian honey which is more expensive; 6.66 % considered that honey price could increase if advertising regarding honey nutritional value and importance for human consumption would be intensified and consumer will be more conscious of its importance in his diet.

Finally, 86.66 % interviewed beekeepers considered that beekeeping is a profitable activity and would like to continue this business increasing the number of bee families and supporting them to produce more honey.

CONCLUSIONS

Beekeeping is a profitable business in all the three counties taken into consideration. The average apiary size in the South part of Romania is 67.4 bee families with variations between 72 bee families in Calarasi and Ialomitza counties and 54 bee families in Prahova county.

About 55 % beekeepers own between 50 and 100 bee families, 38 % less than 50 bee families and the remaining 7 % over 100 bee families. However, apiary size is smaller than in other countries.

The honey produced by the all 4,044 bee families accounted for 105,161 kg, meaning 26 kg in average per bee family, with variations between 9.78 kg/bee family and 48.58 kg/bee family. Honey production could be increased extensively by growing the number of bee families per apiary.

Extracted honey is mainly commercialized in bulk to beekeepers association but also in cans and jars to direct clients.

Honey sale price varied between Lei 7 lei/kg in Calarasi County and Lei 22 in Ialomitza, in average accounted for Lei 11.49/kg, very low compared to honey price in the Western EU countries.

The 60 interviewed beekeepers earned Lei 1,208,566 income from marketed honey in 2011, of which 23.26 % was achieved in Calarasi county, 39.52 % in Ialomitza county and 37.22 % in Prahova county. Average income per beekeeper accounted for Lei 20,142.76, meaming Lei 11.49 per honey kg and Lei 298.85 per bee family. Therefore, income is deeply influenced by honey production and market price.

The major problems in beekeepings are related to low honey sale price, expensive fuels, high tariff for transportation in pastoral, input high price, self polynating crop hybrids and crop spraying, bee diseases, low subsidy and climate change.

To increase economic efficiency in beekeeping, apiculturists have to be focused on the growth of apiary size, production diversification and integration, honey quality, and export intensification on the EU market where demand is continuously increasing.

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CONSIDERATIONS ON THE C.E.E.CS' POSITION IN THE EU-27 POULTRY MEAT PRODUCTION AND FOREIGN TRADE

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Abstract

The paper aimed to analyze the CEECs' contribution to the EU-27 poultry livestock, meat production, export, import and trade balance in the period 2007-2009 based on the FAO Stat data, using the index, score and comparison methods. The CEECs contribute by 25.13 % to the EU-27 poultry livestock, by 22.28 % to poultry meat production, by 14.73 % to meat export and 17.74 % to meat import, by 15.45 % to meat export value and by 12.11 % to meat import value and finally by 54.38 % to poultry meat trade balance. As a conclusion, the CEECs' access to the EU has had a benefic effect on the EU poultry market and also on the coverage of consumer's need.

Key words: *CEECs, livestock, meat, poultry, trade.*

INTRODUCTION

Poultry meat is continuously increasing due to its to high quality, flavor and convenient price (Văcaru-Opris, 2007).

The EU-27 is an important poultry meat producer, exporter and importer in the world, coming on the 3rd position based on meat production, after USA, China and Brazil and consuming 12 % of global poultry meat (Pîrvuţoiu et al., 2012b, Van Horne, 2010, Windhorst, 2011).

The recent accession of 12 new member states in the EU has increased its potential in producing and exporting more poultry meat (Sanda-Costachie et al., 2011a,b).

Romania is the 2nd poultry meat producer in the area after Poland and also is placed on the 7th position as exporter (Pîrvuţoiu et al., 2012a, Popescu, 2009)

In this context, the paper aimed to analyze the development of poultry meat production and trade in the CEECs and their contribution to the EU-27 poultry livestock, meat production, export, import and trade balance, economic efficiency of poultry meat foreign trade in the period 2007-2009.

MATERIALS AND METHODS

In order to set up this paper, the following indicators have been studied: poultry livestock, poultry meat production, amount of imported and exported poultry meat, poultry meat import and export values, as well as economic efficiency of poultry meat foreign trade based on poultry meat foreign trade, export/production ratio, export/import ratio, import and export price.

In this purpose, the data provided by FAO Stat were collected for the period 2007-200 and processed using index and score methods in order to identify the position of each country within the CEECs and also comparison method for showing the CEECs contribution to the EU-27 and differences among the member states.

RESULTS AND DISCUSSIONS

Poultry Livestock in the CEECs-12 registered a decline in the period 2007-2009, by 2.21 %, from 323.56 millions heads in 2007 to 316.36 million heads in 2009. This was a similar trend like in the EU-27, where poultry livestock decreased by 6.10 % from 1,340.14 million heads in 2007 to 1,258.43 million heads in 2009.

Because livestock decline in CEECs-12 was lower than in the EU-27, the share of the

CEECs in the EU's poultry livestock has increased from 24.14 % in 2007 to 25.13 % in 2009.

The highest number of poultry is in 5 countries: Poland, Romania, Hungary, Czech Republic and Bulgaria, which had together 280.72 million heads poultry in the year 2009, representing 88.73% of the CEECs livestock and 22.30% of the EU's poultry livestock. The smallest number of poultry is in Malta, Estonia and Cyprus.

Romania comes on the second position after Poland with 83.84 million poultry in the year 2009.

In the analyzed period, poultry livestock increased in Estonia (+7.19%), Hungary (+2.84%), Romania (+1.59%), Slovakia (+6.47%) and Slovenia (+50.29%) But, in other countries it recorded a decrease: Bulgaria (-2.29%), Cyprus (-5.36%), Czech Republic (-7.91%), Latvia (-2.37%), Lithuania (-4.26%), Malta (-28.58%), and Poland (-6.73%) (Table 1) .

Table 1. Poultry Livestock in the CEECs

Country	2007		2008		2009		2009/ 2007 %	Total score	Position
	Million heads	Score	Million heads	Score	Million heads	Score	%		
Bulgaria	17.96	5	16.43	5	17.55	5	97.71	15	5
Cyprus	3.06	9	3.03	10	2.90	10	97.74	29	10
Czech Republic	26.10	4	25.49	4	24.04	4	92.09	12	4
Estonia	1.64	11	1.48	11	1.76	11	107.19	33	11
Hungary	30.30	3	29.87	3	31.16	3	102.84	9	3
Latvia	4.10	8	4.0	9	4.0	9	97.63	27	9
Lithuania	9.23	7	9.69	7	8.84	7	95.74	21	7
Malta	0.70	12	0.48	12	0.50	12	71.42	36	12
Poland	133.07	1	128.79	1	124.13	1	93.27	3	1
Romania	82.04	2	84.37	2	84.37	2	83.84	2	101.59
Slovakia	12.44	6	10.88	6	13.25	6	106.47	18	6
Slovenia	2.92	10	4.35	8	4.39	8	150.29	26	8
CEECs-12	323.56	24.14	318.86	23.86	316.36	25.13	397.77	-	-
EU-27	1,340.14	100.00	1,335.97	100.00	1,258.43	100.00	93.90	-	-
Share of CEECs	24.14	-	23.86	-	25.13	-	-	-	-

Source: FAO Stat, 2011, Own calculations.

Poultry meat Production increased by 12.62 % in the analyzed period, by around 2 % more than in the EU-27. From 1,956.3 thousand tones in 2007, it reached 2,203.2 thousand tons in 2009 in the CEECs-12, while the EU-27 poultry meat production increased by 10.70 %, from 8,734.8 thousand tons in 2007 to 9,669.6 thousand tons in 2009.

Because of the more dynamic poultry meat production, the CEECs' share in the EU-27 poultry meat production has increased from 22,39 % in 2007 to 22.78 % in 2009.

The highest performance in poultry meat production is reached in 5 countries: Poland, Romania, Hungary, Czech Republic and Bulgaria, which are raising 88.73% of the CEECs poultry livestock. Their poultry meat production accounted for 1,936.7 thousand tons in 2009, representing 87.90% of the CEECs production and 20.02 % of the EU poultry meat production.

The lowest poultry meat production is in Malta, Estonia and Latvia.

In the analyzed period, poultry meat production increased in Bulgaria (+6.13%), Estonia (+29.56%), Hungary (+8.99%), Latvia (+13.17%), Lithuania (+5.38%), Malta (+2.17%), Poland (+18.23%), Romania (+18.96%), Slovakia (+29.34%) and Slovenia (+12,62%).

If one takes into account that the poultry livestock decreased and meat production increased, this means that productivity has increased per slaughtered poultry in the CEECs.

A decline of poultry production was registered in Slovakia (-9.52%), Czech Republic (-6.27%) and Cyprus (-3.24%) (Table 2).

Table 2. Poultry meat production in the CEECs

Country	2007		2008		2009		2009/ 2007 %	Total score	Position
	Thousand tons	Score	Thousand tons	Score	Thousand tons	Score	%		
Bulgaria	97.9	5	98.4	5	103.9	5	106.13	15	5
Cyprus	27.8	9	18.4	10	26.9	9	96.76	28	9
Czech Republic	201.0	3	195.3	4	188.4	4	93.73	11	4
Estonia	11.5	11	13.1	11	14.9	11	129.56	33	11
Hungary	195.7	4	217.2	3	213.3	3	108.99	10	3
Latvia	20.5	10	23.1	9	23.2	10	113.17	29	10
Lithuania	63.2	7	65.4	7	66.6	7	105.38	21	7
Malta	4.6	12	5.0	12	4.7	12	102.17	36	12
Poland	896.4	1	729.8	1	1,059.8	1	118.23	3	1
Romania	312.1	2	316.0	371.3	2	118.96	6	2	
Slovakia	83.0	6	75.5	6	75.1	6	90.48	18	6
Slovenia	42.6	8	52.2	8	55.1	8	129.34	24	8
CEECs-12	1,956.3	-	1,809.4	-	2,203.2	-	112.62	-	-
EU-27	8,734	-	8,704.2	-	9,669.6	-	110.70	-	-
Share of CEECs	32.39	-	20.78	-	22.78	-	-	-	-

Source: FAO Stat, 2011, Own calculations.

The amount of imported poultry meat increased in the analyzed period by 6.48 %, from 313.1 thousand tons in 2007 to 333.4 thousand tons in 2009, to cover consumption need better. The CEECs imports registered a similar trend like in the EU-27 as a whole.

In 2009, the EU-27 imported poultry meat was by 9.18% higher then in 2007. So, it accounted for 1,879 thousand tones in 2009. Due to the higher rate of imported poultry meat amount in the EU-27 compared to the CEECs rate, the share of the CEECs imports in the EU′s imports declined from 18.19% in 2007 to 17.74% in the year 2009.

The main CEECs importers of poultry meat are Romania, Czech Republic, Bulgaria, Lithuania and Latvia. Their import accounted for 239.1 thousand tones in the year 2009, representing 71.71 % of the CEECs imported amount and 12.72 % of the EU-27 imported poultry meat.

The quantity of imported poultry meat increased in the analyzed period in the following countries: Bulgaria (+111.71%), Cyprus (+38.23), Czech Republic (+49.42%), Estonia (+6.35%), Hungary (+3.21%), Malta (+11.90%), Slovakia (+22.64%) and Slovenia (+3.84%).

In other countries the imported amount of poultry meat decreased: Latvia (-12.22%), Lithuania (-30.08%), Poland (-47.46%) and Romania (-1.30%) (Table 3).

The amount of poultry meat exported by the CEECs increased by 56.11%, from 240 thousand tones in 2007 to 375 thousand tones in 2009, showing that export was a dynamic activity in this area of Europe. In the EU-27 as a whole, the quantity of exported poultry meat increased by only 11.31% from 2,287.7 thousand tons in 2007 to 2,544.1 thousand tones in 2009. As a consequence, the contribution of the CEECs to the EU-27 exports increased from 10.50% in 2007 to 14.73% in 2009.

The main CEECs poultry meat exporters are Poland, Hungary, Czech Republic, Slovakia and Lithuania, whose exports accounted for 300.7 thousand tones in 2009, representing 80.18 % of the CEECs export and 11.81 % of the EU-27 exports of poultry meat.

In the analyzed interval, exports of poultry meat increased in Bulgaria (+121.69%), Cyprus (+50%), Czech Republic (+3.24%), Estonia (+50%), Hungary (+68.39%), Latvia (+33.33%), Lithuania (+11.48%), Poland (+53.36%), Romania (+75.29%), Slovenia (+1.67%). But, in Slovakia exported meat registered a decline by 8.70% (Table 4).

Table 3. The amount of imported poultry meat in the CEECs

Country	2007		2008		2009		2009/ 2007 %	Total score	Position
	Thousand tons	Score	Thousand tons	Score	Thousand tons	Score	%		
Bulgaria	22.2	5	3.36	3	47.0	3	211.71	12	3
Cyprus	3.4	12	4.6	11	4.7	11	138.23	34	11
Czech Republic	35.0	2	43.3	2	52.3	2	149.42	6	2
Estonia	12.6	9	12.2	9	13.4	9	106.35	27	9
Hungary	21.8	7	75.2	6	22.5	5	103.21	18	6
Latvia	25.9	5	24.1	7	21.7	6	83.78	18	5
Lithuania	28.0	4	26.1	4	19.3	7	69.92	15	4
Malta	4.2	11	3.9	12	4.7	11	111.90	34	12
Poland	33.5	3	16.4	8	17.6	8	52.54	19	8
Romania	100.1	1	86.4	1	98.9	1	98.70	3	1
Slovakia	21.2	8	25.9	5	26.0	4	122.64	17	7
Slovenia	5.2	10	5.7	10	5.4	10	103.84	30	10
CEECs-12	313.1	-	307.4	-	333.4	-	106.48	-	-
EU-27	1,721.0	-	1,858.8	-	1,879.0	-	109.18	-	-
Share of CEECs	18.19	-	16.53	-	17.4	-	-	-	-

Source: FAO Stat, 2011, Own calculations.

Table 4. The amount of poultry meat exported by the CEECs

Country	2007	2008	2009	2009/2007	Total score	Position
	Thousand tones	Thousand tones	Thousand tones	%		
Bulgaria	10.6	9.4	23.5	221.69	18	6
Cyprus	0.6	0.9	0.9	150.00	33	11
Czech Republic	18.5	18.1	19.1	103.24	11	3
Estonia	1.4	2.9	3.5	250.00	30	10
Hungary	21.2	35.3	35.7	168.39	6	2
Latvia	3.3	3.4	4.4	133.33	26	9
Lithuania	14.8	13.8	16.5	111.48	16	5
Malta	0.005	0.008	0	0	36	12
Poland	140.0	153.3	214.7	153.36	3	1
Romania	1.7	8.9	29.8	175.29	20	7
Slovakia	16.1	15.6	14.7	91.30	15	4
Slovenia	12.0	13.6	12.2	101.67	20	8
CEECs-12	240.205	275.208	375.0	156.11	-	-
EU-27	2,285.7	2,324.4	2,544.1	111.31	-	-
Share of CEECs-12	10.50	11.83	14.73	-	-	-

Source: FAO Stat, 2011, Own calculations.

The poultry meat import value increased in the CEECs-12 by 17.92% from 537.4 USD million in 2007 to 633.7 USA million in 2009. At the mean time, the EU-27 import value of poultry meat registered a lower increase, by 8.45%, from 4,806.7 USD million in 2007 to 5,231.1 USD million in 2009. In consequence, the share of CEECs into the EU-27 poultry meat import value increased from 11.18 % in 2007 to 12.11% in 2009.

Based on the poultry meat import value, on the top positions are 5 countries: Romania, Czech Republic, Slovakia, Bulgaria and Lithuania, whose import accounted for 471.3 USD million in 2009, representing 74.33% of the CEECs import value and 9% of the EU-27 import value.

The value of the imported poultry meat increased in Bulgaria (+192.31%), Cyprus (+35.86%), Czech Rep (+41.08%), Estonia (+8.96 %), Hungary (+17.59%), Malta (+17.21%) and Slovakia (+47.51%) and decreased in Latvia (-7.42%), Lithuania (-24.23%), Poland (-26.6%) and Romania (-0.65%) (Table 5).

Table 5. Poultry meat import value in the CEECs-12

Country	2007		2008		2009		2009/ 2007 %	Total score	Position
	USD million	Score	USD million	Score	USD million	Score	%		
Bulgaria	23.4	4	47.9	4	68.4	4	292.31	15	4
Cyprus	9.2	11	14.4	11	12.5	12	135.86	34	12
Czech Republic	86.4	2	124.1	2	121.9	2	141.08	6	2
Estonia	21.2	8	24.9	9	23.1	9	108.96	26	9
Hungary	29.0	6	46.8	5	34.1	6	117.54	18	7
Latvia	39.1	5	42.8	7	36.2	5	92.58	17	6
Lithuania	42.1	4	45.2	6	31.9	7	75.77	16	5
Malta	12.2	9	13.5	12	14.3	10	117.21	31	10
Poland	39.1	5	34.5	8	28.7	8	23.4	21	8
Romania	170.1	1	174.1	1	169.0	1	99.35	3	1
Slovakia	54.3	3	76.6	3	80.1	3	147.51	9	3
Slovenia	11.3	10	14.6	10	13.5	11	119.46	32	11
CEECs-12	537.4	-	659.4	-	633.7	-	117.92	-	-
EU-27	4,806.7	-	5,434.3	-	5,231.1	-	108.45	-	-
Share of CEECs	11.18	-	12.13	-	12.11	-	-	-	-

Source: FAO Stat, 2011, Own calculations.

The poultry meat export value increased by 32.66 % in the CEECs-12 from USD 661.5 million in 2007 to USD 877.6 million in 2009 as a result of the increased exported meat. In the EU-27, export value raised only by 8.49 % from USD 5,235.1 million in 2007 to USD 5,679.6 million in 2009. As a result, the CEECs-12 contribution to the EU-27 export value increased from 12.63 % in 2007 to 15.45 % in 2009.

Based on the poultry meat export value, the top 5 countries are Poland, Hungary, Czech Republic, Slovakia and Bulgaria, whose export accounted for USD 214.7 million in 2007, representing 81.43 % of the CEECs export value and 12.58 % of the EU export value.

The poultry meat export value increased in the analyzed period in the following countries: Bulgaria (+9.39 %), Cyprus (+40 %), Czech Republic (+39 %), Estonia (+90.24 %), Hungary (+38.52 %), Latvia (+31.46 %), Lithuania (+25.54 %), Poland (+29.88 %), Romania (+45.48 %), Slovenia (+0,56 %), but in Slovakia it decreased by 7.21 % because of the decline of the exported quantity. (Table 6).

The CEECs poultry meat trade balance was a positive one in all the analyzed years, because export value exceeded import value, showing that the CEECs are a net exporting group, with a positive influence on the EU-27 poultry meat trade balance. In 2009, the CEECs balance accounted for USD 243.9 million, being by 96.50 % higher than in 2007 (Table 7).

If in 2007, the CEECs poultry meat trade balance contributed by 28.99 % to the EU-27 balance, in 2009, its contribution was a very substantial one, accounting for 54.38 % (Table 7).

The economic efficiency of the CEECs poultry meat foreign trade is, in general, very efficient regarding export/production ratio, export/import ratio from a quantitative point of view and export/import ratio taking into account its value.

In 2009, the export/production ratio was 0.17, by 41.66 % higher than in 2007.

The export/import ratio regarding the quantity of poultry meat increased by 47.36 % from 0.76 in 2007 to 1.12 in 2009. Therefore, if in 2007 and 2008 imported amount of poultry meat exceeded the exported one, in 2009, the ratio advantaged export.

The average import price increased by 8.94 % from USD 1.90 in 2007 to USD 2.07 while the average export price decreased by 14.49 % from USD 2.90 in 2007 to USD 2.48 in 2009, which is a negative aspect (Table 8).

Table 6. Poultry meat export value of the CEECs-12

Country	2007	2008	2009	2009/2007	Total score	Position
	USD million	USD million	USD million	%		
Bulgaria	42.6	44.5	46.6	39	14	5
Cyprus	0.5	0.7	0.7	140.00	30	11
Czech Republic	42.6	56.7	46.6	109.39	11	3
Estonia	4.1	7.8	7.8	190.34	27	10
Hungary	81.0	119.4	112.2	138.52	6	2
Latvia	8.9	10.5	11.7	131.46	23	9
Lithuania	36.8	39.5	46.2	125.54	16	6
Malta	0.019	0.049	0	0	33	12
Poland	358.4	397.2	465.5	129.88	3	1
Romania	4.2	19.9	61.1	145.48	18	7
Slovakia	47.2	54.6	43.8	92.79	13	4
Slovenia	35.2	39.5	35.4	100.56	19	8
CEECs-12	661.519	790.349	877.6	132.66	-	-
EU-27	5,235.1	5,836.7	5,679.6	108.49	-	-
Share of CEECs 12	12.63	13.54	15.45	-	-	-

Source: FAO Stat, 2011, Own calculations.

Table 7. The CEECs poultry meat trade balance compared to the EU's trade balance (USD million)

	2007	2008	2009	2009/2007 %
The CEECs-12				
Export	661.519	790.349	877.6	132.66
Import	537.400	659.400	633.7	117.92
Balance	124.119	130.949	243.9	196.50
The EU-27				
Export	5,235.1	5,836.7	5,679.6	108.49
Import	4,806.7	5,464.3	5,231.1	108.45
Balance	428.400	402.400	448.500	104.69

Source: FAO Stat, 2011, Own calculations.

Looking at the figures regarding the EU-27 poultry meat foreign trade, one can see that the export/production ratio remained constant at 0.26 from a year to another in the period 2007-2009.

Compared with the CEECs export/production ratio, one can notice the efficiency of poultry meat foreign trade in the EU-27 is lower.

Looking at export/import ratio from a quantitative point of view, one can see that in the EU-27 it has a higher performance than in the CEECs. However, the increase of the EU-27's export/import ratio was only 2.27 % compared to 47.36 % in the CEECs, reflecting a more dynamic development of the exported amount of poultry meat in the CEECs.

Comparing the export/import ratio based on export and import value, one can observe that the CEECs registered a better situation compared to the EU-27.

This ratio registered higher values in the CEECs compared to the ones recorded at the EU-27 level in the analyzed period.

Also, the export/import ratio increased by 12.19 % in the CEECs, while at the EU-27 it decreased by 0.92 %.

The average import price at the EU-27 level registered just a slight increase, 0.71 %, but its level was higher in the analyzed period compared to the CEECs average import price.

The EU-27 export price was lower than the one recorded by the CEECs showing a more efficient foreign trade from this point of view (Table 8).

Table 8. Economic efficiency of poultry meat foreign trade in the CEEcs-12 and the EU-27, 2007-2009

	MU	2007	2008	2009	2009/2007
CEECs-12					
Export/Production	quantity	0.12	0.15	0.17	141.66
Export/Import	quantity	0.76	0.89	1.12	147.36
Export/Import	value	1.23	1.20	1.38	112.19
Average import price	USD/tone	1.90	2.32	2.07	108.94
Average export price	USD/tone	2.90	3.15	2.48	85.51
EU-27					
Export/Production	quantity	0.26	0.26	0.26	100.00
Export/Import	quantity	1.32	1.25	1.35	102.27
Export/Import	value	1.09	1.07	1.08	99.08
Average import price	USD/tone	2.79	2.92	2.81	100.71
Average export price	USD/tone	2.29	2.51	2.21	96.50

Source: Own calculations

CONCLUSIONS

The CEECs accession to the EU has had a benefic impact regarding poultry meat production and foreign trade.

The CEECs poultry livestock represents 22.30 % of the EU-27 poultry livestock and contributes by 22.78 % to the EU-27 poultry meat production.

In the period 2007-2009, while the CEECs share in the EU-27 imported poultry meat declined to 17.74 %, the CEECs contribution to the EU-27 exported meat increased to 14.73 %.

The CEECs have a positive influence on the EU-27 poultry meat trade balance, because of the dynamic increase of the export value compared to import value. In 2009, the CEECs contribution to the EU-27 balance accounted for USD 243.9 million, representing 54.38 % compared to 28.97 % in 2007. This was due to the increased poultry meat export, favorable export/import ratio and better average export price in case of the CEECs compared to the EU-27.

The main CEECs poultry meat producers are Poland, Romania, Hungary, Czech Republic and Bulgaria. The main CEECs poultry meat importers are Romania, Czech Republic, Bulgaria, Lithuania, Latvia, while the main exporting countries are: Poland, Hungary, Czech Republic, Slovakia and Bulgaria.

Romania has a high potential for producing poultry meat, coming on the 2nd position regarding its poultry livestock and meat production. It is on 1st position regarding the amount and value of imported poultry meat, on the 7th position concerning the exported meat amount and value. Therefore, it has to pay more attention to the increase of meat production in order to cover better the domestic market, to improve meat quality in order to grow its competitiveness for export, to decrease imports in order to protect local producers and intensify exports in order the improve poultry meat trade balance and assure a better return to the Romanian producers.

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COMPARATIVE STUDY OF THE QUALITIES OF COWS FROM MOLDOVIAN TYPE OF BLACK SPOTTED AND RED OF STEPPE BREEDS

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Abstract

There were studied the qualities of milk production of cows from Moldovan type of Black Spotted breed. There was proved that cows with three or more lactations of this type have milk production 4016 ± 163.2 kg or 74 kg more than cows of Red of Steppe breed that were in the same conditions of feeding and maintenance. Level of obtained production is less than the genetic potential of new Black Spotted type. There are proposed some measures of a fuller achievement of genetic potential in cattle growth in the household sector.

Key words: *cattle, breeds, Black Spotted, Red of Steppe, production, milk.*

INTRODUCTION

Cattle are, without doubt, one of the most important species of farm animals. In most countries of the world, Cattle farming is the main branch of the livestock sector. These animals are the main suppliers of milk and meat - first necessity food products. From cattle is obtained and the largest amount of leather and organic fertilizers. Cattle exploit large amounts of residues of plant cultivation and processing industry of agricultural raw materials. They also can be used as labor force for agricultural works, transportation of goods, especially in mountainous areas, where it can not be used agricultural machinery and trucks.

Milk and resulting products from its processing: fermented milk, cheese, butter etc., come in 90 - 95% of cattle farming. In addition to milk, beef has a rather high proportion (about 30-40 percent) of total meat consumption of the population, represents an important ingredient in the production of sausages. Milk is the most important product obtained from cattle, because of it there are processed all dairy products and it is very healthy for humans, especially for growing children, sick and old age people.

Main branch of livestock sector in Moldova is cattle farming, which aims to produce milk and meat - valuable food to feed people and raw materials for enterprises of milk and meat industrial processing (Bucătaru N, Radionov V., 2001; Chilimar, 2004).

Although the mass privatization of land and agricultural wealth was completed more than ten years ago to us, the current state did not become much better compared with 2000 year, the year of the completion of reforms. To us, cattle farming had the highest degree of development in the period 1989-1990, when there were recorded the superior indices of cattle herd, of its productivity and of global production of milk and meat. In the coming years, because of failed agrarian reforms, there was a downgrade of all agriculture branches, including cattle farming sector, particularly of economic indices of cattle milk and meat production.

Goal of the conducted research constitutes the analysis of exploitation and growth activity of cattle from Moldovan type of Black Spotted breed from 'south' subtype of Moldovan type of Black Spotted breed by appreciating the qualities of the animals from milk production farm of S.A. Nistru - Olanesti and developing the recommendations to enhance economic efficiency of milk production by using intra-rasial type of Black Spotted breed.

MATERIALS AND METHODS

Cattle are the most important species from livestock animals. Their proper feeding is a prerequisite to their health, welfare and their efficiency. Practitioners of organic agriculture will not be successful without cattle farming and manure using from these farm animals. In

practice of organic farming, farmers often face difficulties in establishing a fair ration of food for their cattle and obtaining organic products of animal origin, very much requested by consumers in all countries (Bucătaru N, Radionov V., 2001; Chilimar S., Miron I., 1999).

Fodders used in feeding of milk producing animals are grouped in: succulent fodders (green fodders, winter succulents, corn silage, the semi-silage of alfalfa, the semi-silage of meadow grass, fodder beet, fodder kohlrabi, fodder potatoes, beet noodles, molasses), fiber and roughage fodders, concentrated fodders (maize grains, barley grains, oat grains, rye grains, wheat grains (non-food), sorghum grains, peas grains, sunflower, soya beans, wheat bran, sunflower meal, soybean meal, flax meal, meat flour, fish flour), synthetic substances.

RESULTS AND DISCUSSIONS

In 2009, from the entire area of agricultural land in Stefan−Voda district 38.7% belonged to enterprises and organizations, 30% to peasant households (farmers), including 13.6% to auxiliary households (lots around the house and gardens), 0.3% to fruit associations and vegetable lots, 17.4% to other landowners. In these circumstances it is not easy to organize dairy farms with modern and advanced technologies. To note that for fodder crops cultivation there was not used arable land, which grains are used as food and fodder cereals.

Red of Steppe breed was formed in the Southern regions of Ukraine by crossing local populations of Grey of Steppe breed with some breeds from group of red breeds brought by Russian and German settlers to the mentioned territories in inadequate conditions of insurance with required quantities of fodders. This influenced the body weight of adult cows, that did not exceed 450-500 kg, milk production no more than 3-5 thousand kg per year and low qualities of meat production. From the second half of the nineteenth century, Red of Steppe breed has spread to the Southern districts of Moldova too. To increase milk production, Red of Steppe breed has been crossed in our country with cognate breeds Angheln from Germany, Red Estonian, Brown Latvian and during the years 1960 - 1974 also with Jersey breed to increase the fat content in milk. Currently Red of Steppe breed is characterized by the production of 3-4 thousand kg of milk per year, it has adapted quite well to the climatic and food conditions from the South.

Creation of Moldovan type of Black Spotted cattle. In the Republic of Moldova, in the first half of the twentieth century, there grew two breeds of cattle: Red of Steppe breed in South and Central districts, Simmental breed in the North districts and in some localities from the Center of republic. Since the early twentieth century until the early sixty, there practiced pure breed cattle farming, using to improve Red of Steppe breed bulls from Angler, Red Estonian, Brown Latvian breeds too. Improvement of Simmental cattle was practiced by pure breed methods, using valuable bulls imported from Ukraine and other parts of the former USSR (Chilimar et al., 2001; Chilimar et al., 2001).

During the sixties and until 1974 to increase the fat content of milk cows from Red of Steppe and Simmental breeds were crossed with bulls from Jersey breed, pure breed and resulting half-breeds from crossing Black Spotted and Jersey breeds. There was achieved an insignificant increase of fat content, but cow productivity remained low (2500 − 3000 kg). For these reasons, and taking into account the need to increase milk production from 1974 there was developed a program for creating Moldovan type of Black Spotted breed (Chilimar, 2003). Principled scheme of creation of Moldovan Black Spotted new type of cattle is shown in Figure 1. Milk production of cows from Moldovan type of Black Spotted breed is shown in table 1.

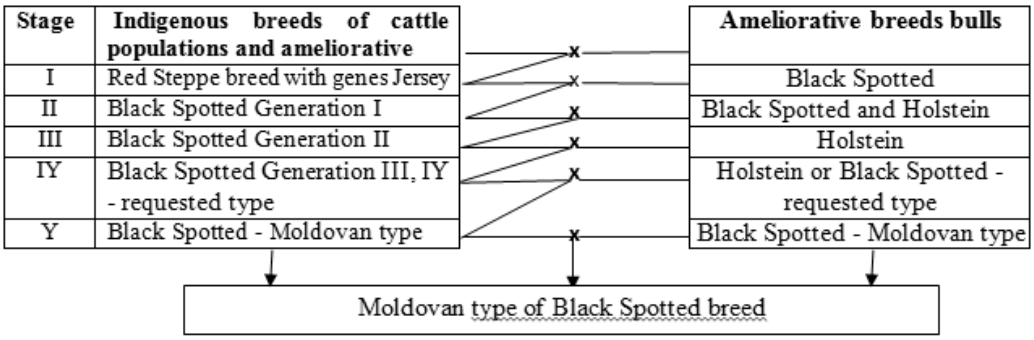

Figure 1. Principled scheme of creation the intrarasial type of cattle

Table 1. Productivity of cows per 305 days of first lactation

The share of Holstein genes breed, %	n	Milk production		± In comparison with the standard of new type of cattle	
		amount M ± m, kg	fat content M ± m, %	milk, kg	fat content M ± m, %
Subtype 'North'					
25-50	75	4249±128.2	3.55±0.010	+ 449	-0.05
51-75	604	5145±38.4	3.58±0.040	+1345	-0.02
76-87,5	1302	5224±26.4	3.58±0.002	+1424	-0.02
90 şi >	342	4578±37.7	3.59±0.004	+ 778	-0.01
Average:	**2323**	**5077±20.0**	**3.58±0.002**	**+1277**	**-0.02**
Subtype 'South'					
25-50	273	4862±72.6	3.69±0.010	+1262	+0.09
51-75	850	4634±58.9	3.71±0.010	+1034	+0.11
76-87,5	487	3938±58.7	3.74±0.007	+ 338	+0.14
90 şi >	68	3423±81.9	3.75±0.018	- 177	+0.15
Average:	**1678**	**4420±37.8**	**3.72±0.006**	**+ 820**	**+0.12**

Researches have shown that cows of the first generation obtained from crossing local populations from Red of Steppe and Simmental breeds with Black Spotted and Holstein ameliorative breeds compared to cows from local populations had higher indices of milk production and approximately the same fat content in milk. First generation cows, obtained from crossing Simmental cows with bulls of Black Spotted and Holstein breeds compared to the first generation cows obtained from crossing Red of Steppe with Black Spotted and Holstein breeds had more increased indices of milk production and almost the same fat content in milk (Chilimar S. et al., 2001; Chilimar, 2004).

With increasing the share of genes of Black Spotted breed cows from second population from crossing between Simmental x Black Spotted compared with cows from local population of Simmental breed had priority on milk production, but something less the fat content in milk. Similar results were obtained in the second generation from crossing Red of Steppe cows with bulls of Black Spotted and Holstein breeds. In this case, cows of second generation had the fat content in milk slightly lower compared to cows of local populations of Red of Steppe and Simmental.

Study of hybrid cattle with different rates of ameliorative breeds genes showed that concurrently with the increase of share of Holstein genes from 50 to 75% and from 75% to 87.5% there is a larger resemblance of new type of cattle with ameliorative breeds.

Researches' results have been obtained at cattle breeding farm of S.A. Nistru - Olanesti, where they grow cattle of Moldovan type of Black Spotted and Red of Steppe breeds. The

investigated livestock according to breed is shown in table 2, from which we see that primary breed in the analyzed household is Black Spotted, Moldovan type.

Table 2. Breed belonging of cattle from farm S.A. Nistru – Olanesti

Specification	Black Spotted		Red of Steppe		Total	
	heads	%	heads	%	heads	%
Cattle livestock	125	73.0	45	27.0	170	100
Mean age, years	6.5	100	7.7	100	6.9	100
including: 3	15	12.0	5	11.1	20	11.8
4	18	14.4	4	8.9	22	12.9
5	15	12.0	5	11.1	20	11.8
6	16	12.8	6	13.3	22	12,9
7	17	13.6	8	17.8	25	14.7
8	15	12.0	4	8.9	19	11.2
9	10	8.0	6	13.3	16	9.4
10	12	9.6	4	8.9	16	9.4
11	7	5.6	3	6.7	10	5.9

From studied livestock of cows 73.0% belongs to Black Spotted breed and only 30.0% to Red of Steppe breed. Cows aged 3-4 years from Black Spotted and Red of Steppe breeds constituted, respectively, only 26.4 and 20.0%. Most of studied cows (76.8%) of Black Spotted breed were aged from 3 to 8 years and of Red of Steppe breed 71.1%. Cows with advanced age (from 9 to 11 years) in Black Spotted breed were 23.2%, and in Red of Steppe breed 28.9%.

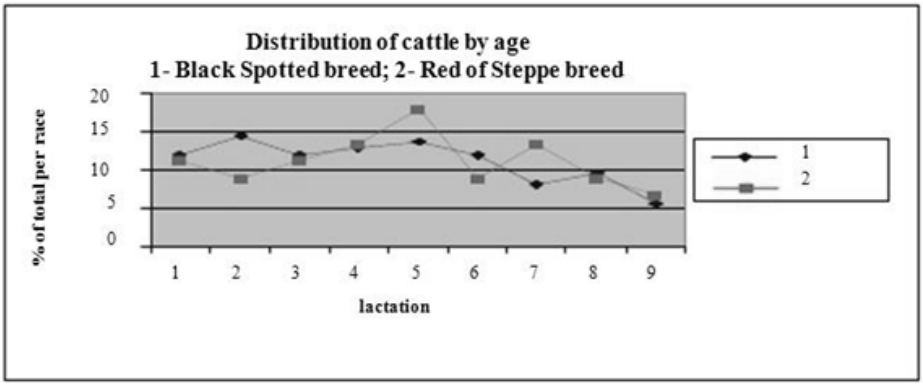

Figure 2. Cows age

It was determined that most of cows had a body weight from 350 to 550 kg and fattening condition – average, below average and weak, which influenced the delayed sowing of cows calved in winter and spring months, increase of duration of service - period up to 102 - 143 days.

Breed	Lactation	n	Body weight, kg		Breed standard,kg
			M ± m	Cv, %	
Black Spotted	I	19	468.5 ± 10.30	7.56	480
	II	32	511.0 ± 5.04	5.53	520
	III	74	521.0 ± 6.97	6.02	550
Red of Steppe	I	6	443.3 ± 5.38	6.78	450
	II	8	481.0 ± 6.16	4.09	490
	III	31	509.4± 9.40	7.98	520

Analysis of data from Table 2 shows that cattle of Black Spotted and Red of Steppe breeds have a bodyweight lower than the breed standard, what it is explained by the low level of feeding and poor quality of feed used in this household.

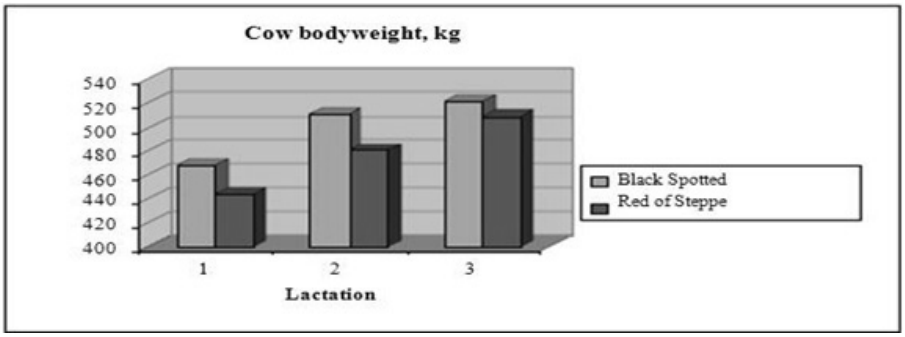

Figure 3. Dynamic of bodyweight based on lactation

From the information provided by the owners of cattle and by selective measuring of milk production from cows in the private sector, there has been established that annual milk production is within the limits of 2640-3975 kg and slightly varies from year to year.

It was found that cows from Moldovan type of Black Spotted and Red of Steppe breeds in first and second lactation had a greater difference in body weight compared with cows with three or more births.

In table 4 it is presented the information on breast resting and service-period at cows from Moldovan type of Black Spotted and Red of Steppe breeds.

Table 3. Characteristic of breast resting and service – period

Breed	Lactation	n	Duration of mammary period, days		Duration of service - period, days	
Breed	Lactation	n	M ± m	Cv, %	M ± m	Cv, %
Black Spotted	I	19	66.5 ± 9.2	49.95	113 ± 11.8	37.65
Black Spotted	II	32	54.4 ± 11.6	35.84	121.2 ±17.4	37.36
Black Spotted	III	74	61 ± 6.2	43.45	134.5 ± 14.7	62.24
Red of Steppe	I	6	63.7 ± 3.4	37.32	160 ± 8.0	29.81
Red of Steppe	II	8	58.4 ± 5.4	41.62	127 ± 12.8	67.55
Red of Steppe	III	31	54 ± 3.7	52.38	137 ± 6.8	58.8

Depending on the length of breast resting there is no significant difference between cows from Black Spotted and Red of Steppe breeds.

This parameter is within physiological norms. Duration of service-period of cows from both breeds is big and demonstrates that in both breeds cows had several repeated insemina-tions. Because of long duration of breast resting and service- period an annual quantity of milk is lost.

Characteristic of milk production, of fat content and of fat amount per normal lactation is presented in table 5 and fig. 4.

Table 4. Milk production per normal lactation

Breed	Lactation	n	Milk production		Fat content,%		Amount of fat	
			M ± m, kg	cv,%	M ± m	cv%	M ± m, kg	cv, %
Black Spotted	I	19	2756.0 ± 103.3	11.1	3.59 ± 0.02	1.63	98.9 ± 5.0	12.1
	II	32	3407.0 ± 136.1	10.4	3.52 ± 0.06	1.61	119.9 ± 6.1	12.0
	III	74	4016.0 ± 163.2	23.2	3.60 ± 0.01	1.63	144.6 ± 6.2	24.5
Red of Steppe	I	6	2456.0 ±148.9	13.7	3.65 ± 0.05	1.37	89.6 ± 2.0	16.2
	II	8	3200.0 ± 125.9	26.4	3.70 ± 0.01	2.46	118.4 ± 1.8	10.2
	III	31	3942.0 ± 77.3	23.2	3.66 ± 0.05	1.64	144.3 ± 3.0	23.6

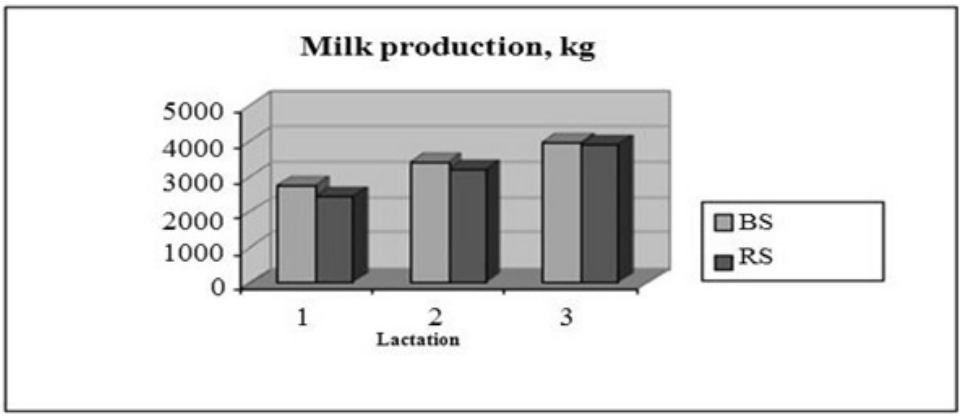

Figure 4. Milk production: BS - Black Spotted breed (Moldovan type), RS - Red of Steppe breed

From the analysis of data from table 4 and figure 4 we can see that there are only some non-essential differences between indices of milk production of cows respectively on first, second and third lactations. One can mention only some trends, because the difference between groups of studied cows, statistically speaking, is not authentic. The biggest differrence in milk production was detected at cows from third lactation (4016 kg), which exceeded by this index cows in first and second lactations.

At Black Spotted breed cows the milk production on first and second lactations is lower than breed standard respectively with 494 and 193 kg or with 14.2 - 5.4%. This can be explained by some insignificant differences in the level of nutrition of cows in lactation one and two. Milk production of cows in lactation III and more lactations is practically at the level of requirements of breed standard.

Indices of fat content and amount in milk from all cows of Black Spotted breed are inferior to breed standard, which shows the insufficient activity of selection and breeding activity of animals in the private sector.

There was too observed a greater variability in milk production at cows from pure breed (lactations II and III) compared with those from three and four generation. Variability of this index at cows with lactation II and III is also higher, compared with first lactation.

Concerning the productivity of cows from Red of Steppe breed, there was found a similar situation to cows from Black Spotted breed. Milk production of cows in lactation I and II is lower compared with breed standard, and at cows with three or more lactations the milk production was with 242 kg higher than standard. Milk fat content practically corresponds to the standard and fat amount only at first lactation cows was lower than standard and those with two-three and more lactations this index insignificantly exceeded the breed standard requirements.

All tested cows had cup and valve shaped udder, but 71% of Moldovan type of Black Spotted breed had valve shaped udder and of Red of Steppe breed only 50% had such a form. Speed of milking at cows of both breeds allows mechanized milking.

Economic efficiency of milk production is influenced by several factors, of which the most important are technology and level of production of cows which depends, primarily on animal breed. In assessing the economic efficiency of milk production there were used the indices of cows productivity from Moldovan type of Black Spotted breed compared with productivity of Red of Steppe cows (table 5).

Table 5. Economic efficiency of using cows from Moldovan type of Black Spotted breed and those of Red of Steppe breed

Indices	Obtained production from 1 cow		Black Spotted compared to Red of Steppe, ±
	Black Spotted	Red of Steppe	
Annual production of milk from a cow, kg	3695	3199	+496
Price of cost of 1 kg, lei	2.63	2.63	-
The cost of milk produced by one cow per year, lei	9717.9	8413.4	+1304.5
The amount of sold milk (80% of total)	2956	2559	+397
The cost of 1 kg of milk on sale	3.15	3.15	2.85
Cost of sold milk, lei	9311.4	8060.9	+1250.5
Profit from 1 cow per year, lei	+1571.1	+1330.7	+240.4

From the analysis of table data it can be concluded that cows growth from Moldovan type of Black Spotted breed compared to Red of Steppe breed cows provides:

- Increase of cows productivity from 3196 kg to 3695 kg or with 15.5%.
- For every cow from Moldovan type of Black Spotted breed it is obtained more production amounted to 1,304.5 lei per year.
- Increase of profit obtained from milk realization, produced by cows from Moldovan type of Black Spotted breed with 240.4 lei from each cow versus Red of Steppe cows.
- Increase of economic efficiency at milk production will be higher in the case of farm completion with animals from Moldovan type of Black Spotted breed.

CONCLUSIONS

1. On research basis it can be concluded that cows from Moldovan type of Black Spotted breed under conditions of farmer households in the private sector have satisfactory qualities of milk production, but more reduced compared with the genetic potential of the new type.

2. Taking into consideration that the fundamental object in animal husbandry, including cattle, it is increasing the products of animal origin and, above all, of milk and meat production for satisfying human food demands, it requires the creation of better conditions for achieving productive qualities of new type of cattle developed in the Republic of Moldova.

3. To achieve this fundamental aim, it is necessary to establish firms with new technologies at new firms from the private sector households, by doing the following ways:

3.1. Orientation on implementation of cattle type from Black Spotted breed with higher milk production compared to Red of Steppe breed from South region of the Republic of Moldova.

3.2. Increase of genetic potential of cattle breeds via genetic improvement of production characters, creating of new lines with superior biological potential, eventually, introduction of some breeds with worldwide recognized biological value for increasing milk production of Moldovan type of Black Spotted breed.

3.3. Improvement of operating technologies according to the practiced system in family type farms from countries with a more developed and performant animal husbandry.

3.4. Creation of a strong forage base, through intensification of forage crops and correlation of necessary areas with cattle livestock and productions that it must achieve.

3.5. Reduction of the losses of young cattle by unjustified slaughter at early ages.

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THE EFFECT OF SPIRULINA SP. POPULATION DENSITIES TOWARD REDUCTION OF BOD5 AND COD OF BEEF CATTLE SLURRY

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Abstract

Spirulina is blue-green microalgae grows abundantly on organic matters rich water bodies. In normal condition the algae uses up the organic matters effectively and improves the quality of water through decreasing of biochemical oxygen demand (BOD_5) and chemical oxygen demand (COD); while in overpopulated condition the algae may worsens water quality. This characteristic has shown that the algae can be used to improve water quality up to certain population density. However, until now this algae has not been used to improve the quality of liquid waste particularly come from beef cattle production. Therefore, the objective of study was to determine the effect of Spirulina population densities toward the reduction of BOD_5 and COD of beef cattle slurry. This study was done experimentally based on Completely Randomized Design with four treatments of population density in term of dilution factors, viz.D1 = 10^0, D2 = 10^{-1}, D3=10^{-2} and D4= 10^{-3}. Each treatment was in four replicates. The effect of the treatments were analyzed by ANOVA, and the effect among treatments were differentiated by mean of Duncan's Multiple Range Test. The result shows that Spirulina reached its maximum growth rates mostly on the 8th day. The treatments significantly ($P< 0.5$) influenced the biomass of the blue-green algae. The 10^{-2} dilution factor (D3) gave the highest biomas production, that is 0.243 g/L. The result also shows that the treatments significantly ($P< 0.05$) gave different effect toward BOD_5 and COD of beef cattle slurry. The 10^{-2} dilution factor also provided the highest reduction rate of BOD_5 and COD, i.e., 85.74% and 75.92% respectively. This findings suggest that Spirulina can be used to treat beef cattle liquid waste, in addition to obtain its biomass production.

Key words: *beef cattle, BOD5 and COD, liquid waste, Spirulina.*

INTRODUCTION

Demand for meat increases continuously with the increase of population and quality of life. This phenomena positively support the growth of local beef cattle husbandry in Indonesia. Unfortunately, the animal husbandry growth also provides some negative impacts to the environment such as water pollution due to unwise disposal of feces, urine of beef cattle either in the solid or liquid form (slurry). Beef cattle feces contains high amount of organic matter principally protein, carbohydrate and fats that represented by biochemical oxygen demand 5 days ($BOD5_5$) and chemical oxygen demand (COD). The oxygen demand can be up to 31,000 mg/L and 268,000 mg/L respectively (Cramer, et al., 1971 in Merkel, 1981). Those amounts of oxygen are required to stabilized the waste biologically and chemically. Therefore, if untreated waste water with high BOD_5 and COD discharged into aquatic environment, the waste may deplete the natural oxygen source of water and develop septic conditions (Tchobanoglous, Burton and Stensel, 2004). This can lead to shortage the aquatic organisms life.

On the other hand, there are alot of aquatic organisms that naturally can grow in the polluted water up to certain condition. One of them is *Spirullina sp*. (*Arthospira sp*.), blue-green microalgae that had been proved having an ability to grow in rich organic matter liquid wastes (Venkataraman, 1983 in Sukmaningrum et al., 1997). This microalgae use nutrient in the liquid wastes to produce its biomass. Consequently, it reduce BOD_5 and COD of wastes. The reduction may up to 95% and 80% respectively (Doke, et al., 2004). Besides, *Spirulina sp.* were also known as a supperfood due its high content of protein (65-70%), polyunsaturated fatty acids, vitamins and minerals (Hongsthong and Bunnag, 2009). Therefore, growing *Spirulina sp.* in beef cattle slurry may solve water pollution problem as well as produce biomass of the microalgae.

However, until nowadays there are no information concerning the use of *Spirulina sp*. to reduce the BOD_5 and COD of beef cattle slurry as well as to obtain its biomass.

MATERIALS AND METHODS

Spirulina sp. used in this study was a pure culture from the Institute of Ecology, while the beef slurry was obtained from the UPPL, Faculty of Animal Husbandry, Universitas Padjadjaran, Indonesia. The experiment was caried out based on Completely Randomized Design with four treatments of population density in term of dilution factors, viz. D1 = 10^0 dilution, D2 = 10^{-1} dilution, D3=10^{-2} dilution and D4 = 10^{-3} dilution. Each treatment was performed in four replicates. Before being diluted, the slurry was filtered to remove the coarse components. After that, every treated slurry (1000 ml) was put into 1500 ml bottle, where 100 unit/ml of *Spirulina sp.* was added afterward. All slurries were aerated and placed on a bench under 40 watt tube lamp. Variables measured in this experiment were temperature, pH, as well as BOD_5 and COD. The effect of the treatments were analyzed by ANOVA, and the effect among treatments were differentiated by mean of Duncan′s Multiple Range Test.

RESULTS AND DISCUSSIONS

The temperature of the slurries during the experiment was relatively constant of 27°C, which was in the range of optimal temperature for *Spirulina sp*, namely 20 – 30°C. The pH of the slurries are varies depend on the dilution factors. The highest pH was found in 10^0 dilution (8.8), followed by 10^{-1} dilution (8.0), 10^{-2} dilution (7.8) and 10^{-3} dilution (7.5). Those pH were also in the range of optimum pH for *Spirulina sp*., that is 7-9 (Couteau, 1996). Furthermore, Haryati (2008) reported that pH lower than 7 and higher than 10.5 will alter the growth of microalgae.

BOD and COD was used to measure the oxygen equivalent of the organic material in waste water that can be oxidized biochemically and chemically. Usually, the BOD/ COD ratio of waste water were 0.3 – 0.8. If the ratio is equal to 0.5 or greater, the waste can be treated easily by biological means, and if lower than 0.3 the waste may contain toxic substances or acclimated microorganisms may required in its stabilization. (Tchobanoglous, Burton and Stensel, 2004). The result shows that the BOD/COD ratios are in the range of 0.37 – 0.68, so that cattle slurry can be treated using *Spirulina sp.* without acclimation to stabilize the waste.

The growth of *Spirulina sp*. affected by dilution factors (Figure 1).

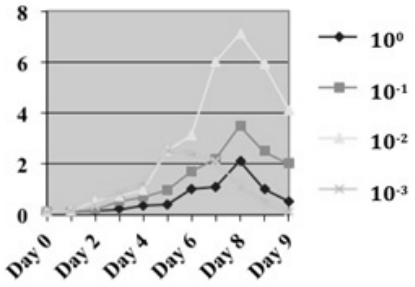

Figure 1. Growth of Spirulina sp. in beef catlle slurries (104 sel/ml)

Figure 1 showed that the highest maximum growth of the microalgae was resulted by 10^{-2} dilution (7.1 x 10^4 sel/ml), and consecutively followed by 10^{-1} dilution (3.5 x 10^4 sel/ml) and 10^0 dilution (2.1x10^4 sel/ml) and 10^{-3} dilution (1.1 x 10^4 sel/ml). In the same order, those dilution produce Spirulina sp. biomas of 0.243 g/L, 0.141 g/L, 0.081 g/L and 0.25 g/L. Slurry with 10^{-1} dilution and 10^0 dilution are slightly more alkaline than 10^{-2} dilution. This increasing pH may reduce CO_2 concentration--the main nutrien for the microalgae in the medium (Jordan, 2001). Therefore, the growth of *Spirulina* in both slurries become slower. While slurry with 10^{-3} dilution may contain less CO_2 due to high dilution. In this case, CO_2 had been used up in shorter period.

The results of BOD_5 measurement are presented on Figure 2. BOD_5 of the slurries are varying by dilutions. Beef cattle slurry with 10^0 dilution indicates the highest BOD_5 (1534.37 mg/L), followed by 10^{-1} dilution (924.94 mg/L), 10^{-2} dilution (595.86 mg/L), and 10^{-3} dilution (223.25mg/L).

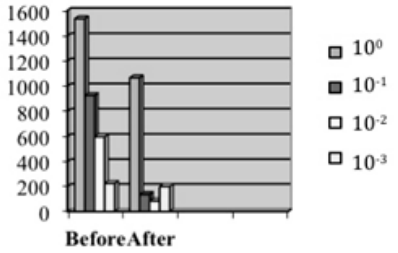

Figure 2. BOD5 of Beef Cattle Slurries differenciated between dilutions

The result of ANOVA indicates that dilutions significantly ($P< 0.05$) influence the reductions rate of BOD_5 and COD in the slurry. The results of Duncan's Multiple Range Test show that the 10^{-2} dilution significantly ($P< 0.05$) reduce BOD_5 of the slurry (85.73%) higher than 10^{-1} dilution (85.33%), 10^{0} dilution (30,56%) and 10^{-3} dilution (10.81%) as presented on Table 1.

Table 1. Reduction of BOD55 content of Beef Cattle Slurry by Spirulina sp.

Treatment	BOD55 before (mg/L)	BOD55 after (mg/L)	Reduct-ion (%)	Signifi-cant 0.05
D1	1534.37	1065.41	30.56	c
D2	924.94	135.7	85.33	b
D3	595.86	84.96	85.74	a
D4	223.25	199.12	10.81	c

Slurry with 10^{0} dilution contains more total solid than the others. Bacteria in this slurry consumed and converted organic matter into bacterial biomass and CO_2. First, the activities of bacteria increased CO_2 concentration which leads to increase the biomass productivity, and then decreased the slurry pH resulting in negative impact upon microalgal physiology. As pH decreased, CO_2 become limited and the growth of microalgae were also altered (Rifka Aisyah, 2012). Therefore, the reduction rate of BOD_5 in no dilution was lower than in other dilutions. While in 10^{-2} dilution which has lower pH and lower total solid as well as bacteria, *Spirulina sp* used the organic matter sparingly with bacteria, so that it grows faster and reach maximum growth higher than in other dilutions. Hence, the BOD_5 reduction was also higher.

BOD_5 of beef slurry resulted from 10^{-2} dilution (84.96 mg/L) lower than its standard according to the Decree of Ministry of Environment, Republic of Indonesia No. 51/MENLH/10/ 1995, that is 100 mg/L. It means that in term of BOD_5 contents *Spirulina sp*. can be use to improve the quality of beef cattle slurry.

The results of COD measurement are presented on Figure 3. Slurry with 10^{0} dilution has the highest COD (3568.3 mg/L), and then 10^{-1} dilution (1465.75 mg/L), 10^{-2} dilution (878.95 mg/L) and 10^{-3} dilution (598.18 mg/L). The result of ANOVA proves that the dilutions significantly influence COD of beef cattle slurry.

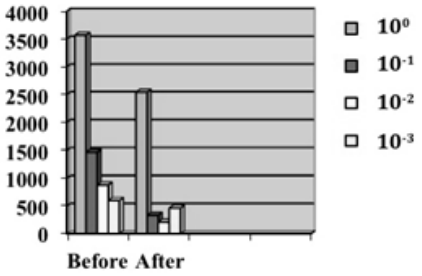

Figure 3. COD contents of Beef Cattle Slurries differenciated between dilutions

Furthermore, Duncan's Multiple Range Test results show that 10^{-1} dilution provides the highest COD reduction (77.30%), which is not significantly ($P> 0.05$) different with 10^{-2} dilution (75.92 mg/L), but significantly ($P< 0.05$) higher than 10^{0} dilution and 10^{-3} dilution (Table 2).

COD and BOD are closely related, because COD measures non biodegradable matter as well as ultimate biodegradable organics (Burke, Singh and Theodore, 2005). Therefore, the reduction rate of COD caused by the dilutions are along with the BOD reductions. According to the Decree of the Ministry of Environment of Republic of Indonesia No. 15/MENLH/10/ 1995, COD of industrial effluent is not allowed to be more than 300 mg/L. Hence, in term of COD, *Spirulina sp*. can be used to improve the quality of beef cattle slurry.

Table 2. Reduction of COD content of Beef Cattle Slury by Spirulina sp.

Treatment	COD before (mg/L)	COD after (mg/L)	Reduct-ion (%)	Significant 0.05
D1	3568.3	2540.15	28.81	b
D2	1465.75	332.64	77.30	a
D3	878.95	211.68	75.92	a
D4	598.18	462.21	22.73	c

CONCLUSIONS

The quality of beef cattle slurry can be improved biologically using *Spirulina sp*. without any acclimation. The population density of *Spirulina sp.* in term of dilution that produced the highest reduction of BOD_5 and COD is 10^{-2} dilution, with the reduction rate of 85.74% and 75.92% respectively. The dilution also produce the highest *Spirulina sp.* biomass of 0.243 g/L.

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PALM SUGAR (*Arenga pinata*) IMPLEMENTATION AS BIOSECURITY PRE-TRANSPORTATION SYSTEM ON BLOOD GLUCOSE AND GLYCOGEN ON BROILER

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Abstract

Transport of broiler chicken pen to the slaughterhouse can lead to stress. Transportation stress substantially unavoidable, but it does not mean that the negative effects of stress can not be reduced. Efforts to suppress the detrimental effects of stress alternative transportation one can do with management prior to transportation, through provision of drinking water containing palm sugar (Arenga pinata). The objective of the study is to obtained the implementation and delivery of palm sugar pre-transportation to maintain the stability of broilers performance. The study was conducted on thirty five days in the Sumedang, West Java Indonesia. The transportation routes are held from the village carried Bentar Cibitung - Cipadung - Nagrag or 2 hours, Village Bentar Cibitung - Cipadung - Nagrag - Cipadung - Tanjungkarta - Cipadung - Nagrag or 3 hours, Village Bentar Cibitung - Cipadung - Nagrag - Cipadung - Nagrag - Congeang - Nagrag or 4 hours. Each car transport speed 50-60 km/hour. Research carried out by using the method of factorial experimental design Completely Randomized Experiment 3 x 3 x 3, Statistical tests performed to the influence of variance and differences between treatments were examined using different test real honest. A factor is the transportation of animal with three replications, namely A_1 is a 2 hour transportation, A_2 is a 3 hour transportation, A_3 is a 4 hour transportation ; anti stress factor B with three replications, namely B_1 is palm sugar 2%, B_2 is palm sugar 3%, B_3 palm sugar 4%. The study found that by implementation of palm sugar in different rations and drinking water makes a decreased of blood glucose ranges from 4.72 up to 8.75 mg/dL, glycogen ranges from 120.27 up to 130.34 mg/g.

Key words: *Palm sugar, transport stress, performance production, broiler chickens.*

INTRODUCTION

Transportation of broiler chickens stricken areas of origin destination can cause stress. Factors that could be cause stress during transport, among others, mileage, time, duration, effects of temperature and humidity during transport. Stress transportation of broiler can make negative effects that cause high shrinkage of weight and slowing even cessation of body weight gain after arriving at the location of dismantling and cutting (Budinuryanto et al., 2000). There are several things can do to reduce the negative effects of transportation stress, namely: provision of adequate nutrition ration before transportation, selection of broilers before transport, density of broiler chickens in a box, setting ventilation and transport time. One function to maintain the biological safety of poultry especially broilers are ready to cut the traffic control system regarding biosecurity, so that all the way until the end of the journey is still obtained optimal performance. Transport to be one of the major factors in trading system is as well as the storage and processing of livestock (Mubyarto, 1982).

Palm sugar as a source of glucose is the energy source of non-ruminant livestock or energy for living organisms, and is absorbed in the bloodstream through the digestive tract. Disaccharide, sucrose or simultaneously absorbed more quickly as glucose and fructose when broken down in the 'brush border' intestinal mucosal cells. Some glucose goes into fuel and then to the brain cells, while the rest to the liver and muscles to be stored as glycogen or animal starch and fat cells are stored as fat. Glycogen is stored in energy source to be converted back into glucose when energy is needed more. Palm sugar release energy slowly or slow energy release, so there will be an increase or decrease in blood sugar

suddenly, too high, or too low, as the main content of palm sugar is sucrose and then glucose and fructose.

The chemical composition content of palm sugar: 9.16% moisture, 84% sucrose, 0.11% fat, 2.28% protein, 1.35% calcium, 1.37% phosphorus, and when palm sugar specificity compared with other sugars because they contain levels of higher sucrose 84%, 20% sugar cane, sugar beet 17% (Burhanuddin, 2005). Palm sugar is believed more lenient towards the stomach or indigestion and expedite metabolism (Susilowati, 2002). Excess glucose can be stored as glycogen. Glycogen is stored in the liver and a lot of muscle. Glycogen any time reformed into monosaccharides and serves as a source of energy through glycogenolysis process. Glucose and fatty acids can be absorbed by the cells of intestinal wall and blood transport it to the liver, then stored in the fatty tissue which contained in the various layers and organs. Glycogen is the stored as energy source that will be converted back into glucose when needed as energy. Some carbohydrates eventually broken down into glucose or other monosaccharides in the small intestine and transported to the liver to be converted into glycogen (Minka and Ayo, 2009).

MATERIALS AND METHODS

Implementation of the research carried out for 35 days (28 days of maintenance and day to 29-35 given treatment palm sugar in drinking water at day 35 before transportation), the amount of which as many as 162 transportation broiler chickens shaped 'straight run' Cobb Strain, obtained from the breeder in Sumedang District. Palm sugar used in this study was purchased a palm sugar makers farmer from the village of Kakas Rinondor District.

Method of administration: Rations (TN-2) given twice a day i.e. morning and afternoon during the study, brown sugar mixed in drinking water supplied ad libitum (300 mL water + 20 grams or 2%, 300 mL + 30 grams, or 3%, 300 mL + 40 grams or 4%) given for 8 days (2 days and 6 days pre-study research) then do transportation for 2 hours, 3 hours and 4 hours treatments.

Broilers were randomly marked in accordance with the treatment then included in the 24 boxes were made from a mixture of bamboo and wood box that has provided 432 broilers. Each box contained 9 broilers for 2 hour transportation research, and has three times replication, and also for 3 hours transportation and 4 hours of transportation.

Treatment of broiler chickens fed palm sugar in water, marked and observed in accordance with existing treatments. The number of broiler cut 54 heads for analyzing the glycogen (liver) 27 heads palm sugar in drinking water (each replication were taken one broiler chicks), the number of treatment studies carried as many as 18 boxes with 162 broiler chickens.

Each treatment unit then marked according to randomized treatment has worn so well treated animals had marked. Placement on the car already randomized treatment according to the serial number on the car is sort from front to back and so on until the top. Variables measured: Decrease in glycogen (mg / g), obtained from liver samples at the end of the study, and analyzed the laboratory (Peungvicha, 1998); decrease in blood glucose (mg / dl), obtained from blood samples beginning with the final blood sample. Blood samples were obtained before the beginning of transportation while final blood sample after transport. Blood sampling on the wing vein with the smallest needle (venojec) heparin the blood does not build up (Barham and Tinder, 1972.).

The study was conducted using a completely randomized design Factorial Experiments 3 x 3 (Gaspersz, 1995). A factor is the transportation of livestock, anti-stress factor B with three replications. Factor A = Transport namely: Livestock Transport A1 = 2 hours. Livestock Transport A2 = 3 hours. Livestock Transport A3 = 4 hours. Factor B = Anti Stress namely: B1 = 2% palm sugar. B2 = 3% palm sugar. B3 = 4% palm sugar.

RESULTS AND DISCUSSIONS

Influence Graph Blood Glucose Treatment of Broiler Chickens.

Results of analysis of variance (Figure 1 and Table 1) shows that the average blood glucose broilers receiving drinking water containing sugar palm (*Arenga pinata*) (G2), (G3), (G4) significantly ($P < 0.05$) decrease in blood glucose. Percentage used of palm sugar levels

in drinking water can lead to differences on blood glucose content due to long transport (T2, T3, T4) in broiler chickens.

Table 1. Test BNJ Treatment Effect of Palm Sugar on the Decrease of Blood Glucose.

Treatment	Decrease Blood Glucose	Significance 0.05
	.....(mg/dL).....	
$G2_{2.2}$	7.11	a
$G2_{2.3}$	7.91	a
$G2_{2.4}$	8.75	a
$G3_{3.2}$	5.40	a
$G3_{3.3}$	7.55	a
$G3_{3.4}$	5.96	a
$G4_{4.2}$	7.91	a
$G4_{4.3}$	5.37	ab
$G4_{4.4}$	4.72	c

Description: Values with different letters significantly (P <0.05).

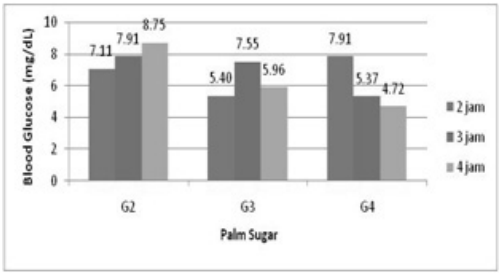

Figure 1. Grafik Influence Blood Glucose Treatment of Broiler Chickens.

Glucose found in the blood stream or blood sugar levels and serves as a provider of energy to all cells and tissues. Glycogen is stored in the liver and muscles as an energy reserve which at times can be converted back into glucose when needed. By order of the most rapidly absorbed are galactose, glucose and fructose (Hutagalung, 2004.). The decrease in average blood glucose results indicates that administration of palm sugar in water before transport can respond to heat stress during broiler chickens transportation. The results of the analysis of the effects of the use of anti-stress between the percentage of palm sugar in treatment of drinking water to the duration of transport showed no interaction (P>0.05) on the decline of blood glucose, but in single-use brown sugar in the drinking water significantly (P<0.05) due to long transportation of broiler chickens (Figure 1).

Hormones epinephrine secreted on the situation when the body is under stress or danger. Ration of carbohydrates consumed through drinking water or through excess body needs and then stored in the muscles as glycogen and the remaining hearts. Capacity is limited glycogen formation or maximum 350 mg / dl, and if in the form of glycogen accumulation has reached its limit, then the excess carbohydrate is converted into fat and stored as fat adipose (Hutagalung, 2004.).

Normal blood glucose levels in chickens is 200-250 mg / dl (Austic and Nesheim, 1990). Palm sugar or brown sugar is an alternative to loss of electrolytes in the body and the physical condition of broiler stable avoid dehydration (Burhanuddin, 2005). The results of this study that the average magnitude was decrease in blood glucose low of 4.72 mg / dl from 159.26 mg / dl before declining transportation and be 154.54 mg / dl after transport, as well as the highest 8.75 mg / dl from 145.25 mg / dl before transport, and then decreased to 136.51 mg / dl after transport in fact is much lower than the results of previous studies.

Decomposition reaction of glycogenolysis or glycogen produces glucose 6-phosphate, the 1-4 bond breaking or glycogen phosphorylase to produce glucose 1-phosphate. By catalyzed with the enzyme fosfo-glucomutase, glucose 6-phosphate can be formed from glucose 1-phosphate. Glucose 6-phosphate is converted into glucose by the enzyme phosphatase catalyzed, thus facilitating the diffusion of glucose from the blood into the cells cause an increase in blood glucose levels (May, 1999).

Table 2. Effect of Treatment of Glycogen Graph Broiler Chickens. Results of analysis of variance (Figure 2) shows that the average glycogen broilers receiving drinking water containing sugar palm (*Arenga pinata*) (G2, G3, G4) had no significant effect (P> 0.05) to glycogen. Mean glycogen is from 120.27 to 130.34 mg / g. The difference in the percentage of palm sugar in water with long transport showed no interaction (P> 0.05) on glycogen levels in the blood (Figure 2). Provision of 2% palm sugar in drinking water is good enough to overcome the transport for 2 hours and up to 4 hours.

Table 2. Test BNJ Treatment Effect Of Palm Sugar Levels in the Blood Glycogen

Treatment	Glycogen	Significance 0,05
	.....(mg/g).....	
$G2_{2.2}$	120.27	a
$G2_{2.3}$	125.70	a
$G2_{2.4}$	130.34	a
$G3_{3.2}$	120.43	a
$G3_{3.3}$	128.97	a
$G3_{3.4}$	126.84	a
$G4_{4.2}$	121.19	a
$G4_{4.3}$	125.84	a
$G4_{4.4}$	127.02	a

Description: Values with the same letter are not significantly different (P> 0.05).

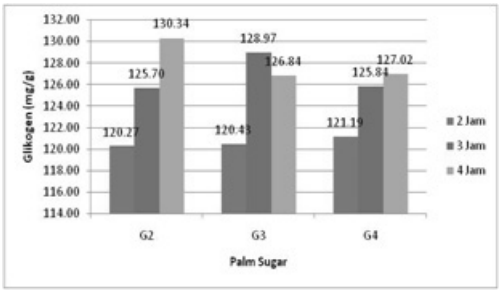

Figure 2. Effect of Treatment of Glycogen Graph Broiler Chickens.

During avian activity, primary energy for muscle contraction are glucose and fatty acids from the blood (Wirahadikusumash, 1985). If the muscles run out of the main energy source in the form of carbohydrate energy reserves in the form of glycogen or intramuscular muscle glycogen utilized. Muscle glycogen is useful as an indicator to evaluate the fatigue or stress on livestock. Plasma glucose is one that is commonly used as an indicator of physiological stress in transportation. The stress of transport has been reported cause an increase in plasma glucose concentrations because the liver glycogen breakdown (Kannan et al., 2000). Increased plasma concentrations of glucose mainly because glycolysis is associated with increased of catecholamines and glucocorticoids released during stress transport (Tadich et al., 2005).

Glycogen is formed through trajectory glycogenesis then stored in the liver and in the muscles, is used as a fuel reserve and outlined through the process of glycogenolysis. On the condition of cattle are stressed, circular system can not carry oxygen and glucose into skeletal muscle at speeds sufficient to meet the needs of such a high muscle to ATP, in the circumstances described glycogen quickly through the process of glycolysis to form lactic acid and ATP as an energy source high (Lehninger, 1994; Aberle et al., 2001).

CONCLUSIONS

Palm Sugar is a source of antioxidants that supplementation into pre-transport drinking water systems can reduce the stress level biosecurity of broilers after transport. Utilization of palm sugar in drinking water 2, 3 and 4 per cent to cope with changes to: blood glucose decreased from 5.37 to 11.13 mg / dL, and serves as an energy reserve or glycogen from 120.27 to 130.34 mg / g, when purposes of cell glucose increases followed the process glyconeolysis decreasing glycogen reserves in the liver and muscle of broiler chickens on a long transport 2, 3 and 4 hours.

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PARTIAL RESULTS REGARDING THE EXPLOITATION AND MORPHO-PRODUCTIVE TRAITS FOR ALPINA BREED GOATS IN SOUTH OF THE COUNTRY

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Abstract

Increasing of goats population, in the last ten years, conduct to the growth of mdern capriculture, based on massive imports of performant dairy goats from Alpine and Saanen breeds. Expectations towards a qualitative and quantitative production led to pretty important imports for specialized breeds like the Alpine, from communitary states, during the last 6 years. This study presents the results of some research started since the last semester of the previous year on some goat livestock from Alpina breed, livestock which was brought to the Garbovi farm, Ialomita county. The livestock presents the following morpho-productive traits, such as: live weight 59.81±0.29 kg, back height 69.35±0.29 cm, croup height 70.36±0.18 cm, oblique body length 75.9±0.20 cm, chest width 19.69±0.19 cm, anterior croup width 20.52±0.2 9cm, thoracical perimeter 91.31±0.38 cm, cane perimeter 9.12±0.04 cm, resulting a dolicomorphe body structure. The medium milk production in is of 1.90±0.26 l/head/day during the stalling months November-February, with average of November 61.07±1.7 l, December 78.56±2.07 l, January 92.34±2.93 l, and February 52.38±1.38 l, only during the first 120 days, and the chemical composition of the main constituent parts: Dry matter nonfat 9.3%, protein 3.53%, fat 4.02%, a significant quantitative and qualitative gain of goat milk especially when the local breeds don′t yield them.The females prolificity is of 146%, being a lot below the breed average over 130% in comparison with the average proven by the breed but also in comparison with the unameliorated breeds, this aspect being is a basical element in selection, next to the milk quantitative aspect. The gain recorded for goat youth over the 90 days is comprised between ADG1 0-30 days is 124.15 g/day, ADG2 30-60 days 209.78 g/day, ADG3 60-90 days 127.32 g/day of males and 106 g/day, 191 g/day, 115 g/day of females, during the whole period which was much over the average of Carpathian breed, especially milk ones, which have typical dolicomorphe structure but with a great productive and somatometrichal variability.

Key words: *goats production, goats somatometers, body structure , milk production.*

INTRODUCTION

Romanian capriculture record a positive evolution, on the last four decades, because of market demands. In Romania, goat was considered as "the poor′s cow", being also a survival niche of the poor families (Vlad et al., 2009).

In this context, the last ten years was considered very important for modern capriculture because of the big imports from most performants dairy milk goats breeds, like Alpine and Saanen. At that moment, we put the foundations of specialized goat farms, in the direction of exploitation for dairy milk.

For more efficiency, for increasing the milk production, but also because of European founds, farmers import individuals from Saanen and Alpina breed from different countries of E.U.

In this way it was supported the initiative of small farmers and goat milk producers participating in both the development of a new branch in the field of manufacturing as well as the development of a new sub-branches specialized in dairy goat exploitation (not mixed, milk and meat).

This study is a part of an ample research concerning Alpina goats breed imported in farm A.F.Cojocaru Country-Gîrbovi-Ialomita.

MATERIALS AND METHODS

The entire herd of goats that were subject to investigation of the livestock stood at the first lactation and the actual imported consisted of young females at first gestation. The biologic material are represented by 85 goats, Alpine breed, imported in summer of 2012 AF Cojocaru farm, Gîrbovi village, Ialomita County.

The main measurements were aimed on highlighting the morpho-productive characterristics regarding performances, especially of especially for milk production, but also on how we can improve other characters of our cultural goats breed Carpatina, whose productions still remain modest. Observations were focused on aspects of conformation, primary production-especially milk quantity and quality, basic reproductive indices and growth process dynamic in young goats. For milk production, as the main objective, we made the control of production, based on the control made only in the first four months, or 120 days, of lactation, during lactation because we hope to finalize the lactation in this summer.

Body measurements were start at the birth age, from October last year. We determined live weight, back height, croup height, length and width of the head, oblique body length, chest width, anterior croup width, thoracic perimeter and canon bone perimeter; body weight of young goats from birth to 3 months old to calculate average daily gain; prolificacy; evolution of milk production on the first four mounts (lactation curve); milk chemical composition fiber determination regarding length and finesse.

RESULTS AND DISCUSSIONS

The biologic material is represented by Austrian Alpine goat breed, at first lactation. It was analyzed through productive performances in actual condition of exploitation.

Somatometrical measurements

The aim of main body size measurements, analyzed in population, was to highlight corporal conformation: live weight 59.81 ± 0.29 kg, back height 69.35 ± 0.29 cm, croup height 70.36 ± 0.18 cm, oblique body length 75.9 ± 0.20 cm, chest width 19.69 ± 0.19 cm, anterior croup width 20,52 ± 0,29 cm, thoracical perimeter 91,31 ± 0,38 cm, cannon bone perimeter 9,12 ± 0,04 cm. All this measurements are the most important characters, with a large grade of variability, as we can see in graphic, representing the morphological body type and offer us the opportunity to calculate bones index (fig.1).

Injury as measured by the number of goats imported from first lactation were found aspects that highlight the type of conformation of the population, if they fall into certain characteristics of the breed on the main body indices determined from the main somatometries for body indicators.

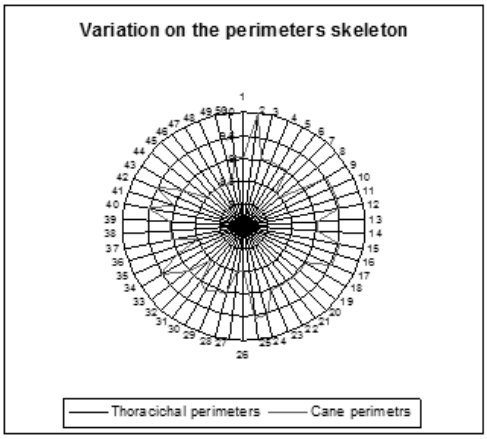

Figure 1. Variation on the perimeters scheleton.

This indices offer us information about corporal conformation, like side body format index (over 110%) and bones index (9.9%). This parameters values reveal a dolicomorphe conformation, typically for this type of breed, with a very good bone structure, but with a large grade of variability due tu the origins of individuals (it cames from different farms). The prolificity of females of goats Alpine was 146%, being a lot below the breed average over 130% in comparison with the average proven by the breed but also in comparison with the unimproved breeds, for example Carpatine breed, this aspect being also a basical element in selection, to next to the milk quantitative aspect. This is another important character fol local bred breeding (Tafta et al., 1993; Vlad et al., 2012).

Dynamic growth process. Its evolution in the young goat reviewed showed that since the birth byproducts Alpina breed have a very large

sexual dimorphism differences in average approx. 18% between the sexes (Vlad et al., 2011; Vlad et al., 2012). The gain recorded for goat youth over the 90 days is comprised between ADG1 0-30 days is 124.15 g/day, ADG2 30-60 days 209,78 g/day, ADG3 60-90 days 127.32 g/day of males and 106 g/day, 191g/day, 115 g/day of females, during the whole period which was much over the average of Carpathian breed. (Figures 2 and 3) (Tafta et al., 1993; Zamfir, 2003).

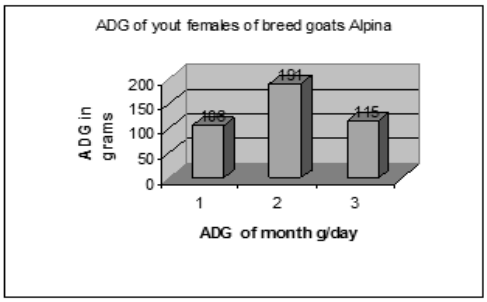

Figure 2. ADG of yout females.

Top or best growth recorded in both sexes of young goat is in the 2nd month of life, thanks to a good potential female lactogen and precocity of youth work and other feed consumption due to early development of enzyme equipment. Comparing with Carpatina breed or with half blood Saanen, we can say that, the average daily gain is good, with same evolution, recording the highest value in the second month, having a slow involution then because of weaning stress, phenomenon justified by a good precocity (Figure 3) (Tafta et al., 1993; Vlad et al., 2012).

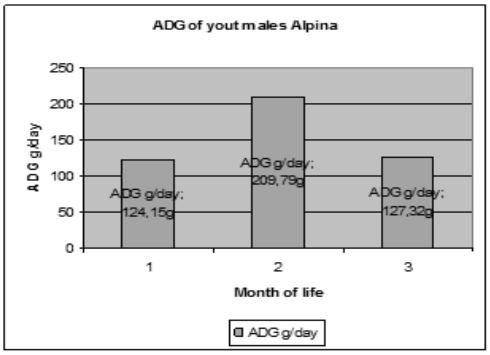

Figure 3. ADG of yout males

Milk production.

The medium milk production is 2,19 l/head/day during the stalling months November-February, with month average of: November 61.07 ± 1.7 l., December 78.56 ± 2.07 l., January 92.34 ± 2.93 l, and February 52,38 ± 1.38 l, only during the first 120 days (Figure 5). This represents the most important character that must be followed during lactation. The character was analyzed on the first half of lactation, aspect who can demonstrate only the beginning of lactation and also the evolution of lactation curve, fact who reveal the high potential of Alpine goats, but who, from some reasons (poor management), have, in this condition, a decreasing curve of lactation after 4 months. Other motivation can be lack of knowledge in dairy goats exploitation and a poor nutrition (Figure 6).

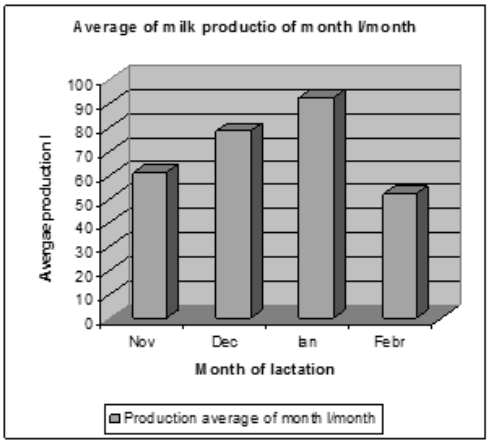

Figure 4. Milk production on month.

Lactation curve.

In this breed, the lactation curve must be slower, to decrease slowly, and after the sixth or seventh month of lactation, to record app. 70 l/head/month (Figure 5).

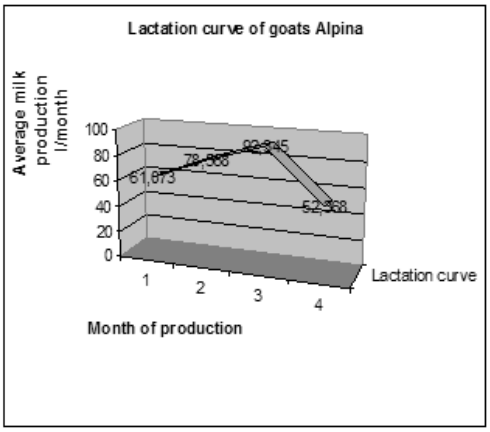

Figure 5. Lactation curve of milk in winter.

The main component elements of goat's milk were determined on the control day (Figure 6). The milk chemical composition during the lactation of may month was: non-fat dry matter 9.33%, protein 3.54%, fat 4.03%, representing a good qualitative production for winter period. On the analysis of histograms for milk production both in the morning and in the evening of the first month of production, data are normally distributed, however, spotlighted differences of the extreme series allow a better selection with animals distribution in stocks but also with specific production (Figure 6) (Vlad et al., 2011; Vlad et al., 2012).

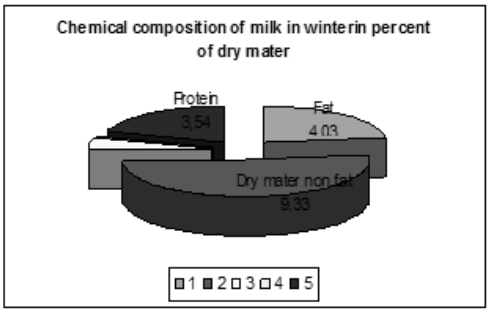

Figure 6. Chemical composition of milk in winter.

CONCLUSIONS

Our analysis, on primiparous Alpine goat females, imported in this farm from Gîrbovi village, reveal these:

The female primiparous livestock presents the following morpho-productive traits, such as: live weight 59.81 ± 0.29 kg, back height 69.35 ± 0.29 cm, croup height 70.36 ± 0.18 cm, oblique body length 75.9 ± 0.20 cm, chest width 19.69 ± 0.19 cm, anterior croup width 20.52 ± 0.29 cm, thoracical perimeter 91.31 ± 0.38 cm, cannon bone perimeter 9.12 ± 0.04 cm, resulting a dolicomorphe body structure. We determinate some indices like side body index (over 110%) and bones index (9.9%), who demonstrate a typically dolicomorphe conformation for dairy goats breeds. Also, we observe, a large grade of variability, in analyzed livestock, because of the different origins of goats (same breed but from different exploitation).

The prolificity of females of Alpine breed, was 146%, being a lot below the breed average over 130%.

The gain recorded for young goats over the 90 days is comprised between ADG1 124.15 g/day, ADG2 is 209.78 g/day, ADG3 is 127.32 g/day of males and 106 g/day, 191 g/day, 115 g/day of females, during the whole period which was much over the average of Carpatina breed performances.

The average milk production was at 2.19 l/head/day during the stalling months November-February, with monthly averages at: November 61.07 ± 1.7 l, December 78.56 ± 2.07 l, January 92.34 ± 2.93 l, and February 52.38 ± 1.38 l, only during the first 120 days. This production, recorded only on first 4 months of lactation, demonstrate a good start of lactation curve evolution, but who decrease instantly after this 4 months because of defectuous management. This production with only the first 4 months of lactation curve shows an evolution of lactation with a good debut performance as Alpina race but for some reason of mismanagement and also because of lack of knowledge regarding dairy goat breeds.

All this characteristics, of imported goats breeds, are superior comparing with Carpatina breed, but also with a higher requirements regarding exploitation, breeding, and, most important-feeding condition, totally different from our Carpatian breed requirements.

ACKNOWLEDGEMENTS

This work represents a synthesis of a survey and the analysis still from the second part of the

previous year on the performance of morphologically productive livestock of Alpin goats breed imported in Ialomita County, Gîrbovi village. On behalf of the collaborators, we thank to Mr. Cojocaru Ovidiu as farmer, for his support and cooperation

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USING FEED ADDITIVE (COMPLEX SYMBIOTIC) IN STIMULATING FEEDING OF BEES

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Abstract

Honey bees use as food nectar, honey, and pollen and bee bread. They collect nectar and pollen on flowers, that process in food-honey and bee bread. Food provides bees body with energy due to carbohydrates, proteins, enzymes, lipids, vitamins, minerals. One of the methods by which it is possible to increase growth and productivity of bee families is early stimulating nutrition. The purpose of research is to use feed additive Premix Bionorm P (symbiotic complex) in bees′ development. It was found that the optimal dose of feed additive Premix Bionorm P (symbiotic complex) is 100 mg / l sugar syrup administered in spring time to boost bee families using one liter over every 10-12 days, beginning with the first days of April until early harvest of white acacia. It was found that the use of feed additive (symbiotic complex) in the nutrition of bees′ families stimulates increasing power of families from 29.2 to 39%, queens′ prolificacy and capped juvenile with 41.9 to 50.2%. It is reasonable the stimulation of bee families in the spring when there is no natural harvest, which ensures honey increase production by 43.2%.

Key words: *bee′s families stimulates, feed additive, honey bees, sugar syrup.*

INTRODUCTION

Honey bees use as food nectar, honey, pollen and bee bread. They collect nectar and pollen on flowers, that process in food - honey and bee bread. Food provides the bees body with energy due to carbohydrates, proteins, enzymes, lipids, vitamins, minerals.

Bee's family needs a considerable amount of food - honey and bee bread for vital processes. Strong family during the year consumes 90 kg honey during the winter rest - about 10 kg and during active vital period - spring, summer and autumn - about 80 kg (for life maintaining of adult individuals, feeding larvae, wax secretion, energy consumption during the flight and processing nectar in honey).

In cases where family amount of food reserve is insufficient, bees must be fed. For juvenile growth stimulation there is using sugar syrup with concentration of 50% (1 kg sugar 1 liter water) (Eremia, 2009; Буренин et al., 1977; Кривцов, et al., 2000).

When artificial supplement was tested in production circumstances it showed its superiority over sugar syrup used as a nutrition stimulant, what increases the growth of juveniles in the absence of harvest during the preparation of bee families for main harvesting (Билаш, 2000).

One of the methods by which it is possible to increase growth and productivity of bees' families is early stimulating nutrition.

The purpose of research is to use feed additive Premix Bionorm P (symbiotic complex) in bees' development.

MATERIALS AND METHODS

To achieve the aim of the experiments, as an object of investigations served bees families, of Carpathian breed from apiary 'Albinarie' Straseni district, Republic of Moldova.

There were formed six groups of bees' families to study the influence of feed additive Premix Bionorm P (symbiotic complex) on growth, development and productivity of bees' families. Bees' families of I control group I had developed during spring time using honey reserves of the nest without additional feeding, the bees families of control group II-II were given one liter of pure sugar syrup , bees' families of experimental group III were administered every 1 liter of sugar syrup and feed additive 1:1 (symbiotic complex) Premix Bionorm P by 50 mg feed additive, group IV - correspondingly 100 mg / l of syrup, group V - 150 mg / l of syrup, group VI - 200 mg / l of syrup.

Bees' families were fed on 22.04.11, 05.07.11, 19.05.11 using one litre of syrup.
The syrup was prepared as follows: Water was heated until boiling, and then the sugar had been added in a 1:1 ratio to 1 liter of water with one kilogram of sugar, the solution was stirred until the sugar was completely dissolved. When the syrup had cooled to a temperature of 30 C there was added feed additive (symbiotic complex), which was dissolved in 80-100 ml of water and was stirred together.
To determine the influence of feed additive on growth and productivity of bees' families during the active season the bees' families control was made over every 12 days until main harvest of white acacia.
There were studied productive characteristics of the bees' families such as: strength, number of capped juveniles and honey productivity.
The data obtained were processed by statistical variation method after Меркурьева, 1970; Плохинский, 1971 and using computer programs Microsoft Excel.

RESULTS AND DISCUSSIONS

The results of the research have shown at the time of experimental groups forming (01/04/2011) the bees family power was from 6.0 to 6.67 areas between honeycombs populated with bees.
While testing bees families on 22.04.2011, it was found that the strength of families varied from 6.33 (group II) and 7.67 areas between honeycombs populated with bees (group VI) (Table 1). There was found in bees nest from 63.33 hundred of capped cells (group II) to 83.67 (group VI), and food reserve ranged on average from 2.0 kg (group I) and 6,67 kg of honey (group V).

Table 1. The status of bees′ families on 22. 04. 2011

Group	The power of bees families, areas between populated honeycombs	Capped juveniles, hundred cells	Honey, kg
I. Honey (control I)	6.67 ± 0.667	77.33 ± 10.651	2.0 ± 0.577
II. Sugar syrup (control II)	6.33 ± 0.882	63. 33 ± 4.702	4.0 ± 1.000
III. Sugar syrup + feed additive (symbiotic complex), 50 mg/l de syrup	6.97 ± 0.465	79.57 ± 8.347	3.24 ± 0.432
IV Sugar syrup + feed additive (symbiotic complex)., 100 mg/l of syrup	7.00 ± 0.001	80.00 ± 12.342	2.67 ± 0.667
V. Sugar syrup + feed additive (symbiotic complex), 150 mg/l of syrup	7.00 ± 0.527	68.67 ± 11.289	6.67 ± 0.888*
VI. Sugar syrup + feed additive (symbiotic complex), 200 mg/l of syrup	7.67 ± 0.33	83.67 ± 13.383	6.0 ± 1.00*

Next control on 07/05/2011 there was found that the best had developed bees families what received syrup feed additive (symbiotic complex). Compared with the control group (I) bees families in groups III-VI had higher strength with 1.0 to 2.34 spaces between honeycombs populated with bees and those that received sugar syrup-0.34 spaces between honeycombs populated with bees (Table 2).
The highest number of capped juveniles was found in bees families that received feed additive (symbiotic complex) 100 mg / l of syrup-191.33 hundred cells, or with 59 hundred cells more than the control group (II).
At the control made on 19.05.2011 at the beginning of the main harvest of white acacia there was found that families of group IV had increased their power and it was on average of 13.33 spaces between combs populated with bees or higher with 26.9-37.8% than in control groups I and II (figure 1).
The maximum number of capped brood was in bees′ families of group IV-199.33 hundred cells, with 41.9 hundred cells more than the control group I and respectively 66.66- in II control group, or with 41.9-50.2% more than those in both control groups (Table 3).

Table 2. The status of bees′ families on 7. 05. 2011

Group	The power of bees families, areas between populated honeycombs	Capped juveniles, hundred cells	Honey, kg
I. Honey (control I)	8.33 ± 1.453	152.67 ± 26.235	2.67 ± 0.333
II. Sugar syrup (control II)	8.67 ± 1.202	132.33 ± 21.835	4.33 ± 0.822
III. Sugar syrup + feed additive (symbiotic complex), 50 mg/l de syrup	9.45 ± 1.324	167.29 ± 5.628	3.89 ± 0.746
IV Sugar syrup + feed additive (symbiotic complex)., 100 mg/l of syrup	10.0 ± 1.000	191.33 ± 6.227	2.0 ± 0.577
V. Sugar syrup + feed additive (symbiotic complex), 150 mg/l of syrup	9.33 ± 0.333	183.00 ± 4.163	5.0 ± 0.577*
VI. Sugar syrup + feed additive (symbiotic complex), 200 mg/l of syrup	10.67 ± 0.882	177.33 ± 8.838	4.33 ± 0.333*

The significance of differences between average: (I-V), (I-VI) *B=0.95

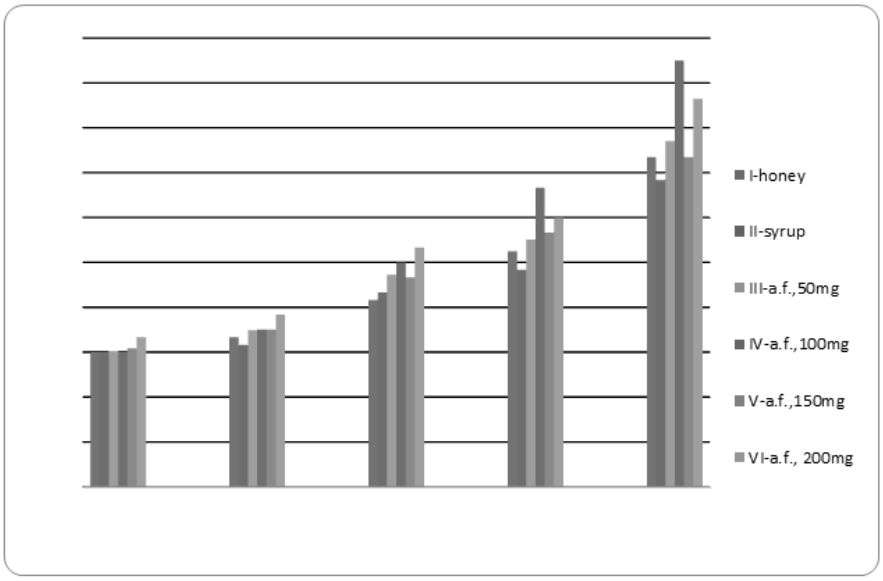

Figure 1. Dynamic of growth of bees′ families′ power, areas between populated honeycomb with bees

Table 3. The status of bees′ families at the beginning of white acacia harvesting (19. 05. 2011)

Group	The power of bees families, areas between populated honeycombs	Capped juveniles, hundred cells	Honey, kg
I. Honey (control I)	10.5 ± 2.500	140.5 ± 13.59	2.00 ± 1.000
II. Sugar syrup (control II)	9.67 ± 1.202	132.67 ± 10.806	3.33 ± 0.667
III. Sugar syrup + feed additive (symbiotic complex), 50 mg/l de syrup	11.02 ± 0.453	155.69 ± 2.637	3.02 ± 0.459
IV Sugar syrup + feed additive (symbiotic complex), 100 mg/l of syrup	13.33 ± 1.856	199.33 ± 12.143*	2.33 ± 1.333
V. Sugar syrup + feed additive (symbiotic complex), 150 mg/l of syrup	11.33 ± 0.333	169.33 ± 1.856*	3.33 ± 0.333
VI. Sugar syrup + feed additive (symbiotic complex), 200 mg/l of syrup	12.0 ± 1.732	173.33 ± 7.965	3.33 ± 0.333

The significance of differences between average: (I-V), (I-VI) *B=0.95

Honey reserve of bee families ranged from 2.0 kg (group I) to 3.33 kg (group II, V and VI), confirming that this time around the apiary, in the useful radius of bees flight was not productive for harvesting of families maintenance and bees had consumed family reserve of honey.

Maximal prolific queens were found in group IV, in this period it was 1661.1 eggs during 24 hours, in group VI-1444, in group V-1411.1 and in group III-1297.4 eggs. In control group I bees families were not additional fed, but bees used honey reserves, and queens prolificity was -1170.8, while those in group II that were fed with pure syrup sugar, their prolificity was-1105.6 eggs during 24 hours, or with 5.6% lower than in control group I.

Queens of the experimental groups had the prolificity with 126.6 (group III)-490.3 (group IV) eggs during 24 hours, or with 10.8 to 41.9% higher than in control group I and with 191.8-555.5 eggs during 24 hours, or with 17.3-50.2% higher than control group II.

Before extracting honey from white acacia (06/08/2011) bees′ families had the power in control groups I and II from 13.67 to 14.7 areas between honeycombs populated with bees. Better development had the bees families of group IV, that received feed additive (symbiotic complex), 100 mg / l of syrup, with an average of power of 19 spaces between honeycombs populated with bees with 4.3 to 5, 33 spaces between combs populated with bees or with 29.3 to 39.0% more than in control groups I and II (Table 4).

Table 4. The status of bees′ families before recolted the honey to 8. 06. 2011

Group	The power of bees families, areas between populated honeycombs	Capped juveniles, hundred cells	Honey, kg
I. Honey (control I)	14.7 ± 4.41	113.3 ± 12.72	24.9 ± 8.396
II. Sugar syrup (control II)	13.67 ± 2.728	139.0 ± 10.693	25.0 ± 2.266
III. Sugar syrup + feed additive (symbiotic complex), 50 mg/l syrup	15.4 ± 3.426	161.4 ± 11.22	29.3 ± 2.523
IV. Sugar syrup + feed additive (symbiotic complex), 100 mg/l syrup	19.0 ± 2.517	182.0 ± 21.794	35.8 ± 4.073
V. Sugar syrup + feed additive (symbiotic complex), 150 mg/l syrup	14.7 ± 0.88	152.7 ± 12.02	28.5 ±4.320
VI. Sugar syrup + feed additive (symbiotic complex), 200 mg/l syrup	17.3 ± 1.45	122.0 ±7.21	34.7 ± 6.570

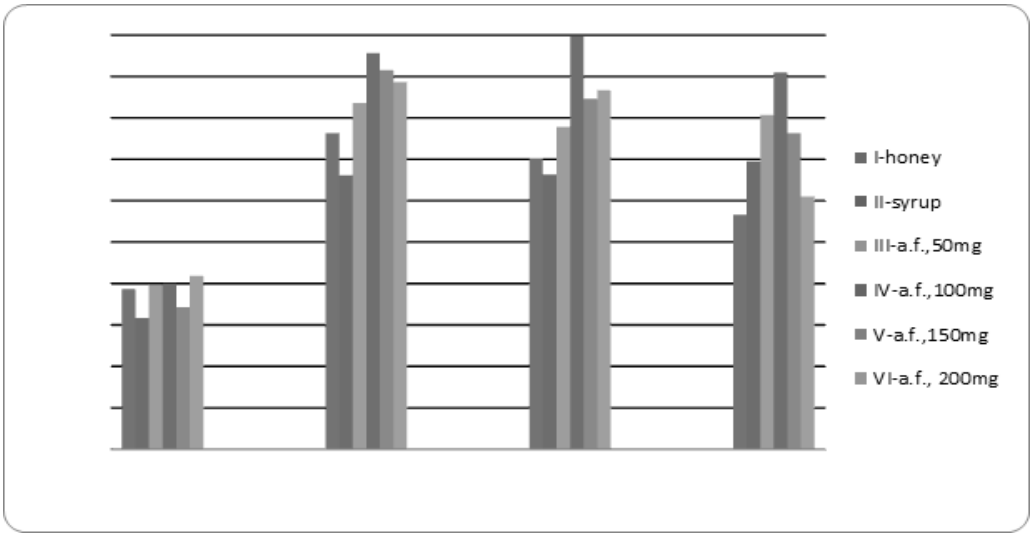

Figure 2. Dynamic of capped juveniles, hundred cells

Increasing the amount of preparation per liter of syrup 150 or 200 mg did not significantly affect the growing power of bees' families.

The highest number of capped juvenile during this period had grown the bees families of group IV-182 hundred cells (Figure 2), or more by 60.6% compared to the control group I and 30.9% compared to control group II.

There had been deposited 24.9-25 kg of honey by bees families of the control groups from White acacia (Figure 3). The maximum amount of honey had stored bees families of group IV, which received feed additive (symbiotic complex), 100 mg / l of syrup-35.8 kg or more by 10.8 kg (43.2%) compared to control group, in group III-29.3 kg, or more by 17.2%, group V-28.5 kg, or more by 14%, in group VI respectively 34.7 kg or more by 38.8% than in the control groups.

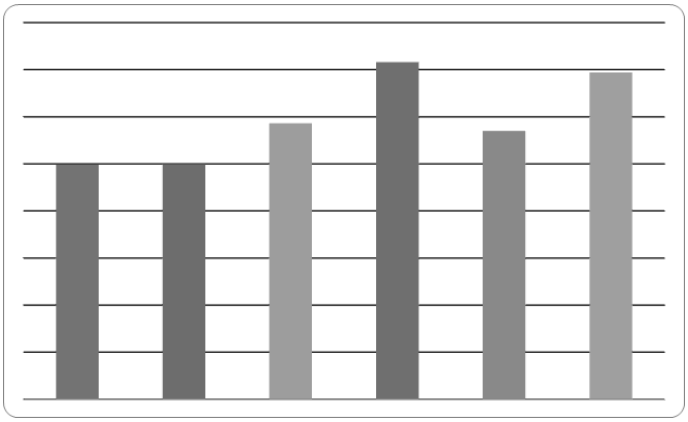

Figure 3. Dynamic of honey storage, kg

During four controls, on 22.04.2011 and until 06.08.2011 bees families in control groups had grew each 467.33-479.8 hundred of capped cells. The highest number of capped juveniles had grew the bees families of group IV-652.66 hundred cells, or more by 39.7% than the control group II and 36.0% than in control group I (Table 5).

Table 5. Number of capped juveniles

Control date	Experimental groups					
	I	II	III	IV	V	VI
22.04.11	73.33	63.33	79.57	80.00	68.170	83.67
7.05.11	152.67	132.33	167.29	191.33	183.00	177.33
19.05.11	140.5	132.67	155.69	199.33	169.33	173.33
8.06.11	113.3	139.00	161.40	182.00	152.70	122.00
Total	**479.8**	**467.33**	**563.95**	**652.66**	**573.20**	**556.33**
±,%	102.7	100.0	120.68	139.70	122.70	119.00

Bees families in group V had grew respectively-573.2 hundred cells, or more by 22.7%, in group VI-556.33 hundred cells, or more by 19.0% than in the control group II. So, using of feed additive (complex symbiotic) in nutrition stimulating of bees during spring time it is possible to increased power, queens' prolificity of bees' families.

CONCLUSIONS

1. It was found that the optimal dose of feed additive Premix Bionorm P (symbiotic complex) is 100 mg / l sugar syrup administered in the spring time to stimulate the bees' families using one liter over every 10-12 days, from the

first days of April until early harvest of white acacia.

2. It was revealed that the use of feed additive (symbiotic complex) in the nutrition of bees' families stimulates the increasing of the family power from 29.3 to 39%, queens' prolificity and capped brood with 41.9 to 50.2%.

3. It is reasonable stimulation of bees' families during spring time when there is no natural harvesting that ensures increasing of honey production by 43.2%.

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DEVELOPMENT OF HEAT-STABLE FRUIT FILLINGS USING GELLAN GUM AS STABILIZER

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Abstract

This research was designed in aim to determine the optimum percentage of gellan gum for the development of heat-stable fruit fillings with a wide range of soluble solids – from 40 to 70°Brix, which maintain its sensory and textural characteristics before, during and after baking on the basis of Response Surface Methodology. The low acyl gellan gum Kelcogel F was selected to be used in heat-stable fruit filling′s development due to its excellent thermal and freeze-thaw stability. This high-viscosity hydrocolloid possesses curative properties for sufferers of hypercholesterolemia, high blood pressure, and diabetes and forms a firm gel in as little as two hours, delivering full strength in less than 5 hours.
The fruit fillings′ samples with the same pH and different soluble solids content were prepared locally from fruit pulp, sugar, low acyl gellan gum Kelcogel F and citric acid. The produced fruit fillings were put through standard bakery test to evaluate their heat-stability by determining bakery index (BI) through measuring the diameter of a fruit filling′s sample before and after baking process performed under exactly fixed conditions: at a temperature of 220°C for 20 minutes. The rheological behavior of the fruit fillings prepared on the basis of gellan gum has been investigated by performing experimental measurements at the rotational rheometer Rheotest RV2.
There were obtained the final equations in terms of actual factors in order to describe the influence of soluble solids and gellan gum on fruit filling's heat-stable, sensory and rheological properties. The adequacy of the regression equations was evaluated by the F-test for analysis of variance (ANOVA) using statistical package STATISTICA v.6 and has shown that the models were statistically significant.

Key words: *heat-stable, gellan gum, filling.*

INTRODUCTION

Nowadays extended demand for palatable, cheap and ready on-the-go snacks and meals containing eco-friendly fruit-based foodstuffs has resulted in development of various confectionery and bakery products with fruit fillings. Each fruit filling has different designations and requirements, depending on the customer′s demands, product type, technological process of preparation and the manufacturer′s capabilities. For example, a fruit pie filling baked with the dough, which can further be stored, frozen and then thawed or even rebaked, certainly needs not only high oven and freeze/thaw-stability, but also texture control. The high thermo-stability of fruit filling insures that after baking the pastry remains intact when cut and the filling retains "fresh-made" characteristics. There are three main types of bakery fillings: heat-stable, limited bake-stable and non heat-stable fillings (*Herbstreith & Fox KG)* . The melting behavior of fruit fillings depends on the duration and temperature of the baking process. Fruit compositions start melting and flow if they are exposed for a short time to a temperature much higher than their melting point or if they undergo high temperatures in the range of melting point in a long time. However, some bakery products, such as doughnuts that are filled after frying, stored for a few days at room temperature and then consumed, don't require fillings with high heat-or freeze/thaw stability. Fruit fillings for cookie bars require a solid consistency to hold their shape and volume in the finished product, and low aw to ensure long-term shelf life and decrease moisture migration. These mostly need to be formulated with dehydrated fruit, such as low-moisture apples, apricots or prunes or concentrated fruit-stuffs with high soluble solids content and stabilizing agents, to achieve a low finished-product moisture level. Stabilizers and dehydrated fruit can highly increase the viscosity and prevents "boil out" during baking.

One of the restrictive factors in the utilization of natural fruit fillings for bakery products is their tendency not only to become softer but also to degrade thermally at high oven temperatures. Unfortunately at the moment fruit jams and jellies rich with pectin are mostly used as bakery fillings. But they also do not behave well at high temperatures during baking, being not heat-stable. Food manufacturers can currently buy ready-to-use fruit bakery fillings from canning or ingredient suppliers, but the percent of fruit part there is very low, because formulations for heat-stable fruit filling with high sensory characteristics face technological difficulties that impose some constraints. Fruit – whether it's fresh, frozen or dehydrated – adds flavor, texture and natural nutrients to the finished fruit fillings. However, it can vary in size and quality as a natural ingredient. Thus, in order to create a smooth and consistent product, manufacturers have to make adjustments during production. They may include the following manipulations: blending the fruit or fruit concentrate, adding sugars, acids or buffers, using stabilizing agents to adjust the product's stability and texture, within legal guidelines. Generally, the requirement for using high percentage of fruit-based raw materials can't be matched with heat-stability and low water activity and thus, manufacturers have to resort to imitation or application of high amounts of various food additives.

Therefore, in this research we decided to experiment with adding gellan gum to fruit fillings' compositions, seeking for the heat-stable fillings which will not drip out of the cake during baking.

Our objectives were to study the correlation among heat-stability, sensory and rheological properties of the fruit fillings prepared with different ratio of gellan gum within a wide range of soluble solids – from 40 to 70°Brix on the basis of experimental design technique.

There are a lot of advantages gained from the use of gellan gum in heat-stable fruit fillings preparation, such as:

- no 'boil-out' of filling;
- good volume fill throughout shelf life;
- excellent flavor release;
- decrease of moisture transfer into the dough;
- fluidity control of the filling if consumed hot;
- perspective to create new bakery products, such as mini-turnovers, which would be extremely difficult using a more conventional filling thickener.

The removal of "boil-out" and "tailing" of fruit fillings keeps the processing lines free from burned on deposits, and thus maintains the standard of hygiene of the line high, without a requirement for extra labor.

Further, since the heat-stable fruit fillings don't melt, the addition of hot custard beyond fruit filling's sheet in a multilayer desserts' preparation, for example, can also be accepted. These properties permit energy savings and more rapid processing of ready-to-eat layered desserts prepared with fruit fillings.

The application of Response Surface Methodology (RSM) as one of the most commonly used techniques of experimental design allows predicting the heat-stable and rheological properties of the fruit fillings prepared with gellan gum as stabilizer.

MATERIALS AND METHODS

Raw materials

Apple aseptic puree was produced at the canning plant 'Conserv-E' (Chisinau, Republic of Moldova). Sugar was acquired from a local supermarket (Chisinau, Republic of Moldova). Citric acid solution (50%) was prepared locally in the Laboratory of Functional Foods of the Practical Scientific Institute of Horticulture and Food Industry (PSIHFT) of the Republic of Moldova. Low acyl gellan gum (KELCOGEL F) was purchased at the Moscow International Exhibition for Food Ingredients, Additives and Flavorings – "Ingredients Russia" (Moscow, Russian Federation).

Sample preparation

The fruit fillings samples were prepared locally in the Laboratory of Functional Foods of the PSIHFT from apple puree (12°Brix), sugar, low acyl gellan gum powder (KELCOGEL F) and citric acid (50% concentration).

The whole amount of the sugar was shared out in two parts, and the first one was introduced to the smooth apple puree, and heated till the sucrose has dissolved. This prior apple-sugar mix served as the basis for four different fruit fillings' formulations presented in the study. The amount of added sugar was calculated on the basis of the final required soluble solids for

each fruit filling sample, in order to prepare fruit fillings within a wide range of soluble solids – from 40 to 70°Brix. After that, the gellan gum was dissolved with the rest of the sugar in the ratio 1:10 in hot water using a high speed stirrer. This dispersion was heated from 90 to 98°C and the temperature maintained for 1 min to give a clear solution. The resulted gellan gum solution was added to the apple blend by taking in account four different formulations and followed by intensive mixing at a temperature of more than 80°C. After obtaining a homogeneous mixture, citric acid was introduced to the final apple filling′s composition, leading to lowering pH. With the initiation of gelling, mixing time became critical, and mixing was only continuing for one to two minutes after adding citric acid.

The prepared fruit fillings were preserved in glass jars after sterilization for 2 days before testing.

Physicochemical, rheological and sensory analysis

The physicochemical, rheological and sensory analysis of the fruit fillings samples were carried out at the Laboratory of Functional Foods of the PSIHFT.

The soluble solids of the fruit fillings were determined using benchtop refractometer ABBE and expressed in°Brix. The pH was measured by a potentiometric method, introducing the electrode directly into the fruit fillings.

The fruit fillings′ viscosity before baking was investigated through experimental measurements at the rotational rheometer Rheotest RV2.

Sensory analysis of the fruit fillings' samples after baking was conducted by 10 randomly selected members of the panel. Each of the key sensory characteristics (color, taste, flavor, general appearance and texture) was evaluated by a numerical estimation, ranging between 1 (for extremely bad parameters) and 5 (excellent parameters). Average value based on estimates of each parameter was calculated to receive overall evaluation of the sample.

Determination of heat-stability

Standard bakery test was used to determine the heat-stable properties of the fruit fillings′ samples in the following way: a certain amount of prepared fruit filling was given into a base of the filter paper type 'Blue ribbon' with a diameter of 120 mm by a metal ring with defined geometry (50 mm diameter and 10 mm height) and then baked under exactly fixed conditions: at a temperature of and 220°C for 10 minutes (*Herbstreith & Fox KG)*. During and after this baking process all changes in physicochemical, textural and sensory attributes of the tested fruit filling were estimated. The bakery index was determined by measuring the sample diameter before and after baking by placing a line across the sample and calculating via the following formula:

$$BI = 100 - \frac{D_2 - \bar{D}_1}{D_2} \cdot 100 \quad (1)$$

where

BI – bakery index, %;

D_1 – average diameter of sample before baking, mm;

D_2 – average diameter of sample after baking, mm.

Diameter of a fruit filling sample before baking as a diameter of the metal ring is 50 mm. For measuring the sample diameter depending on its shape, from two to four lines were drawn, and the average was calculated.

For validation experiments we used not only the filter paper type 'Blue ribbon', but also pastry samples with a diameter of 60 mm. For the pastry samples we selected another metal ring with the following dimensions: 30 mm diameter and 10 mm height.

Statistical analysis

In order to establish the optimal percentage of gellan gum introduced into fruit filling's composition for attributing high thermo-stable properties, Response Surface Methodology (RSM) was applied. It was revealed that mostly percentage of stabilizer and soluble solids influence fruit fillings′ thermal stability and rheological behavior (at the same high temperature and for the same baking duration). Thus, only gellan gum content and soluble solids were used as independent variables in two-level factorial design. The levels of these variables were set at: 0.1 and 1.0 for percentage of gellan gum and 40 and 70 for soluble solids,°Brix. The heat-stability of fruit fillings

as one of the response variables was expressed through the bakery index (BI, units). All experiments adjusted by the design planned in coded and encoded form of process variables, were conducted randomly. The results obtained through application of RSM were verified by conducting the validation experiments under the optimized conditions of all factors. Statistical package STATISTICA v.6 and MATCAD v.15 was used to evaluate the adequacy of the regression equations through analysis of variance (ANOVA) and to visualize the influence of all factors on response variables by drawing 3D surface plots.

RESULTS AND DISCUSSIONS

Physicochemical and sensory characteristics of the fruit fillings analyzed before baking under laboratory conditions have revealed that they meet the international food standard CODEX STAN 296-2009 FOR JAMS, JELLIES AND MARMALADES.

All fruit fillings prepared with different soluble solids and gellan gum content had the same low pH – 3.1.

The fruit fillings baked along with pastry samples at a temperature of 220°C for 20 minutes were evaluated for sensory quality. The main organoleptic parameters of the fruit fillings with gellan gum after baking are shown in Table 1.

Table 1. Organoleptic parameters of the fruit fillings with gellan gum after baking

Product name	Average sensory scores			
	color	taste	flavor	texture
Fruit filling with 40 ºBrix and 1% gellan gum	5	5	5	4
Fruit filling with 70 ºBrix and 0.1% gellan gum	4	4	4	3
Fruit filling with 40 ºBrix and 0.1% gellan gum	4	4	4	3
Fruit filling with 70 ºBrix and 1% gellan gum	4	4	4	4

The statistical analysis of sensory scores demonstrated that no significant difference was found ($p > 0.05$) for the color, taste, flavor and texture of the fruit fillings with low soluble solids (40°Brix) and 1% gellan gum before and after baking. However, sensory score of listed characteristics for the fruit fillings with low content of gellan gum (0.1%) for both low and high soluble solids was significantly different from initial values after baking process. Mean values ranged from 4.0 to 5.0, 4.0 to 5.0, 4.0 to 5.0, and 3.0 to 5.0 for color, taste, flavor and texture respectively indicating moderate acceptability of the product after baking.

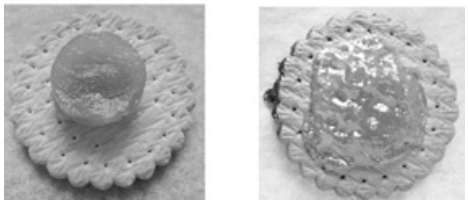

Figure 1. Fruit filling′s appearance after baking at 200ºC for 20 minutes: 40 ºBrix sample prepared with 1% gellan gum (a) and with 0.1% gellan gum (b)

According to the Figure 1, it is clear that the fruit filling sample prepared with 1% gellan gum didn′t become runny or caramelized and stayed well on its place, maintaining its original color, shape and volume. It also didn′t make the biscuit sample under and around it wet or burnt (such as fruit filling sample prepared with 0.1% gellan gum) during baking. The consistency of the fruit filling after baking is nice, smooth but not sticky or gooey. The fresh apple taste and aroma was also well preserved for both of the fruit filling samples shown in the figure above.

Table 2. 2k design matrix for fruit fillings development on the basis of gellan gum

No	X_1 gellan gum content,%		X_2 soluble solids,°Brix		Y_1 bakery index expressing thermal stability	Y_2 viscosity before baking Pa·s	Y_3 overall acceptability after baking, scores
	Code value	Encode value	Code value	Encode value			
1	1	1	-1	40	96.15	521.7	4.75
2	1	1	1	70	40.0	88.2	4.00
3	-1	0.1	-1	40	50.0	14.5	3.75
4	-1	0.1	1	70	30.0	30.5	3.75

The elaboration of heat-stable fruit fillings was conducted through application of the design expert software package STATISTICA v.6 and MATCAD v.15. The experimental design with different independent variables in coded and encoded form i.e. soluble solids and gellan gum content and bakery index, viscosity and overall acceptability of the fruit fillings after baking as responses are presented in Table 2 above.

After processing the experimental data, the following regression equations (2, 3 and 4)

describing fruit fillings′ heat-stability, viscosity and overall acceptability in terms of actual values were derived:

$$BI = 66.18 + 104.83 \cdot G - 0.53 \cdot SS - 1.34 \cdot SS \cdot G \quad (2)$$

$$V = -129.78 + 1229.48 \cdot G + 2.19 \cdot SS - 16.65 \cdot G \cdot SS \quad (3)$$

$$A = 3.5277 + 2.2222 \cdot G + 0.0003 \cdot SS - 0.0253 \cdot G^2 \cdot SS \quad (4)$$

where
BI – bakery index, units;
V – viscosity, Pa·s;
A – overall acceptability, scores;
G – gellan gum content,%;
SS – soluble solids,%.
The obtained models were statistically significant according to the data of the F-test for analysis of variance. The validation experiments′ data closely agreed to the predicted values of the developed models with acceptable percentage errors.

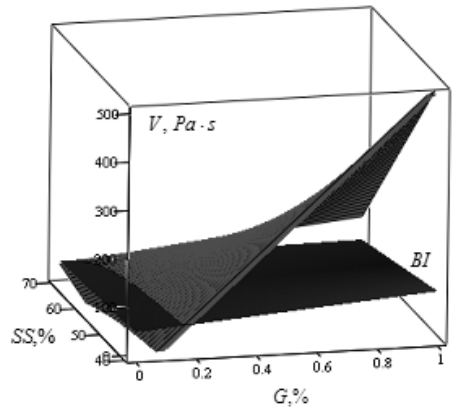

Figure 2. The influence of soluble solids and gellan gum content on bakery index (blue plot) and viscosity of fruit fillings (red plot)

The response surface plots of the polynomial equations represented above have been plotted using MATCAD v.15 as a function of two variables i.e. soluble solids and gellan gum content (Figure 2) in order to visualize their common effect on bakery index that expresses heat-stable properties (blue plot) and viscosity of fruit fillings.

CONCLUSIONS

There were derived three statistically adequate regression equations in terms of actual factors describing the common effect of soluble solids and gellan gum content on fruit filling's heat-stable, rheological and organoleptic parameters.

Judging from the present study, it is obvious that low acyl gellan gum can be definitely used in the development of heat-stable fruit fillings with low soluble solids because of the two major benefits of this hydrocolloid. The first advantage is that gellan gum reduces heat transfer, by forming gelled phase, thereby keeping the temperature of fruit fillings′ compositions lower during the baking time. This also decreases moisture loss and eliminates boil-out. The second main advantage is a reduction in the rate of moisture loss for an increase in shelf life of the finished product. During the investigation it was also revealed that gellan gum would be more advantageous try to use in combination with other hydrocolloids (by finding their common synergetic effect) for the development of heat-stable fruit fillings with high soluble solids, while reducing the standard doses of adding gellan gum as stabilizer.

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FERMENTATION OF SUGARCANE (*SACCHARUM OFFICINARUM L.*) BAGASSE HYDROLYZATE BY *PICHIA STIPITIS*, *SACCHAROMYCES CEREVISIAE*, *ZYMOMONAS MOBILIS* TO ETHANOL

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Abstract

Sugarcane bagasse (Saccharum officinarum L.) is a readily available waste product of cane-sugar processing. The major components of bagasse are cellulose and hemicellulose. The objective of the research was to produce bio-ethanol from hydrolizate of sugarcane (Saccharum officinarum L.) bagasse which hydrolyzed by combination of acid and enzymeand further fermented by three types of microorganisms, respectively P. stipitis CBS 5773, or S. cerevisiae D1/P3GI, or Z. mobilis 0056 FNCC. The experiment employed descriptive analyses in triplicates. The result were as follows: effectiveness of Z. mobilis 0056 FNCC was highest for producing bio-ethanol, respectively 18.99 g/L within 3 hours. S. cerevisiae D1/P3GI produced 17.05 g/L bio-ethanol within 12 hours, and P. stipitis CBS 5773 13.03 g/L bio-ethanol within 24 hours.

Key words: *bagasse, P. stipitis, S. cerevisiae, Z. mobilis, ethanol, fermentation.*

INTRODUCTION

Sugarcane bagasse (*Saccharum officinarum*) is a byproduct of the extraction process or milking cane liquid. From one mill generated bagasse approximately 35-40% of the weight of the milled sugar cane. Bagasse contains most of ligno-cellulose are composed of lignin, cellulose and hemicellulose. Lignocellulose is a substrat that can be renewed, most are not used and is available in abundance (Taherzadeh and Karimi, 2007). Chemical hydrolysis with sulfuric acid can be formed on lignocelluloses materials with specific time and temperature will produce four mayor components namely carbohydrate polymers (cellulose, hemicellulose), lignin, extractive materials, and ash. Further elaborate polysaccharide polymers into a single sugar monomers (Morohoshi, 1991), so the enzyme is more easily hydrolyze these compounds into monomers more simple sugars (Taherzadeh and Karimi, 2007). Enzyme hydrolysis of sugarcane bagasse can be done with the addition of cellulase and hemiselulase to hydrolyze cellulose and hemicellulose into sugar monomers. But using a combination of acid hydrolysis and enzyme more effectively and efficiently produce DE value of about 65% (Tjokroadikoesoemo, 1986). The last stage is fermentation of hydrolizate with culture *P. stipitis, S. cerevisiae,* and *Z. mobilis*. Sugar cane bagasse hydrolysates containing C-5 and C-6. P. stipitis and Candida sheatae they use xylosa and able to ferment hexoses and give a high yield, low tolerant, to ethanol and can produce ethanol concentrations above 30-35 g/ l. According Rouhollah (2007), *S. cerevisiae* has the ability to ferment glucose, maltose, and trehalosa to ethanol. However, *S. cerevisiae* is not tolerant to high concentrations of ethanol produced during fermentation. In addition, *S. cerevisiae* is not able to ferment xylosa because they do not have xylosa xyilitol reductase and dehydrogenase (Gaur, 2006). *Z. mobilis* is more tolerant to ethanol with the concentration levels of 2.5-15% since its membrane plasma structure containing a hopanoid and sterols (Gunasekaran et al., 1986). *Z. mobilis* is more

tolerant to high of concentration of sugar and give higher ethanol production, fermentation at low pH (Kompala et al., 2001).The aim of this research was to study ability of microbes to produce bio-ethanol from hydrolyzate of sugarcane (*Saccharum officinarum L.*) bagasse. In this study, sugar cane bagasse was hydrolyzed by a combination of acid with enzyme then fermented by *P. stipitis, S. cerevisiae*, and *Z. mobilis* to ethanol.

MATERIALS AND METHODS

Preparation of hydrolyzates

Sugarcane bagasse (*Saccharum officinarum*) were powdered by size of 30 mesh and dried in an oven at a temperature of 80^{o}C for 10 minute then add water at a ratio of 1: 20; (w/ v). Further baggase is heating in steam at a temperature of 120^{o}C for 30 and 45 minutes at a pressure of 1 atm using the autoclave. In erlenmeyer, 250 ml, suspension of baggase is added with H2SO4 as much as 2% (w/w) of the weight of sugar cane bagasse. Then it heated by autoclave at a temperature of 120 º C, for 60 minutes. After its cooling at 25 º C temperature and pH is ajusted to 6.0. Hemicellulase with dose 0,001 g/g, is added then it incubated at 55 º C temperature for 4.5 hours with agitation speed of 150 rpm. Further, hydrolysates is cooling to 25 º C and add cellulose enzymes with dose 0,83 µl/g pH to 4.8 then hydrolysates incubated at 60 º C for 48 hours with agitation speed of 130 rpm. DE (Dextrose Equivalent) was measured and type of sugar is formed is analyzed by HPLC.

Preparation of culture starter

The microorganism used was S. D1/P3GI *S.cerevisiae, P. stipitis* CBS 5773, and *Z. mobilis* FNCC 0056. S. cerevisiae, and P. stipitis was cultured in YEPD agar, containing per liter: 3 g yeast extract, 10 g Peptone, 20 g dextrose, 15 g agar-agar, and 1 L aquades. For the culture of *Zymomonas mobilis* used medium containing per liter: 10 g yeast extract, 10 g Peptone, 20 g glucose, 15 g agar-agar, and 1 L aquades (Atlas, 1993). Starter was cultured in agar slant and incubated at 30^{o}C for 24 hours. For starter, cultures was growing in YEPD broth at pH 7, incubated at 30^{o}C using a shaker with agitation speed 100 rpm, culture is incubated 39 hours for *P. stipitis* for 39 hours, 18 hours for *S. cerevisiae*, and 9 hours for *Z. mobilis*.

Fermentation

Sugarcane bagasse hydrolyzate with high DE is filtered using filter paper no.4 Whatman. Hydrolyzate added with medium containing (/ L): 4 g yeast extract, 2 g KH_2PO_4, 3 g $(NH_4)_2SO_4$, 1 g MgSO4.7H_2O, and 3.6 g peptone, pH was adjusted at 7,0 (Sanchez et al., 2002), then hydrolyzate is sterilized by autoclave for 15 minutes. After sterilized, hydrolyzate is cooling and add with starter with concentration of starter 10%. Fermentation is incubated at 30^{o}C, at pH 7, and agitation speed of 150 rpm for 84 hours for *P. stipitis*, and *S. cerevisiae* and 21 hours to *Z. mobilis*.

Analitical Methode

pH is measured with a pH meter. Total reducing sugars is measured by DNS (Apriyantono et al., 1989), and type of sugar is measured and analyzed by HPLC column HPX-87H AMINEX. The ethanol concentration is measured by bichromate oxidation method (Caputi et al., 1968). Type of organic acids is formed during fermentation is measured by HPLC column HPX 87H Aminex.

RESULTS AND DISCUSSIONS

Fermentation was conducted to determine the ability of each straint to ferment the sugar cane bagasse hydrolyzate to ethanol.

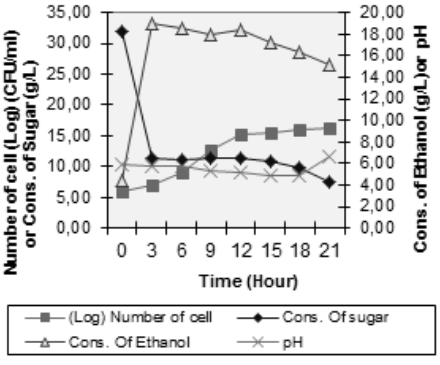

Figure 1.a

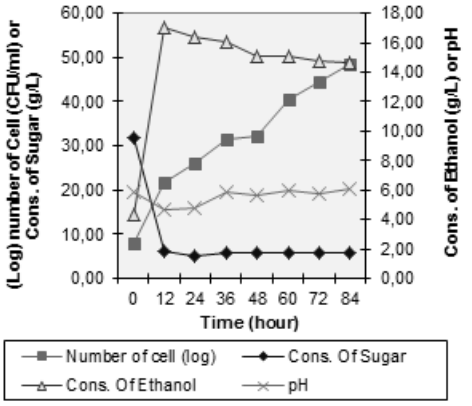

Figure 1b.

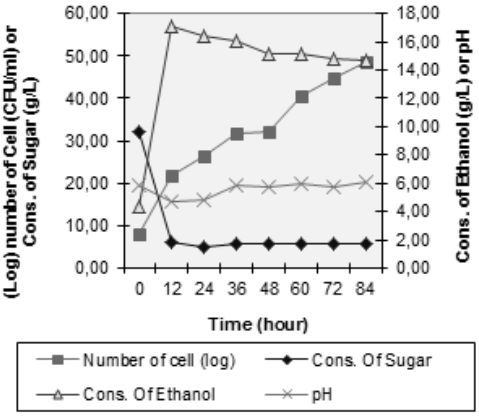

Figure 1 c

Figure 1 a, b, c. Production of ethanol from sugarcane bagasse hydrolysates by (a) *Z. mobilis* (b) *S. cerevisiae* D1/P3GI, (c) *P. stipitis* CBS 5773.

Based on the results shown in Figure 1, show that *S. cerevisiae* D1/P3GI, *P. stipitis* CBS 5773, and *Z. mobilis* FNCC 0056 need adaptation in three hours than begin to log phase. It proves that the three microorganisms capable of using reducing sugar from sugarcane bagasse hydrolyzate as a nutritional source of growth. Based on the results of HPLC analysis of hydrolysed cane is used for the fermentation process containing 23.78% glucose, 56.91% xylosa, and 19.31% arabinose. Based on the results of HPLC, the final stage, fermentation produced lactic acid and acetic acid using a culture of *S. cerevisiae* D1/P3GI and acetic acid from culture *Z. mobilis* 0056 FNCC. Concentration of ethanol produced in line with the growth of each culture and the amount of reducing sugar consumed. In this study, *Z.* 0056 FNCC *mobilis* able to ferment sugar into ethanol hydrolyzate with highest yield of ethanol compared to *P. stipitis* CBS 5773 and *S. cerevisiae* D1/P3GI. Fermentation was more fast, which is 3 hours produced ethanol at 18.93 g / L. The results of this study in line with more reference stated that *Z. mobilis* can convert mixture of sugar into ethanol 92%-94% is greater compared to the yeast which only reached 88-90% (Silalahi, 1987). This possibility is caused that ethanol fermentation of *Z. mobilis*, conversion of glucose into two molecules of ethanol produced one molecule of ATP. The low energy generated ATP resulted in cell mass produced low and high ethanol produced. From Fig. 1c also show that in the first 12 hours, fermentation is constantly increasing. This means that *S. cerevisiae* capable of using sugar cane bagasse hydrolysates as a source of nutrition. This can be seen from the decrease in the amount of sugar in the hydrolyzate accompanied with the production of ethanol amounted to 17.05 g / L. While the research Rouhollah et al. (2007), fermentation using microorganisms Fermentative *S. cerevisiae* in sugar mixture to produce ethanol 0.32 g / L per gram of glucose or by 62% with ethanol concentrations of 14.25 g / L and ethanol productivity was 0.88 g / L / hour in 48 hours fermentation time. This proves that *S. cerevisiae* able to ferment sugar cane bagasse hydrolysates with a shorter time which is 12 hours with levels of ethanol produced 17.00 g/ L. Okur and Nurdan (2006) conducted research on the production of ethanol from waste and the fermentation process needs 55 hours and produced of ethanol concentration 9.66 g / L. While the fermentation of sugar mixed media using *P. stipitis* produces ethanol 0.40 g / L per gram xylose and ethanol concentration 30.23 g / L with productivity of ethanol is 0.95 g / L / hour in 72 hours fermentation time (Rouhollah et al., 2007). While in this study, *P. stipitis* CBS 5773 is able to ferment sugar cane bagasse hydrolyzate to ethanol as much as 13.03 g / L in 24 hours fermentation time. Overall, *P. stipitis* CBS 5773, *S. cerevisiae*, and *Z.* 0056 FNCC *mobilis* use sugarcane bagasse hydrolyzate as a source of nutrition in the period 0-24 hours in line with the ethanol production.

CONCLUSIONS

Z. mobilis 0056 FNCC able to ferment sugar cane bagasse hydrolyzate to ethanol 18.99 g L in 3 hours, *S. cerevisiae*, is able to ferment the sugar cane bagasse hydrolyzat to ethanol amounted to 17.05 g / L in 12 hours, whereas *P. stipitis* CBS 5773 is able to ferment sugar cane bagasse hydrolyzate to ethanol 13.03 g / L in 24 hours.

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BLOOD GLUCOSE AND TRIGLYCERIDEPROFILE USING *ALPINIA GALANGA* (L.) / LENGKUAS JUICE

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Abstract

Alpinia galanga (L) is a tradisional medicine is also known as lengkuas in Indonesia. The World Health Organization (WHO) estimated that almost 80% of world population relies mainly on traditional medicines, mostly plant drugs in their health care. Alpinia galanga L..) /Lengkuas found all over the world. Chemical components which give the distinctive aroma is asetokskhavikol acetate. The active compounds are galangin, eugenol, kaempherol, and quercetin. These compounds can lower the blood lipid level.The objective of the study was to investigate the Profile blood glucose and triglyseride level using Alpinia galanga (L) /lengkuas Research has done at Biochemistry Laboratories, Animal Husbandry Faculty, Universitas Padjadjaran, and Biofarma Laboratory in Bandung, from October until December 2012. This research used an experimental method with a Completely Randomized Design. There were five treatments (R0 = control ration , R1= control ration + 0.01% Alpinia galanga (L), R2 = control ration + 0.02% Alpinia galanga (L) , R3= control ration + 0.03% Alpinia galanga (L), and four replications. From the statistical analysis indicated that effects of using Alpinia galanga (L) /lengkuas, has potencial effect to.decline trigliseride level i.e: R1= 21,15% , R2= 8.65%, R3= 6.73%, although the blood glucose and triglyseride level showed not significant effect (P> 0.05)

Key words: *Alpinia galanga (L), triglyseride, blood glucose, broiler.*

INTRODUCTION

Capability of broiler growth accompanied by relatively rapid high fatty. High fat content is one of the constraint in broiler chicken meat, so most people limit in taking it. Broiler chicken blood triglyceride levels according to Lovita et.al (2013) is 35.20 ± 16.45 mg / dL, while according to Freeman (1984) in Sunarto (2012) levels of broiler blood triglyceride is 27 mg / dL. The high content of cholesterol in the blood affects to high cholesterol content of broiler meat. One of the effort for lowering cholesterol and blood triglycerides broiler by giving langkuas (*Alpinia galanga* L.) juice. Data from the Central Statistics in 2011 reported that *Alpinia galanga* L. production in Indonesia reached 59,332,313 kg or 2.98 kg/m2 and in 2011 is 57,701,484 kg / year. Based on previous research, it is known that *Alpinia galanga* L. contains a variety of active ingredients such as alkaloids, saponins, flavonoids, terpenoids, atsiri oils, and quinone (Iswantini et al., 2010). Flavonoids and essential oils can inhibit the synthesis of cholesterol biosynthesis by inhibiting the enzyme 3-hydroxy-3-Metilglutaril-CoA (HMG-CoA reductase) that play a role in the synthesis of cholesterol as well as LDL receptor through SERBP so integrally suppress cholesterol synthesis and absorption. In addition, saponins and tannins also play a role in inhibiting the absorption of cholesterol by inhibiting the activity of lipase, binds cholesterol and lowers the surface tension, through the mechanism of binding of cholesterol by saponin in the lumen of the intestine that affects the metabolism of fat in the body (Malinow et al., 1981 in Morehouse et al., 1999). The role of the active compound found in lengkuas, able to reduce the cholesterol level and triglycerides in the blood Lipid are primarily triacylglycerol will hydrolyse to monoasilgliserol and fatty acids in the intestine, and then by re-esterification in the intestinal mucosa. Lipid together with protein and secreted into the lymph system and then into the bloodstream as chylomicrons, the largest plasma lipoproteins. Chylomicrons also contain other lipid-soluble nutrients. Unlike glucose and amino acids, triacylglycerol

chylomicrons are not absorbed directly by the liver. This compound is initially metabolized by tissue containing lipoprotein lipase that hydrolyze triacylglycerol, and free fatty acids, Another major source of long-chain fatty acids are synthesized from carbohydrates, in adipose tissue and liver (Murray et al,.2009).Fatty acids synthesized through a new process of lipogenesis to form triglycerides in the liver. Lipoprotein help to release these material from liver, especially the very low density lipoprotein (VLDL), and then deliver and stored in adipose tissue. The main function of triglyceride that have been stored in adipose, until use as energy in the body (Piliang, 2006). The synthesis of triglycerides, fatty acids or diestereficase combined with glycerol molecule. Triglycerides are a glyceride, the esters of glycerol and three fatty acids. Triglycerides are not cholesterol, but a fat type found in the blood as lipoprotein particles. Two acyl-CoA molecules are formed through the activation of fatty acids by acyl-CoA synthetase, binds to glycerol 3-phosphate to form phosphatidate. This takes place in two stages, which are catalyzed by glycerol-3-phosphate and 1-asilgliserol asiltransferase-3-phosphate asiltransferase. Phosphatidate changed by phosphatidate asiltransferase phosphohydrolase and diacylglycerol (DGAT) to 1,2-diacylglycerol and then into triglycerides. In mucosa intestine, monoasilgliserol asiltransferase transform into 1,2-diacylglycerol in the path monoasilgliserol. Most of the enzyme activity was found in reticulum endoplasmic, and in the mitochondria (Murray, et al. 2009). Triglycerol holds a very important role in produce energy in animals. Triglycerides are stored in cells as fat grains are almost pure and can be stored in very large amounts in adipose tissue. There is also store in fat tissues such as liver and tendons. Beside as a source of energy, triglycerides can be converted into cholesterol, phospholipids and other lipid forms when it needed (Linder, 1992). This group provides more than half of the energy needs of multiple organs, especially the liver, heart and skeletal muscle during rest (Lehninger, 1982). Glucose is very important, required to supply as energy source of the cell. Glucose is carbohydrate that is absorbed in the intestine. Glucose is the only carbohydrate found at nutritionally relevant concentrations in the blood also will form of energy currency, which can be transported in different tissues.

MATERIALS AND METHODS

In this research used 60 straight run broilers, and randomly divided into 20 unit, so each unit contain three heads. In this research,broiler was given *Alpinia galanga* juice since the fourteenth day, the content of Nutrient and metabolisable energy of the rations can be seen in Table 1.

The body weight on fourteen days broiler is + 400 grams with variations + 9%. Blood Sampling was taken on 5 weeks, at the end of the experiment. The Experiment using completely Randomied Design (CRD) which concist of four treatments, and 5 times repeated :P0 = without *Alpinia galanga* L. juice P1 = *Alpinia galanga* L. juice 0.01% from body weightP2 = *Alpinia galanga* L. juice 0.02% from body weightP3 = *Alpinia galanga* L. juice 0.03% from body weight Broilers were divided into following four groups of three animals each, and repeated five times. The treatment was continued for 5 weeks.

Document in research

Alpinia galanga

Prepare Alpinia galanga for juice

Given Alpinia galanga per oral

Body weight 5 weeks old

Take a blood from vena pectoralis

Figure 1. Document in research

Table 1. Nutrient content and Energy Metabolism of the ration

Nutrient	Content
Water (%)	Max14%
Crude Protein (%)	21.50 – 23.80
Crude Fiber (%)	Max 4%
Crude Fat (%)	Min 2.5%
Ash (%)	Max 6.5%
Calcium (%)	0.90 – 1.1%
Phosphor (%)	0.7 – 0.9%
Energy Metabolism	3025-3125 kg/kcal

RESULTS AND DISCUSSIONS

Table 2. There are the effect of sweet citrus waste flour on broiler triglyceride and blood glucose

Treatment	Triglyceride (mg/dl)	Glucose (mg/dl)
P0	20.8	244.6
P1	16.4	238.4
P2	19.0	247.6
P3	19.4	235.6

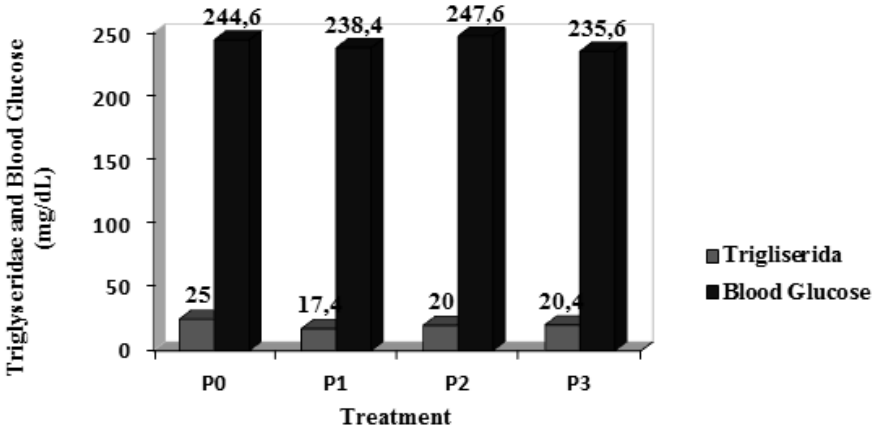

Figure 2. Effects of *Alpinia galanga* Juice on Triglyceride and Blood Glucose

Triglyceride

From Table 2, blood triglyceride levels on P0 (without lengkuas) is higher than P1, P2 and P3. According to Lovita A. et al (2013) blood triglyceride levels is 35.20 $\pm$ 16.45 mg/dL, while according to Freeman (1984) in Sunarto (2012), the content of triglyceride is 27 mg/dL Blood triglyceride levels in broiler decreased by adding lengkuas juice, because of the atsiri oil in lengkuas, inhibit the formation of triglycerides compounds of the early work of glycerol-3-phosfat derived from glycerol, dihidroxiacetone phosphate, and the NADH help to synthesize Glycerol-3-phosphate for triglycerides. In addition atsiri oil is capable of lowering the activity of Glycerol-3-Phosphate (GPDH) enzyme in the biosynthesis of triglycerides (He et al., 2009).

The active ingredient of antioxidant flavonoids is 0.21%, it will inhibit the early stages of the reaction by release 1 hydrogen atom forming and reducing associated with one free radicals, this bond will stabilize the radical peroxy that makes energy activity reduced, and finally the content of triglycerides will decline (Reynertson, 2007). Decreasing blood triglycerides also affected by saponins content for delaying the absorption of fat in the small intestine by inhibiting lipase activity, through the mechanism of binding triglyceride-and saponins in the intestinal lumen, and affect the metabolism of fat in the body (Malinow et al., 1981 in Morehouse et al., 1999).

Dalimartha, (2003) stated a nutritious kolagoga saponins that improve production and increase the secretion of bile, bile for solid particles are removed, so that the fat metabolism is able to decrease blood triglycerides. Decreasing triglyceride levels are also influenced the content of tannins in the *Alpinia galanga* L. juice, that will bind to the protein of the body and will coat the intestinal wall and digestive tract mucous layer compaction so that inhibits the absorption of dietary substances including triglycerides and cholesterol.

Low levels of blood triglycerides broiler proves that in line with provision of the liquids galangal previous research conducted Padikkala and Achuthan (1997) in mice, where the ethanol extract of in vivo galangal can effectively decrease blood triglyceride levels of serum, gave 20 mg/day/body weight (0.008%/day/body weight).

Blood Glucose

Blood glucose levels on P0 (without *Alpinia galanga* L. is almost the same with P1, P2 and P3. According to Riesenfeld et al., 1982, in Lovita A. (2013), blood glucose will circulate in the blood and will stabile in the bird, regardless of different dietary level. Much of this regulation is due to the interplay of many variety of hormones, including glucagon, pancreatic polypeptide, insulin and thyroxine. These hormones can regulate in the glucose metabolism. Usually, more than a third of glucose absorbed during a meal and is

converted to lactate in the intestinal wall, buffering the peak influx.

Glycogen synthesis. Glucose is not oxidized within minutes after a meal, it can be stored as glycogen. The major glycogen storage areas are the liver and glycolytic muscles. The muscle and liver pools are available for flight or other activities, whereas, during fasting, the liver pool is depleted prior to use of muscle glycogen. Because glycogen is very hydrated, it is a physically bulky (low kJ g^{-1}) energy storage molecule relative to triglycerides. This bulk precludes storage of large amounts and surfeit dietary carbohydrates are converted to fatty acids and stored as triglycerides. The glycogen content of the liver is usually less than 4% and is depleted within a few hours of fasting (Blem, 1990; Swain, 1992).

CONCLUSIONS

Using lengkuas (*Alpinia galanga* L.) juice, on giving 0.01% from body weight, can decrease the level of triglyceride broiler blood until 21.15% for P1, P2= 8.65%, P3 =6.73 mg/dL, although the level of blood glucose is almost the same in all treatment.

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HYDROLYSIS OF SAGO PITH POWDER (METROXYLON SAGO ROTTB.) IN ENZYMATIC AND FERMENTATION OF HYDROLYZATE BY *PICHIA STIPITIS CBS 5773, SACCHAROMYCES CEREVISIAE D1/P3GI,* AND *ZYMOMONAS MOBILIS FNCC 0056* TO ETHANOL

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Abstract

Sago (Metroxylon sago Rottb) is an abundant source of biomass and have a high starch content, which can be used as a source of renewable energy (bioethanol). The purpose of this study is to obtain the best microorganisms to ferment sugars of the enzymatic hydrolysis yield of sago pith into bioethanol. The research method is experimental in the laboratory consisting of starch hydrolysis and fermentation of sugar pith of sago pith of sago starch hydrolyzate by Pichia stipitis CBS 5773, Saccharomyces cerevisiae D1/P3GI, and Zymomonas mobilis FNCC 0056 in single culture. Pith of sago flour used in this study contained 77.5% starch, 4.86% hemicellulose, cellulose 4.63%, 3.07% lignin and 10.12% water. Through gelatinization and enzymatic hydrolysis of sugar, the sugar concentrations obtained in this study was 47.95% and 61.66% DE. Hydrolysates were used containing 10% sugar, fermented ethanol showed that ethanol production by Pichia stipitis of 2.45% without pH adjustment, 2.19% with a pH adjustment treatment, the concentration of ethanol by Saccharomyces cerevisiae at 3.65% without adjusting the pH, 2.17% with a pH adjustment, and the concentration of ethanol by Zymomonas mobilis at 1.89% for those without pH adjustment and 0.37% with a pH adjustment.

Key words: *Ethanol, Hydrolisis, Sago Palm Pith Powder, Fermentation, Pichia stipitis CBS 5773, Saccharomyces cerevisiae D1/P3GI, Zymomonas mobilis FNCC 0056.*

INTRODUCTION

Pith of sago starch has a complex structure, composed of cellulose, hemicellulose, and starch. Starchy pith is also difficult to dissolve in water because it contain amylopectin more than amylose (Hidayat et al., 2006).

Cellulose is a long polymer of D-glucose and has a very regular shape with ß-1, 4 glycosidic bonds. Cellulose hydrolysis yields D-glucose relatively large (Lehninger, 1997). Unit composed of hemicellulose sugars (sugar anhidro) which can be divided into groups of pentose, hexose, hexuronat acid and deoxy-hexoses. Hydrolysis of hemicellulose in particular are composed of hexose sugar units also produce simpler compounds of ß-D-glucose and D-galactose (Fengel, 1995).

To produce bioethanol from stem pith of sago starch it is necessary to do gelatinization. The purpose of gelatinization is to open up the structure of lignocellulose so that the cellulose can be hydrolyzed by the enzyme.

After starch, cellulose and hemicellulose is hydrolyzed to C6 and C5 sugars then fermented into ethanol. Hemicellulose is a heterogeneous polysaccharide, it can be produced relatively easy to hydrolyze into sugar hexoses and pentose sugars that ready to be fermented into ethanol. Hexose sugars include glucose, mannose, galactose, and a little ramnosa. While the pentose sugars include xylose and arabinose (Taherzadeh and Karimi, 2007). Thing that Starch, cellulose, and hemicellulose have in common is they composed of simple sugars D-glucose which ready to be fermented by microorganisms to produce ethanol (Said, 1987).

Enzyme hydrolysis aims to hydrolyze cellulose and hemicellulose into sugar monomers. Enzymatic hydrolysis can be carried out using a hydrolase enzyme a-amylase enzyme, hemicellulase, cellulase, and amiloglucosidase (Gerhartz, 1990). Enzyme hydrolysis process is divided into three stages; the first is the

liquefaction, the liquefaction process pith of sago starch gel using the enzyme a-amylase (Judoamidjojo et al., 1992). The second stage is the hydrolysis of hemicellulose using hemicellulase enzymes. Purpose of hemicellulase enzymes is to convert hemicellulose into simple sugars units in the form of units of hexose sugars and pentose sugar unit. Then proceed with saccharification, which is split into glucose likuifikasi results using cellulase enzymes and enzyme amiloglucosidase (Judoamidjojo et al., 1992).

Stem pith of sago starch hydrolysis using α-amylase, hemicellulase, cellulase, and amiloglucosidase. Based on the previous research Flour pith of sago starch content was found to have 77.5%, 4.63% cellulose, 4.86% hemicellulose, lignin 3.07%, and 10.12% water. Starch hydrolysis process enzymatically provide value of DE (Dextrose Equivalent), at around 95 percent (Judoamidjojo et al., 1992). Hydrolysis of hemicellulose to produce two types of sugar, which is sugar pentose and hexose sugars. The resulting hexose sugars are glucose, galactose, and mannose, while the resulting pentose sugar is xylose and arabinose (Taherzadeh and Karimi, 2007).

Process of ethanol fermentation using fermentative microorganisms influenced by pH condition and the concentration of simple sugars. P. stipitis can grow well at pH 4.0 to 7. *S. cerevisiae* can survive at pH 4.0 to 7.0 (Marx, 1991), while *Z. mobilis* can grow well at pH 6.0 to 7.0 (Gunasekaran et al., 1986).

According Obire (2005) pH range for *S. cerevisiae* is optimum at pH 3-7 and 5, to *P. stipitis* has an optimum pH range of 4-7 and at pH 5, and to *Z. mobilis* has an optimum pH range of 4.5 to 7 and at pH 7. Glucose fermentation using *Saccharomyces cerevisiae* to produce high concentrations of ethanol, tolerant of high concentrations of alcohol [12-18% (v / v)], resistant to high sugar levels and remain active in the fermentation temperature 4-320C. While *Zymomonas mobilis* is superior because, it is more resistant to high temperatures and acidic pH (Aziz, 2002). Based on the Haagensen (2005) research, *Saccharomyces cerevisiae* only capable to fermenting hexose sugars, while *Pichia stipitis* could ferment pentose sugars Haagensen (2005) xylose. Productivity and the amount of ethanol produced by P. stipitis xylose fermentation is greater than *S.cerevisiae*. *Z.mobilis* is a type of bacteria that can produce ethanol. As with *S. cerevisiae*, these bacteria can only convert hexose sugars to ethanol, but the ability fermentation is faster than *S. cerevisiae* (Davis et al., 2006).

MATERIALS AND METHODS

Materials

The usedequipments were HPLC (High Performance Liquid Chromatography), shakers, spectrophotometers, Stopwatch. The materials used in this study are the stem pith of sago starch (sago Metroxylon Rottb.), Culture *Pichia stipitis* CBS 5773, *Saccharomyces cerevisiae* D1/P3GI, and *Zymomonas mobilis* FNCC 0056. The enzyme a-amylase liquozyme supra, dextrozyme amiloglukosidase enzyme, the enzyme hemicellulase (Sigma), Sigma cellulase enzyme (Novozyme).

Methods

The method used in this research is descriptive like experimental methods in the laboratory. This research is generally divided into two stages, the stem pith of sago starch hydrolysis into reducing sugars and fermentation of hydrolyzate into ethanol formed. Sago trunk in the process in the form of powder size of 100 mesh. Tepung in gelatinization by stem pith of sago starch in the suspension and then incubated at a temperature of 120^{o}C for 20 min with a pressure of 1 atm. Subsequently the pH was adjusted to 6.0 using a solution of Hydrochloric Acid (HCl) and 1 N sodium hydroxide (NaOH) 1 N. The enzyme a-amylase is added at 0.17 mL / g (volume enzyme / g substrate). After it was incubated at 104^{o}C for 60 min with a pressure of 1 atm.

The results obtained are first measured the concentration of sugar hydrolyzate and dextrose equivalent (DE). I hydrolyzate is heated at a temperature of 121^{o}C with a pressure of 1 atm for 10 minutes. Hemicellulase enzyme is then added as much as 1/3 dose. Hydrolyzate subsequently incubated at 55^{o}C with agitation at 150 rpm for 270 minutes. Then the measured parameter is the concentration of reducing sugar and dextrose equivalent (DE). Hydrolyzate has cooled to 25^{o}C temperature. Then the pH was adjusted to 4.8 using 1 N HCl and NaOH 1 N.

Followed by the addition of cellulase enzymes as much as 0.55 mL / g and 0.37 mL enzyme as much amiloglucosidase / g (volume enzyme / g substrate). Subsequently incubated at 60°C for 48 h with agitation speed of 130 rpm.

At this stage, the measured parameter is the concentration of reducing sugar. Before entering the stage of fermentation, and cellulase enzyme used amiloglukosidase heated at 100°C for 10 minutes (Gerhartz, 1990). Sugar hydrolyzate saccharification results then set to a concentration of 10% and 20%. Each hydrolyzate plus sugar fermentation medium containing (per liter): yeast extract 4 g, 2 g KH_2PO_4, $(NH_4)_2SO_4$ 3 g, 1 g $MgSO_4.7H_2O$, and pepton 3, 6 g (Sanchez et al., 2002). After the substrate pH adjusted using HCl 0.83 N and 0.83 N NaOH. For fermentation by *P. stipitis* CBS 5773 and *S. cerevisiae* D1/P3GI made pH 5 medium, and fermentation by *Z. mobilis* 0056 FNCC made medium pH 7. Fermentation substrate sterilized in an autoclave at a temperature of 1210°C for 15 min with a pressure of 1 atm. The parameters used were: concentration of total sugar concentration, Dextrose, bacterial cell number, pH and the concentration of ethanol.

RESULTS AND DISCUSSIONS

Enzymatic Hydrolysis of Sago Pith Flour

Hydrolysis process begins with gelatinization, is a mechanism of entry of water into the starch granules that will facilitate the work of the hydrolyzing enzyme substrate.

Table 1. Sugar DE (Dextrose Equivalent) value of Hydrolysis Enzymatically of Sago Pith Flour

Enzyme	Cons. Of Sugar (%)	DE
Alpha amylase	40.75	52.41
Hemicellulase	42.69	54.90
Amyloglucosidase + cellulase	47.95	61.66

Alpha-amylase break down amylose molecules produce glucose and maltose, into the amylopectin, work of this enzyme produces glucose, maltose, and dextrin. Based on reducing sugar measurement results obtained DE value of 52.41%. Further provision of hemicellulase, hydrolyze hemicellulose into glucose and xylose (Howard et al., 2003), and the obtained value of 54.90% of DE.

Giving of cellulase will convert cellulose into glucose, while amyloglucosidase convert amylose and amylopectin into glucose monomers, since this enzyme is able to break the bonds of alpha-1,4 glycosidic and 1,6 alpha-glycosidic so dextrin become glucose and at this stage DE values generated by 61.66%. Increase in value due to Dextrozyme DX DE, breaking the glycosidic bond α-1, 4 and α-1,6 Dextrozyme DX, is a combination of amyloglucosidase, and pullulanase so the process of bond breaking α-1,6-glycosidic become faster and more effective. Overall, the pith of sago starch hydrolysis enzyme producing reducing sugar concentration of 47.95% with a DE value of 61.66%, its mean that there is about 38.34% has not been hydrolyzed.

Fermentation of sugar hydrolizate of Sagu pith Flour by *Pichia stipitis* CBS 5773 *Saccharomyces cerevisiae* D1/P3GI, and *Zymomonas mobilis* FNCC 0056

pH of Fermentation

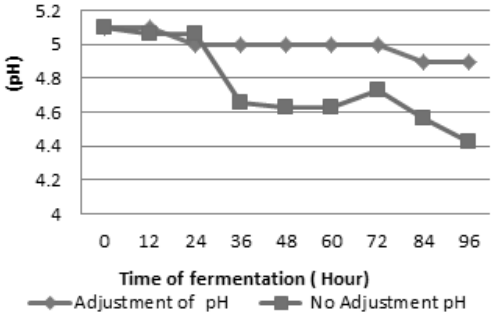

Figure 1. Changes in pH during fermentation by Pichia stipitis

Figure 1 showed that during the fermentation process, the pH has decreased; due to the formation of acid compounds results byproducts of metabolism. Initial acidity of the fermentation medium is 5, where the pH is an optimum pH for growth of *P. stipitis* for growth processes. Treatment pH adjustment is done to maintain optimum conditions for the growth of *P. stipitis*, where the pH is maintained between 4.8 to 5 settings. Whereas untreated pH adjustment pH decline, which was initially 5.1 to 4.43, this means that the pH has decreased by 13.1%.

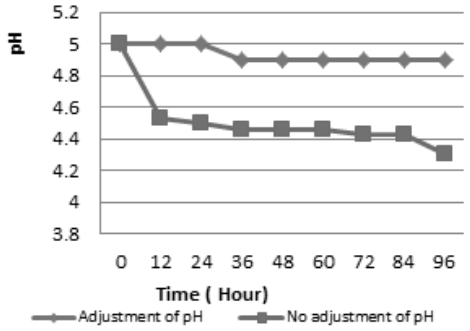

Figure 2. Changes in pH During Fermentation by Saccharomyces cerevisiae

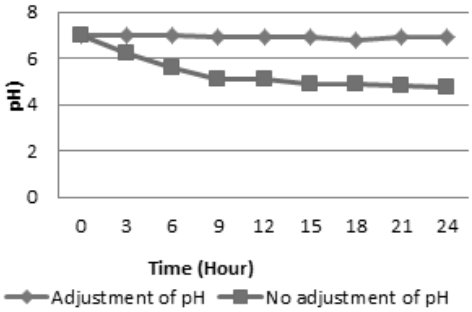

Figure 3. Changes in pH During Fermentation by Zymomonas mobilis FNCC 0056

On fermentation by *S. cerevisiae* of this study, the pH of the fermentation medium is set by 5, due to a pH range of 4-7 pH optimum (Obire, 2005). During the 96 hours fermentation pH decreased from the initial pH of 5.0 to 4.3, however, the pH was produced the optimum pH range. PH changes during fermentation by *Z. mobilis* down from pH 7 to 4.76, a decrease in pH by 42%. The decrease in pH due to *Z. mobilis* produce lactic acid as a product of metabolism (Buchanan and gibbons, 1994). Changes in pH caused by fermentation substrate that cells use ammonia as a nitrogen source is converted into NH4+. NH4+ molecules will merge into the cell as R-NH3. In this process H + left in the media, so that more biomass and fermentation time increased H^+ ion is causing more and more in the substrate which causes the lower the pH of the media (Fardiaz, 1988).

Fermentation ability of *P. stipitis*

Fermentation medium with initial sugar concentration of 10% containing 8.34% glucose and 1.20% maltoheptase. Changes in sugar concentration and ethanol concentration during fermentation can be seen in the following figure 4.

pH treatment adjustment effect the pattern of sugar consumption by microorganisms, fermentation with pH adjustment is able to consume more sugar than without pH adjustment, this is because the growth is at the optimum pH range is between 4-6.4-6. Sugar concentration for pH adjustment treatment, down from 8.41% to 2.68%, resulting in a decrease of 68%. While fermentation without pH adjustment only sugar concentration decreased by 55.2%, with 8.34% initial sugar concentration drops to 3.73%. The decrease in the concentration of sugar because sugar is used for metabolism can also be due to the low pH of the medium .

Efficiency of fermentation by *P. stipitis* with pH adjustment is smaller than that without pH adjustment, pH adjustment to the treatment efficiency of fermentation by 26% and for the culture without pH adjustment treatment efficiency of fermentation by 29.3%. Value fermentation efficiency by *P. stipitis* is low due to its ability to ferment glucose is very low, because the best is the ability to ferment pentose sugars, namely xylose (Almaeda et al., 2008).

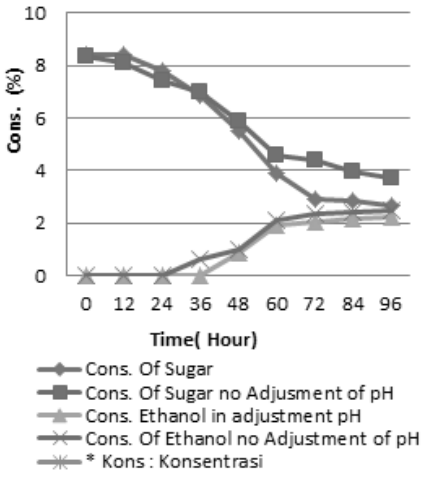

Figure 4. Concentrations of Sugar and Ethanol During Fermentation by P. stipitis

Result of lower concentrations of ethanol produced during fermentation is also due for

the addition of NaOH pH adjustment, and led to an influx of oxygen into the bottle fermentation, so it can affect the stability of the fermentation process, the ethanol fermentation process when it should be in a state of anaerobic (Almaeda et al., 2008).

Ability ethanol fermentation by *Saccharomyces cerevisiae* D1/P3GI. Fermentation medium with initial sugar concentration of 10% containing 8.48% glucose and 1.52% maltoheptaosa. Percentage of sugar concentration and ethanol produced during fermentation (96 hours) by *S. cerevisiae* by treatment setting and without pH adjustment can be seen in Figure 5.

Concentration of ethanol produced in fermentation by *S. cerevisiae* without adjusting the pH at 3.65%, and the pH adjustment of 2.17%. Efficiency ethanol fermentation by *S. cerevisiae* without pH adjustment of 43.3%, whereas the treated fermentation pH adjustment has an efficiency of 25%. According Periyasamy et al (2009) states that the maximum ethanol that can be produced in theory is 51%, meaning that 1 gram of glucose can produce 0.51 grams of ethanol. *S. cerevisiae* produces a maximum of 53% ethanol with optimum conditions, at pH 4 and temperature of 35^{o}C.

No fermentation efficiencies up to 51% due to the formation of acetic acid, lactic acid, and the formation of heat and steam, thus inhibiting *S. cerevisiae* to produce ethanol, while the lower fermentation efficiency by adjusting the pH due to the addition of sodium hydroxide, so that it can inhibit the growth and affect the concentration of ethanol produced (Rahman, 1992).

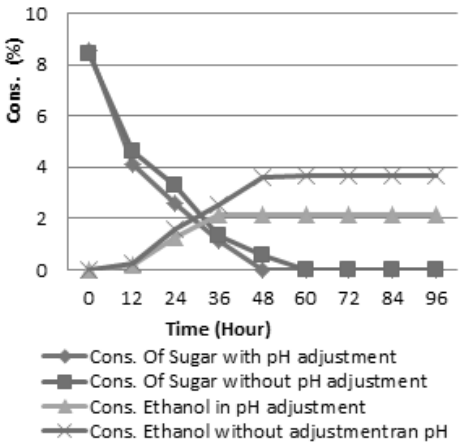

Figure 5. Concentrations of Sugar and Ethanol During Fermentation by Saccharomyces cerevisiae D1/P3GI.

Ethanol Fermentation Capabilities By *Zymomonas mobilis*.

Initial concentration of reducing sugar used in the fermentation of glucose is composed of 8.13% glucose and 1.87% maltoheptase. Consumption pattern of sugar fermentation showed the pH adjustment is able to consume more sugar than without pH adjustment. Sugar concentration during the final 24 hours of fermentation by *Z. mobilis* can be seen in Figure 6.

During fermentation sugar concentration decreased by 72.1%, the initial sugar concentration of 8.13% to 3.37% in the fermentation without pH adjustment, and with pH adjustment in sugar consumption by 58.5% where the initial sugar concentration amounted to 8.22% down to 2.29%. According Obire (2005), *Z. mobilis* not able to ferment the sugar arabinose and maltose, arabinose contained in the medium is only used to add biomass to produce ethanol instead, *Z. mobilis* is a bacterium that has the rate of glucose consumption, ethanol production and a high tolerance to ethanol.

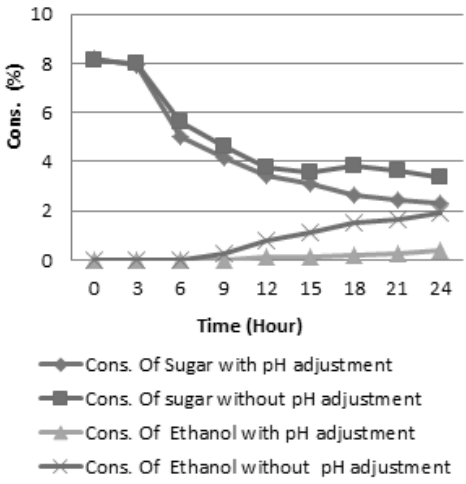

Figure 6. Concentrations of Sugar and Ethanol During Fermentation by Z. mobilis

CONCLUSIONS

Saccharomyces cerevisiae D1/P3GI is best microorganisms to ferment sugars in the enzymatic hydrolysis results pith of sago starch to ethanol with no pH adjustment is obtained with a concentration of 3.65% ethanol and fermentation efficiency of 43.3%, while the pH adjustment with the highest concentration of ethanol produced by *Pichia stipitis* CBS 5773 by 2.91% with a fermentation efficiency of 29.3%.

pH adjustment provides increased consumption of sugar, but the produced ethanol concentrations did not differ with fermentation without pH adjustment.

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SPARKLING WINE QUALITY IN A ROMANIAN WINE PROCESSING UNIT-STUDY CASE

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Abstract

Based by the law of Vineyard and Wine, the wine quality category represents the level resulted upon its chemical, physical and sensorial features, established by the natural conditions, cultivated species and the applied technology. Wines designated to the public consumption have to be obtained by authorized treatments and practices and correspond to some features required by the hygiene standards. Due to the more and more increased importance of the quality of food products in the modern society, the present study proposes to assess the quality of five sparkling wine sorts processed in a Famous Romanian wine processing unit. There were analyzed the chemical and physical parameters and also the sensorial ones. The sensorial parameters referred to were: colour, smell, taste, aspect, pearling. The physical and chemical parameters referred to were: alcoholic concentration, total acidity g/l tartric acid, volatile acid g/l acetic acid, pressure at 20^0C, reducing carbohydrates. The result samples of the five wines were statistically compared and there were concluded upon the best quality. As a major conclusion it is noticed that the wine quality in all the studied sorts keeps the quality standards in food production.

Key words: *chemical, physical, sparkling wine, Romania,. sensorial parameters,.*

INTRODUCTION

Wine technology or oenology is the science linked to the study of the processes, methods and preparing and conditioning of wines and the other products obtained by wine and grapes juice to achieve some final products to the market (pop, 2004). Sparkling wines represents the most important and appreciated group of the special wines (Banu, 2000). These are effervescent wines which contain whole carbon dioxide, endogenous origin that means it comes from the second alcoholic fermentation of added sugar or residual one, due to the activity of the yeast in close recipients (Antoce, 2005; Marin, 2006). At the bottle opening and pouring in the glass, the sparkling wine produces a strong and long effervescence, due to the carbon dioxide releasing, as large foam, which disappear and appear again at the surface of the liquid (Cotea, 2005; Pomohaci et al., 2001). The quality of these wines is appreciated by fine savour, the richness and continuing foam and the finesse of pearls (Petcu, 2006).

MATERIALS AND METHODS

The present paper had as aim a study regarding the quality of the sparkling wine produced in a top unit of the Romanian industry. There were studied five sorts of sparkling wine: D.O.C. Extra Brut White, D.O.C. Brut White, D.O.C. Semidry White, D.O.C. Semidry Red and D.O.C. Brut Pink. There were carried out 50 samples of each sort. The quality of the wine products has in view two major aspects: the chemical and physical composition of the wine and its sensorial aspects. The quality control consisted in checking, examining, analyze and measuring the stipulated by standards parameters. These activities consisted in the studied unit in permanent checking on the technological flow and adopting the necessary measures to avoid different inconvenient. By the physical and chemical assessment of wine it was analyzed the sparkling wine quality by establishing the main parameters as: alcohol percentage, total and volatile acidity, reducing sugar, pressure and also the sensorial parameters.

The alcoholic concentration represents the content in ethylic alcohol, expressed in volume percents (% vol.), at 20ºC. It was used the

steam distillation method and the concentration establishing with the aid of hydrostatics balance.

The total acidity represents the sum of all the titrable acidities to pH =7, by adding a titrable alkaline solution. It was used a solution of NaOH 0,1 N, after anterior elimination of CO_2.

By volatile acidity it is understood the part of fat acids owing to acetic series in wines, free or as salts. The method principles is water steams releasing of the volatile acids and titrating the distillate with NaOH 0,1N in the presence of phenolphthalein as indicator.

The sensorial analyze of wine, called wine tasting is based upon the biologic senses of the peripheral organs of the organisms. This analyze solicits the five biologic senses: view (limpidity, colour, oxidative status), smell (intensity and purity of odour), hear (effervescence), taste (acidity, sweetness, astringency, bitterness) and tactile sense (temperature, consistence, fluidity). After sampling and analyze there were calculated the main variability parameters.

RESULTS AND DISCUSSIONS

The following chart presents our results regarding the alcohol percentage of the analyzed sparkling wines.

It may notice the low variation of this parameter among 11,5% and 12,5%.

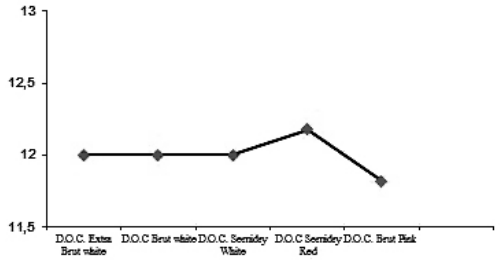

Figure 1. Alcohol percentage in the mean sample of the analyzed wines.

The total acidity expressed in g/l in tartric acid of the analyzed sparkling wines is presented in chart number 2. It may also noticed the variation of this parameter in the five analyzed wines, being recorded a decreasing of the values, starting from almost 6.31 g/l to 5,86 g/l. the lowest total acidity was recorded in D.O.C. Semidry Red.

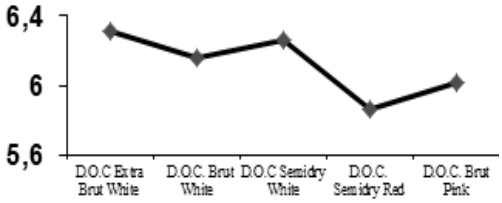

Figure 2. Total acidity percentage in the mean sample of the analyzed wines

The volatile acidity expressed in acetic acid g/l in the analyzed sparkling wines is presented synthetically in chart number 3. It may notice the different values of this parameter, starting from 0.55 to 0.65.

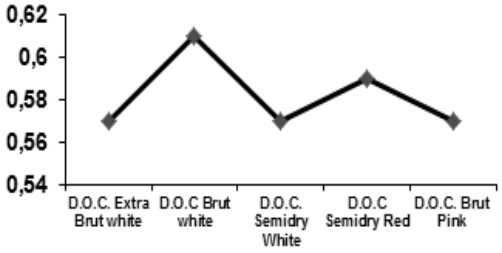

Figure 3. Volatile acidity percentage in the mean sample of the analyzed wines.

Chart number 4 presents the amount of reducing sugar expressed in g/l of the analyzed sparkling wines.

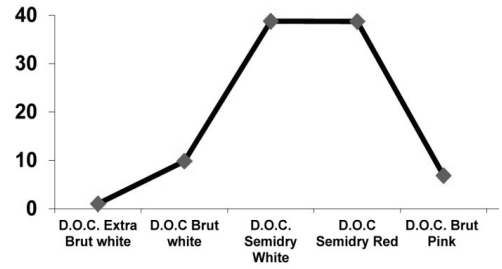

Figure 4. Reducing sugar in the mean sample of the analyzed wines.

Following the comparison of the five analyzed sparkling wines regarding the pressure at 20°C chart number five was made. The highest value

was recorded in D.O.C. Semidry Red and the lowest in D.O.C. Brut White.

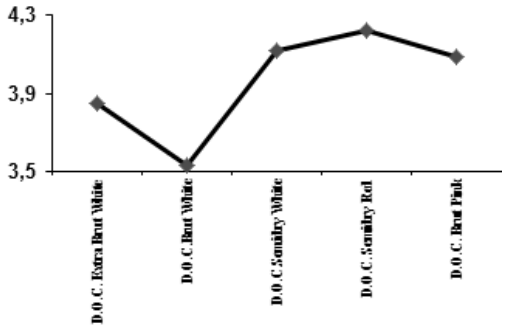

Figure 5. Pressure at 20°C in the mean sample of the analyzed wines

CONCLUSIONS

Upon the studies achieved to assess the quality of the five sparkling wines sorts we may conclude the following:

1. The all five sorts of sparkling wines analyzed in the elite unit in the Romanian industry are framed in the maximum admitted limits regarding the physical and chemical parameters, as in the sanitary veterinary standards.
2. Analyzing the content of alcohol it may notice that in all three white wine sorts, DOC Extra Brut, Brut White and Semidry White, the alcohol percentage was 12.00%, while in DOC Brut Pink, or DOC Semidry Red, the value of the alcohol percentage easily increased, it recording average values of 12.18% in DOC Semidry Red, respectively 11.82% in DOC Brut Pink.
3. The total wine acidity, expressed in g/l tartric acid had a mean value of 6.12 g/l, with small differences among the samples analyzed in the five sorts, varying between 5.86 g/l in DOC Brut Pink wine and 6.31 g/l in DOC Extra Brut white wine.
4. As the total acidity, the volatile acidity of wine, expressed in g/l acetic acid, a had a low variation, recording an average value of 0.58 g/l, with oscillations between 0.57 g/l in DOC Extra Brut wine, Semidry white wine and DOC Brut pink wine and 0.59 g/l in DOC Semidry Red, respectively 0.61 g/l in Brut white wine.
5. Reducing sugar content established for the five analyzed wine sorts recorded larger variations, explained by the sugar content of the sorts. Thus, it may notice that in the white dry wines, the concentration in reducing sugars was low, it having an average value of 1,02 g/l in white wine DOC Extra Brut and 9,82 g/l in Brut white wine, besides them being DOC Brut pink wine, with an intermediary average value of the reducing sugar of 6,84 g/l. The demi dry wines, the white one and the red wine recorded higher values, reaching 38,71 g/l in DOC Semidry Red and 38,77 g/l in Semidry white.
6. As in every sparkling wine, one of the analyzed physical feature was the pressure at 20°C. Conformingly the carried out analyzed, this feature had an average value of 3.96 atm, with low oscillations between the five analyzed sorts. It may notice that the superior value was recorded in DOC Semidry Red wine, 4.22 atm, followed by 4.12 atm in DOC Brut pink wine, then decreasing to 3.53 atm in Brut white wine.

Following the physical and chemical quality control in this unit, we may say that the studied unit is a processing unit which respects the stipulated standards and keep itself within the top units in Romania.

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A BETTER START IN BREAD QUALITY-ULTRA FIBER AND OTHER PLANT INGREDIENTS

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Abstract

Bread is considered to bea fermented confectionery product produced mainly from wheat flour, water, yeast and salt by a series of process involving mixing, kneading, proofing, shaping and baking. The consumption of bread and other baked goods such as biscuits, doughnuts and cakes produced from wheat flour is very popular, but the low protein content of wheat flour, which is the most vital ingredient used for the production of different kinds of baked goods has been major concern in its utilization. That is why, most of the bread producers tried to enrich the bread composition adding different ingredients so the nourishing quality of the final product being superior and best for the human being life. The present study has as view the comparison of the quality of two sorts of bread, special ones, enriched with ultra fiber and plant ester so that the consumers improve their health status. The first sort of bread is a major help to the diabetic people due to its high content in fibers and the second bread sort is a better support to human beings affected by cardiovascular disorders, due to the plant esters which reduce the serum cholesterol. The study tried to emphasize the main physical and chemical parameters of the two sorts of bread and to compare them to other frequent sorts in the Romanian market.

Key words: *sparkling wine, chemical, physical, sensorial parameters, Romania.*

INTRODUCTION

In every people history and civilization, bread is considered as symbol of a spiritual matter, a link between God and human being. All the early advanced civilizations knew bread and honored it like a gift of God. In Romania, it could say about the industrial processing of bread at the end of the IXth century and the beginning of the XXth century, this doing in the army, in towns and workshops. The importance of bread products in population requiring is a factor that determines the development of the Romanian bakery sector, in an accelerate trend. Lately, there were built new modern facilities, of large capacities, with a wide range.

MATERIALS AND METHODS

The material researched in the present paper is represented by five batches of two bread sorts with rye flour, which have contributions in cholesterol reducing by added plant stenol, being recommended to the people with cardiovasculary disorders and diabetes, but also to the ones who want a healthy style of life. We wish to emphasize their quality aspects, regarding sensorial and chemical parameters, as humidity, acidity, porosity, NaCl content, fat and insoluble ashes. The research methods were made by stipulated standards, also having in view the checking of wrapping, packaging, and marking, including the checking of the final products. The sensorial parameters represent for the large mass of consumers the most important factors of a food product analyses. The main sensorial features are: appearance, volume, color, smell, crust appearance, foreign corps and taste. The examining was done with the aid of senses, at natural light, in clean rooms, with specialized staff. The chemical and physical parameters were made by classical methods, conformingly SR.91/2007.

RESULTS AND DISCUSSIONS

Based on the carried out study on the recorded data and the analyze bulletins we have established the mean values for the chemical and physical parameters in five batches of two different sorts of bread. The first sort is represented by a specific sort with plant oil added (sort A), and the second one is

represented by a sort of bread with a low content in sugar (sort B).
The sensorial exam demonstrated that the bread in the five batches is framed in ISO standards, having no alternative disorders and normal sensorial parameters during the control analyses. The physical and chemical exam proved low variations of the analyzed parameters, comparable with the ones in the special standards (Petcu, 2006; SR.91/ 2007).
Humidity percentage carrying out is made in most of the bread products, and the method of drying in the oven represents the most precise and compulsory method (Petcu, 2006; SR.91/2007; Tapaloaga, 2008).
The mean value of the humidity percentage in the sort A is 44.16%, a value framed within the maximum admitted limit by the standard. (45%). Regarding the sort B, the mean value of humidity percentage is 46.96%, with a maximum value of 48%, as in the standards. In the chart 1, he Humidity dynamics in the analyzed sorts is shown.

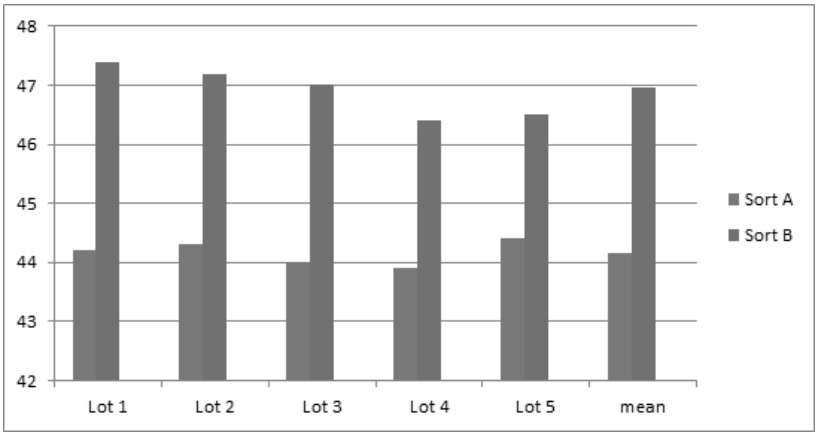

Figure 1. Humidity dynamics in the analyzed sorts

Sort A bread is framed within the ISO admitted limited, having a mean value 3.22°/100g product. During the laboratory analyzes there were recorded oscillation within the normal parameters, the maximum value being 3.5°/100g product. Sort B bread is framed within the ISO admitted limited, too, having a mean value 5.08°/100g product. During the laboratory analyzes there were recorded oscillation within the normal parameters, the maximum value being 5.4/100g product. These values are shown in chart 2.

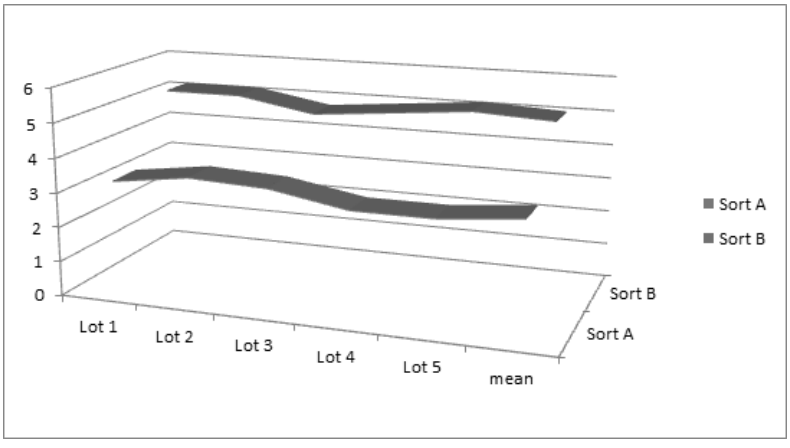

Figure 2. Acidity dynamics in the analyzed sorts

The elasticity is framed within the admitted limit, the mean recorded value on the five batches is 91.2, for sort A. The variations of this parameter are low, the minimum standard value being 89. For sort B, the mean recorded value is 97.4, being framed within the normal values, the minimum standard being 95. The graphic presentation of this parameter is illustrated in chart 3.

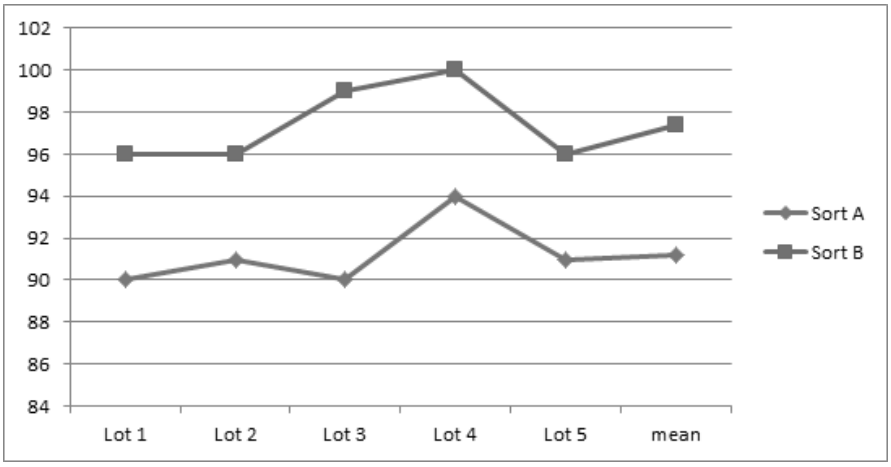

Figure 3. Elasticity dynamics in the analyzed sorts

Analyzing the total volume of the pores in the total core volume, knowing the density and the weight of core, we noticed that the porosity is 86.7%, framing within the standard, the minimum value being 85%, for the A bread sort. The sort B recorded the mean value of 87.12%., with a minimum standard of 82%. The graphic interpretation is presented in chart 4.

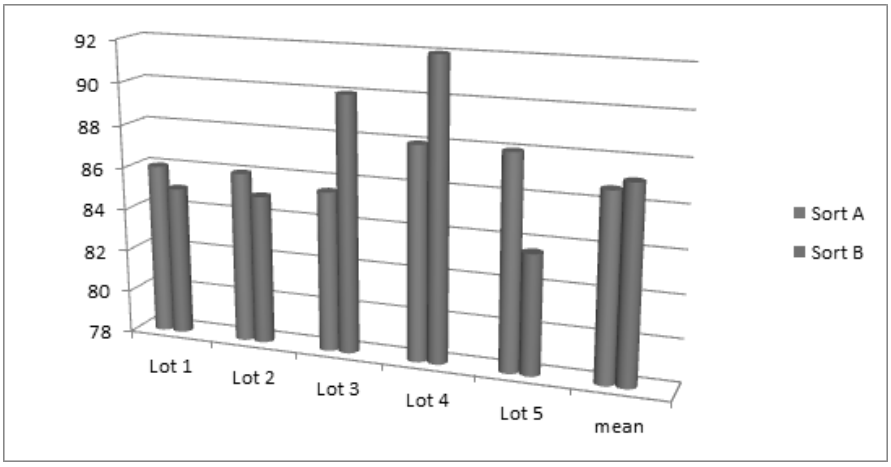

Figure 4. Porosity dynamics in the analyzed sorts

Following the laboratory analyses, it was noticed that the natrium chloride content is in normal parameters, with a mean value of 0.54%, for the sort A bread and 1.128% for the sort B bread, the maximum value being 1.2% for both bread sorts. The variation of this parameter in the five batches of sort A and B is presented in chart 5.

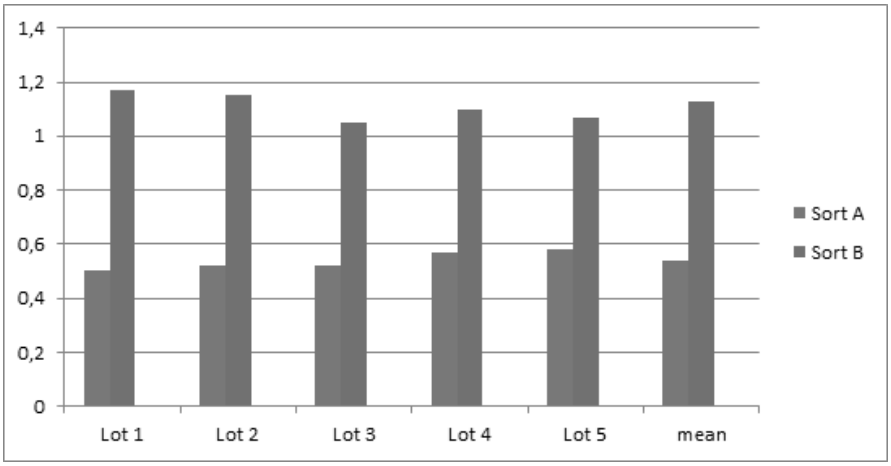

Figure 5. NaCl concentration dynamics in the analyzed sorts

By the sample calcinations and filtering of the insoluble substances in HCl, we noticed that the mean value of the ashes content is 0.17% for sort A bread and 0.176% for sort B, both mean values being framed within the standard norms, the maximum admitted limit being 1.2% for the two sorts. The graphic expression is shown in chart 6.

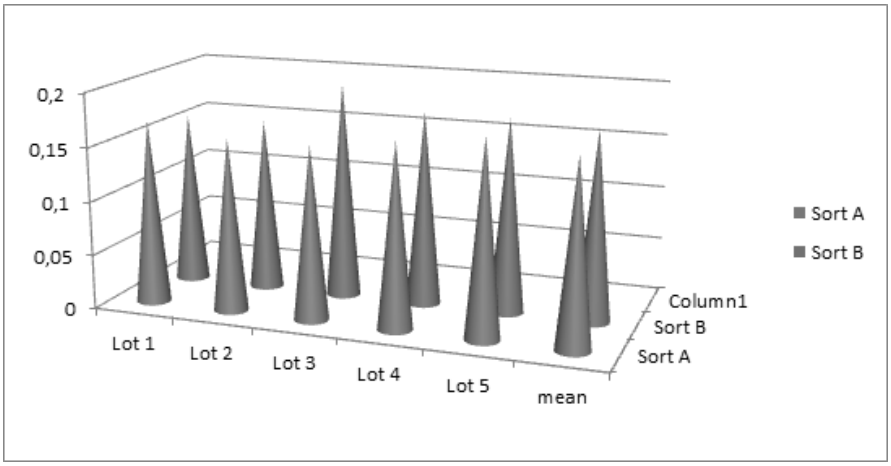

Figure 6. Insoluble ashes content dynamics in the analyzed sorts

The average value of the volume determination is 574 cm^3 for the five batches of sort A bread, with a minimum standardized values of 500 cm^3, and 480 cm^3 for the five batches of sort B, with a minimum standard of 520 cm^3. The graphic presentation of these parameters in all the studied batches is shown in chart 7.

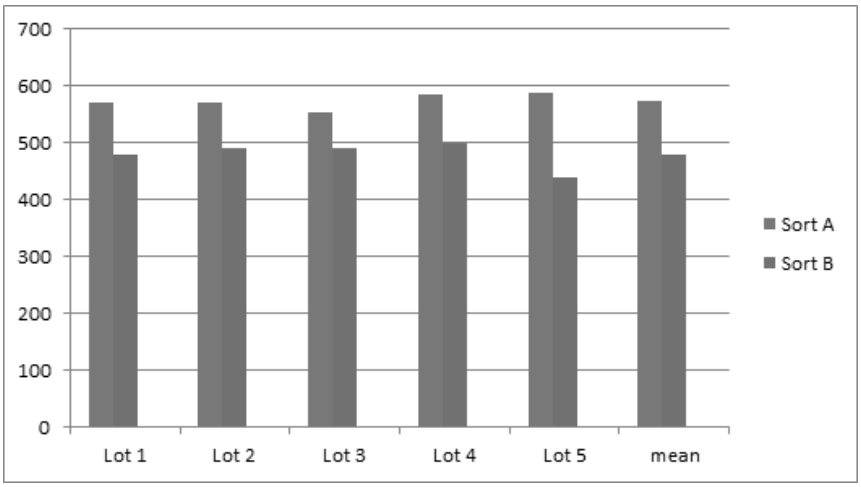

Figure 7. Volume dynamics in the analyzed sorts

The sensorial exam of the studied samples demonstrated that the analyzed bread does not present alterations regarding the shape, appearance, color, smell, taste, core, having all the sensorial qualities in normal conditions during the whole checking period.
The chemical and physical analyses demonstrate that the analyzed parameters are conformingly the reference standard.

CONCLUSIONS

After the sensorial, physical and chemical laboratory analyses we can conclude the following:

The taste in both bread sorts is a pleasant one, characteristic to the used ingredients, without foreign smell.

The core in the sort A bread is lighter brown and no wet, while the one in sort B is darker and a little wet.

All the values of the sensorial parameters are framed within the SR 91/2007 standard.

The humidity percentage in sort A bread is higher due to the technologic process.

The volume is smaller in sort A bread beside sort B bread.

The bread porosity percentage is higher in sort A bread due to the larger content in gluten, the mean recorded value is 87.12%, with a minimum of 82%, while sort b bread had a mean value of 86.7%, with a minimum value of 85%. Both values are framed in SR 91/2007.

The bread elasticity is lower in sort B bread, due to the added plant esters, even the recorded values framed within SR 91/2007, with a mean value of 86,7% and a minimum imposed value of 85%. In sort A bread, the mean value is 97.4%, with a minimum of 95%;

-bread acidity is higher in sort A, with a mean value of 5.08°/100g, beside 3.22°/100g, in sort B, with a maximum value of 3.5°/100g in this last sort.;

-NaCl content is higher in sort B bread beside sort A bread, the recorded mean values being1,128%, beside 0,54%, due to the necessity of a large content because the dough is more sticky and needs more salt.

-the ashes content in sort B bread is 0.176%, with a maximum value of 1.2% and in sort A is 0.170% with the same maximum imposed value. This thing is explained by the larger content in flour 650 in the composition.

Sort A bread is a healthy bread, with a large availability term, without preserving. Due to the controlled carbohydrates content and also fibers, is a real help to the overweight people and the ones with diabetes. The sort B bread is the most low carbohydrates content bread made by rye flour, having a plus of plant esters. So, for avoiding some organism′s disorders due to less healthy food, it is indicated to replace normal bread with special created sorts.

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PARTICULARITIES REGARDING PROCESSING TECHNOLOGY IN A CATERING UNIT

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Abstract

Food safety has to be assure and proven along all the stages of the food chain, starting from raw materials and ingredients obtaining, their transport, final products processing, marketing and consumption. The present paper has as major aim the emphasizing of the correct establishing of the processed and marketed product groups, and also of the correct and complete monitoring of the processing technology in catering type units.
It is implemented a food safety management system based on HACCP principles in the studied unit where was made the analysis. There were established five large groups, depending on the technological stages necessary for their processing. There were established the flow diagrams which include all the stages of the processing technology and after a documented analysis of safety food risks there were established the monitoring and keeping under control methods.
In the studied unit two critical points were identified and monitored and also two operational preliminary programs. The recorded made by the responsible designated people proved that the technological flows are kept under control, the control critical points are kept and the keeping of the operational preliminary systems could guarantee safe food products delivery to the consumers.

Key words: *catering, monitoring technology, processing stages.*

INTRODUCTION

Food safety has to be assure and proven along all the stages of the food chain, starting from raw materials and ingredients obtaining, their transport, final products processing, marketing and consumption.
The present paper has as major aim the emphasizing of the correct establishing of the processed and marketed product groups, and also of the correct and complete monitoring of the processing technology in catering type units. For all food products designated to human consumption it should be monitored the production stages, especially of critical control points and pre-operational programs (Savu and Petcu, 2002).

MATERIALS AND METHODS

The study was developed in 2011-2012 in a catering establishment in Bucharest. In the studied unit is implemented a food safety management system based on HACCP principles (Hazard Analysis and Critical Control Points).
There were established five large groups, depending on the technologic stages necessary for their processing. There were established the flow diagrams which include all the stages of the processing technology and after a documented analysis of safety food risks there were established the monitoring and keeping under control methods.

RESULTS AND DISCUSSIONS

In the studied unit, there were identified five major food groups: soups, main dishes, side dishes, salads and desserts. Flow charts include all necessary steps for food production technology. Functional circuits are provided, without any crosses between wholesome and unwholesome stages (Tapaloaga D., 2012).
The flow diagrams checking and validation were performed by food safety team.
As proof of validation it was drawn the meeting food safety team within the unit. For all technological stages are identified and analyzed physical, chemical and biological dangers. Potential risks were analyzed taking into account three important aspects: identifying potential contaminants, assessing the significance of potential risk and establish appropriate control measures to prevent,

eliminate and / or reduce potentially significant risk to an acceptable level.

When analyzing risk factors there were taken into working ingredients (Marin M., 2006) and used raw materials, each stage of the technological process, technological particularities, storage and serving products (EFSA, 2005).

The determination of critical control points was reviewed by modernizing production equipment (Petcu, 2006). In determining the critical control points performed a requirement analysis of all stages of the technological process, taking into account the performance of work equipment, staff training and level and the complexity of manufacturing recipes. In the study unit two critical points were identified and also two operational preliminary programs.

In 2011, the stage storage material that requires controlled heat treatment there was recorded an exceeding of the temperature values as a result of repeated open storage enclosure. For 2012, after analyzing the previously recorded event purchased a refrigerator compartment. Thus, there recorded fewer cases of non-fixed temperature (Figure 1). The monitoring was performed by the responsible staff.

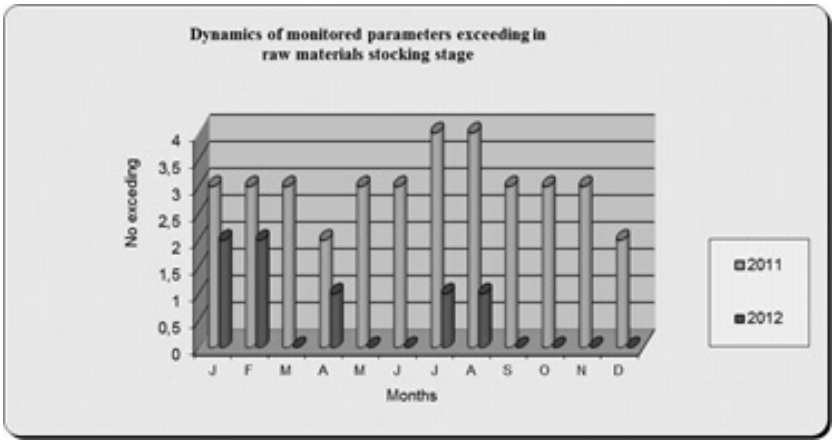

Figure 1. Dynamics of monitored parameters exceeding in raw materials stocking stage

The records made by the appointed officers demonstrated that technology flows are respected, that the parameters are kept under control settings in the critical control point of heat treatment.

There were no deviations from the temperature values set in the stage of thermal treatment products, and thus it can guarantee the delivery of safe products to consumers.

CONCLUSIONS

The correct grouping of foods is essential to meet manufacturing technology catering unit.

Production equipment plays an important role in maintaining the working parameters and the acquisition of cold chambers established the decreasing of nonconformities number with 80.55% in 2012 compared to 2011. The catering units have a complex mission in delivering food. These issues are directly influenced by the large number of food products to process and deliver requiring constant monitoring of technological stages.

The staff training is an objective standard which catering establishments have to pay attention.

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RESEARCHES CONCERNING SOME MEAT PRODUCTS CONTROL IN A SPECIALIZED UNIT

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Abstract

Meat products are going on to be on an important place in human being nutrition, because they are available in a large variety and could be consumed fresh, without any thermal processing. The present study has in view the legal requiring keeping range applicable to some meat products. During July 2011-April 2012 there were analyzed samples of meat and there were established the following parameters: water content, salt, nitrites, fat and protein. The obtained results proved that the recipes and processing technologies are kept, excepting a few samples which recorded an exceeding of the maximum admitted limit for salt content. Concluding, it is necessary the permanent monitoring of food industry units regarding keeping of the imposed parameters for the processed foodstuffs.

Key words: *meat, salt content, physical and chemical parameters.*

INTRODUCTION

Human nutrition is a constantly acting factor that determines the development of metabolic processes. Currently, food takes its toll on human pathology as a result of the imbalance between intake and requirement of biologically active substances. Animal foods, unlike those of vegetable origin (Cristea, 2012; Tapaloaga, 2012) have a higher biological value determined by content rich in almost all essential amino acids. Because time has become an issue of all, we tend to buy ready-to-eat foods that do not require additional training. Therefore, meat products industry have diversified, being available a wide range of meat products for consumption date (Petcu, 2006).

MATERIALS AND METHODS

This study aims to tracking compliance with the legal requirements applicable to certain categories of meat products. In the period July 2011-April 2012 there were analyzed meat samples, which were made sensorial tests and there were determined the following parameters: water content, salt, nitrite, fat and protein (Marin, 2006; Savu, 2002). Samples were collected so that more accurately represent the group which they originated from. A total of 23 samples, representing varieties of cooked and double smoked meat (sausages, salami summer), cooked meat (pork sausage), smoked and cooked meat products (Italian salami, Bucharest salami, smoked sausages) (Ord 210/2006), were collected and analyzed for physical and chemical examination carried out during July 2011-December 2011. In the second study period, January 2012-April 2012 there were analyzed 15 samples of meat products harvested at the end of the process flow. Analyses were performed in the program's self-profile unit and there were performed by internal laboratory. Used methods of analysis are the common methods performed to quickly highlight any nonconformity of products (Savu, 2002) .

RESULTS AND DISCUSSIONS

Results showed that compliance recipes and manufacturing technologies, except for samples with exceeding of the maximum permitted limit of salt. In the first study period there were recorded fair values of the analyzed parameters (Table 1). Following the laboratory, the water content was appropriate, which proves that they respect the manufacturing networks. Also, NaCl content ranged between 1.3 and 2.9, falling within the maximum limit for this parameter. Nitrite and easily hydrolysable

nitrogen have raised all relevant evidence. Also, the fat content and protein were suitable for all the analyzed samples previously mentioned.

Table 1. The results of physical and chemical examination of meat products analyzed during July 2011-December 2012

No	Sample	Water %	Fats %	NaCl %	Nitrites mg%	Protein %	mg NH_3 / 100g
1	Bucharest salami	52.3	28.6	2.3	6.2	16.8	32.6
2	Smoked sausages	54.8	25.9	2.2	5.9	17.1	38.5
3	Smoked sausages	58.1	23.4	2.3	4.6	16.2	41.2
4	Bucharest salami	54.2	25.7	2.3	5.1	17.8	43.2
5	Pork salami	59.2	22.2	2.4	3.3	16.2	37.8
6	Bucharest salami	51.6	27.7	2.6	5.8	18.1	39.5
7	Smoked sausages	48.6	30.7	2.6	4.3	18.1	29.8
8	Italian salami	51.2	29.2	2.1	5.7	17.5	32.4
9	Cabanos	49.8	33.8	1.9	5.4	16.5	35.4
10	Smoked sausages	51.9	28	2.9	5.2	17.2	34.5
11	Bucharest salami	52.4	26.7	2.8	4.6	18.1	36.8
12	Italian salami	49.8	30.6	2.7	5.3	16.9	31.8
13	Bucharest salami	52.4	27.9	2.9	4.9	16.8	32.9
14	Pork salami	56.8	23.1	2.8	5.8	17.3	39.2
15	Italian salami	51.6	29.7	2.3	5.7	16.4	35.2
16	Pork salami	52.8	27.4	2.3	4.6	17.5	39.5
17	Smoked sausages	51.3	28.3	2.3	5.6	18.1	32.6
18	Bucharest salami	52.1	30.3	2.4	5.4	15.2	35.6
19	Cabanos	52.3	26.5	2.8	5.2	18.4	35.6
20	Smoked sausages	51.6	27.3	2.9	5.1	18.2	37.8
21	Italian salami	51.6	28.2	2.7	5.4	17.5	34.6
22	Cabanos	49.5	32.8	1.3	5.8	16.4	32.3
23	Smoked sausages	48.9	29.8	2.9	5.6	18.4	33.2
		P.N.=0%	P.N.=0%	P.N.=0%	P.N.=0%	P.N.=0%	P.N.=0%

During January 2012-April 2012 there were analyzed 15 samples of meat products and the results of the physical and chemical determinations are presented in Table 2. There were exceeded, the salt content in 1 sample, representing 6.66% of the total samples analyzed between two of the study.

Table 2. The results of physical and chemical examination of meat products analyzed during January 2012-April 2012

No.	Sample	Water%	Fats%	NaCl%	Nitrites mg%	Protein%	mg NH_3 / 100g
1	Smoked sausages	54.2	25.5	2.5	5.6	17.8	29.5
2	Bucharest salami	52.6	26.9	2.3	3.5	18.2	35.6
3	Smoked sausages	57.4	22.8	2.4	5.6	17.4	38.6
4	Bucharest salami	51.5	29.5	2.3	5.6	16.7	35.9
5	Summer salami	49.2	31.2	2.8	4.8	15.8	36.9
6	Pork salami	57.3	27.5	3.3	4.1	13.1	37.6
7	Bucharest salami	56.3	27.4	2.5	6.2	13.8	34.5
8	Italian salami	58.2	26.1	2.9	6.7	12.8	36.8
9	Smoked sausages	57.2	28.9	2.1	5.8	11.8	34.6
10	Bucharest salami	60.2	24.4	2.3	6.2	12.1	34.5
11	Italian salami	57.3	27.6	1.9	6.2	13.2	35.6
12	Smoked sausages	58.2	27.2	2.1	4.9	12.5	31.6
13	Summer salami	37.5	39.5	1.9	5.1	21.1	34.8
14	Italian salami	51.2	34.2	2.5	4.8	11.8	42.5
15	Pork salami	62.3	23.6	2.3	5.1	11.8	33.2
Total		P.N. = 0%	P.N. = 0%	P.N. = 6,66%	P.N. = 0%	P.N. = 0%	P.N. = 0%

CONCLUSIONS

It is important that sampling be performed on homogeneous lots and representative of a third person to ensure the reliability of the results. The samples analyzed in the second study period, although they were fewer, have revealed that the proportions are not always that all constituents, behind the salt content in one sample. For the next period, it was recommended the analyses of a larger number of samples and personnel training to respecting

the manufacturing network. The continuous monitoring of food establishments is required in order to ensure safe products on the market, and if overtaking identification of parameters imposed by legal regulations must be followed the traceability.

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A WAY TO PREVENT SYNERESIS IN FRUIT FILLINGS PREPARED WITH GELLAN GUM

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Abstract

The present research investigates the effectiveness of incorporating amylopectin starch with gellan gum in fruit compositions to develop heat-stable fruit fillings resistant to syneresis. The heat-stable and water-retaining properties of the fruit fillings prepared in a wide range of soluble solids (30-70 ºBrix) and containing amylopectin starch and gellan gum in different concentrations were investigated. The novel amylopectin starch Eliane BC-160 was studied in bake-stable fruit fillings' development not only as water-binding agent but also for a partial replacement of gellan gum used to improve heat-stable properties. The fruit filling samples with the same pH and various soluble solids were produced locally from apple puree, sugar, low acyl gellan gum, amylopectin starch and citric acid. The degree of syneresis wad determined for all fruit fillings along with control samples prepared without amylopectin starch. During the study it was revealed that amylopectin starch could be applied in combination with gellan gum for the development of bake-stable and syneresis-free fruit fillings with high quality characteristics, while reducing the standard doses of each of the ingredients by finding their common synergetic effect on heat-stable properties.

Key words: *filling, gellan gum, syneresis, starch.*

INTRODUCTION

Hydrocolloids are high-molecular-weight biopolymer substances soluble in water which create viscous colloidal solutions. They are mainly used to influence functional properties of foods (structure, texture, etc.), improving heat-stable characteristics, adjusting the microstructure, prolonging shelf-life and maintaining taste firmness in frozen products (Mikuš et al., 2011). Hydrocolloids posses neutral taste and flavor, which enables them free access to food insertion. Natural hydrocolloid gums represent a good source of soluble dietary fiber (up to 85% of dry mass), which reduces the concentration of cholesterol and improves gastrointestinal functions and glucose tolerance (Sozer, 2009), while their energy value is minimal to none.

The most common hydrocolloid used in canning and confectionery industry is high methoxyl pectin because of its simplicity of gelatinization in low acidity in high soluble solids content conditions (Basu and Shivhare, 2010). However, other food hydrocolloids with strong heat-stable properties, like gellan gum, can be used in bake-stable fruit fillings formulations as pectin substitutes.

Every fruit filling should have a characteristic fruit flavor, with good mouth-clearing properties and no artificial or chemical aftertaste. Some fruit fillings need added flavor to compensate that which is lost or changed during processing or to make up for low fruit content. Gellan gum incorporated into fruit filling's composition can accent and intensify fruit flavor notes and aromas, which could be impossible to make with other hydrocolloids such as pectin, agar or gelatin – and acts so even in low quantities (from 0.1%).

It was also established in previous research that baking stability of a fruit filling could be enhanced by applying gellan gum as heat-stabilizer. Although this hydrocolloid was used at concentrations less than 1% (from 0.1 to 0.9%) in fruit fillings formulations, it possessed substantial effect on heat-stable, textural and sensory properties of final products. However, gellan gum requires the presence of another stabilizer with good water-retaining properties, because fruit fillings made only with gellan gum as stabilizer exhibit a strong tendency to syneresis, which negatively affects the quality of pastry after baking and leads to a finished product with a less desirable appearance.

The name and description of syneresis was given by Graham in 1864 to the phenomenon of the breaking up of jellies on long standing or when disturbed. The jelly product, instead of consisting of one homogeneous mass, becomes segregated into solid lumps surrounded by a thin liquid (Graham, 1876).

Starches are widely used in fruit fillings, creams, toppings and glazes alone or in combination with hydrocolloids such as pectin, gelatin, agar, alginates and others. In cold prepared fruit fillings, starches are applied to retain free water avoiding syneresis, and to ensure also clarity, quick viscosity built-up and a smooth shiny appearance of the final product.

Some modern types of starches exhibit more shear-resistance, more freeze/thaw and heat stability and a certain type of texture compared to ordinary native starch. The novel amylopectin starch Eliane BC-160 is worth to be studied in heat-stable fruit filling development along with gellan gum, because it not only can prevent water release, but can also provide proper texture, eliminate bake-out of the filling while baking, improve appearance and ameliorate freeze/thaw properties of the finished product. This starch can also resist breakdown from shear, low pH and heat attack. For extremely high-solid fillings, the amylopectin starch is especially required due to its good cold-water-swelling properties.

The aim of this study consists in the development of heat-stable and completely syneresis-free fruit fillings may be improved by applying gellan gum in conjunction with a special starch with a high temperature resistance, such as amylopectin starch, in order to minimize the doses of each of the ingredients.

MATERIALS AND METHODS

Raw materials

Sugar was purchased at a local supermarket (Chisinau, Republic of Moldova). Apple aseptic puree was manufactured at the canning plant 'Conserv-E' (Chisinau, Republic of Moldova). Citric acid solution (50%) was prepared locally in the Laboratory of Functional Foods of the Practical Scientific Institute of Horticulture and Food Industry (Republic of Moldova). Low acyl gellan gum powder (KELCOGEL F) was acquired from the Moscow International Exhibition for Food Ingredients, Additives and Flavorings – "Ingredients Russia" (Moscow, Russian Federation). Amylopectin potato starch Eliane BC-160 (AVEBE) was kindly supplied by the Trading House AVERS (Sankt-Peterburg, Russian Federation).

Sample preparation

The fruit filling samples were produced locally in a wide range of soluble solids (30÷70 ºBrix) from apple puree (12 ºBrix), sugar, low acyl gellan gum KELCOGEL F, amylopectin potato starch Eliane BC-160 and citric acid on the basis of two-level factorial design. In this research gellan gum and amylopectin starch were selected in two concentrations according to the levels established in design of experiments. The levels of these concentrations (%) were set at: 0.5 (min) and 1.0 (max) for amylopectin starch and 0.1 (min) and 1.0 (max) for gellan gum.

The fruit filling samples were prepared according to the procedure for jam manufacturing presented by Basu et al., i.e. mixing the ingredients, evaporation to reach the desired Brix level, finally cooling (Basu et al., 2011). The total soluble solids were monitored through the fruit filling making process with a benchtop refractometer ABBE to reach the required soluble solids content according to the filling formulations. All samples of fruit fillings were prepared with the same pH (3.35) established by a potentiometric method, introducing the electrode directly into the fruit fillings.

To achieve proper structure formation through gel setting, the fruit filling samples were placed in refrigerator (after sterilization in glass jars) for up to 24 hours at (4 ±1ºC). After that filling samples were removed from the refrigerator for further testing. The test results obtained through application of two-level factorial design were verified by conducting the validation experiments under the optimized conditions.

Determination of syneresis

Degree of syneresis in fruit fillings samples was measured before bakery test for thermal stability determination, as follows: a specific amount of prepared fruit filling was given into

a base of special filter paper named 'Blue ribbon' with a diameter of 120 mm by a metal ring with defined geometry (50 mm diameter and 10 mm height) and the diameter of released liquid was measured after 30 minutes. Then the filling samples were baked under exactly fixed conditions: at a temperature of and 220°C for 20 minutes (*Herbstreith & Fox KG) to determine their thermal stability*. The syneresis degree was determined by measuring the released liquid diameter by placing a line across the sample and calculating using the following formula:

$$S = \frac{D_2 - D_1}{D_2} \cdot 100$$

(1)

where

S – syneresis degree,%;

D_1 – average sample diameter, mm;

D_2 – average released liquid diameter, mm.

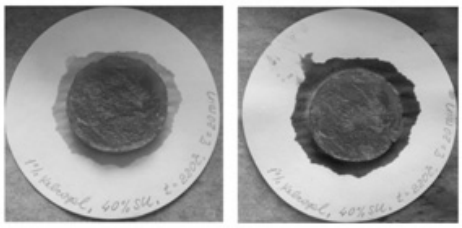
Diameter of the filling sample before baking is 50 mm, because it′s the diameter of the metal ring used in bakery test. For measuring the released liquid diameter depending on its shape, from two to four lines were drawn, and the average was calculated.

the pastry samples we selected another metal ring with the following dimensions: 30 mm diameter and 10 mm height.

RESULTS AND DISCUSSIONS

Quality characteristics of the fruit fillings analyzed under laboratory conditions have demonstrated that they meet the international food standard CODEX STAN 296-2009 FOR JAMS, JELLIES AND MARMALADES.

The lowest achievable syneresis degree is 0 when the sample is completely stable and no water releasing from the fruit filling is observed. Fruit filling is considered syneresis-free if its syneresis degree is in the range 0÷5.



a) b)

Figure 1. Appearance of a fruit filling prepared only with gellan gum as stabilizer: before (a) and after baking at 220ºC for 20 minutes (b)

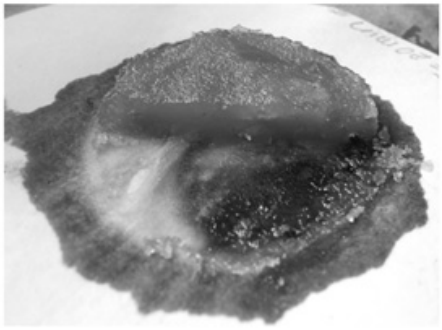


Figure 2. Cross-sectional view of a fruit filling prepared only with gellan gum after baking at 220ºC for 20 minutes

Judging from the first picture, it is evident that fruit fillings prepared with gellan gum Kelcogel F (0.5 – 1%) are characterized by high thermal stability (BI = 90÷100), maintaining their original shape, volume and initial sensory characteristics after baking under a temperature of 220^{o}C for 20 minutes. But despite this, gellan gum requires the presence of one more ingredient with water-binding properties, because fruit fillings made with only one gellan gum exhibit a strong tendency to syneresis, which may negatively affect the quality of pastry after baking. Formation of the 'absorption band' on the filter paper around the filling (with a characteristic color after baking process) indicates the separation of moisture (Figure 1 and 2).

a) b)

Figure 3. Appearance of fruit fillings prepared with gellan gum and amylopectin starch: before (a) and after baking at 220ºC for 20 minutes (b)

Adding the amylopectin starch even at low quantities (0.5 – 1.0 w/w) into fruit fillings prepared with gellan gum, not only prevents them from syneresis, but also improves heat-stable properties of the finished products (Figure 3).

The table 1 below shows the relationship between syneresis degree and percentage of gellan gum and amylopectin starch in fruit fillings prepared with various soluble solids.

Table 1. Relationship between gellan gum and amylopectin starch contents and syneresis degree of fruit fillings prepared within a large range of soluble solids

Soluble solids, °Brix	Gellan gum content, w/w	Starch content, w/w	Syneresis degree
30	0.1 – 1.0	0.6 – 1.0	0 – 5
40	0.1 – 1.0	0.5 – 1.0	0 – 5
50	0.1 – 1.0	0.5 – 1.0	0 – 5
60	0.1 – 1.0	0.5 – 1.0	0 – 5
70	0.1 – 1.0	0.5 – 1.0	0 – 5

According to the data presented in table 1, for the same low percentage of gellan gum (0.1 – 1.0%) used as heat-stabilizer, the least amount of amylopectin starch is required to eliminate syneresis in bake-stable fruit fillings prepared with 40, 50, 60 and 70 soluble solids. For the production of heat-stable fruit fillings with 30°Brix a slightly more amylopectin starch is needed to achieve the lowest syneresis degree than in previous examples.

Therefore, it is more advantageous to use this type of starch as water-binding agent for production of heat-stable fruit fillings prepared within 40-70°Brix with addition of gellan gum.

CONCLUSIONS

At present time consumers demand high-quality foods in various innovative forms at competitive prices. Developing the novel heat-stable and syneresis-free fruit fillings will lead to the emerging of new high quality products for baking, confectionery and canning industries.

As a result of conducted investigation it was established that gellan gum could be applied in combination with amylopectin starch for the development of bake-stable and syneresis-free fruit fillings with high quality characteristics, while reducing the standard doses of each of the ingredients by finding their synergetic effect on heat-stable properties.

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HELMINTH COMMUNITIES OF FISHES FROM THE RIVER DANUBE AND LAKE SREBARNA, BULGARIA

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Abstract

Ecological monitoring from water of the River Danube and Srebarna Lake was performed using freshwater fishes and their parasites and parasite communities as bioindicators. For an ecological evaluation of the situation of the analyzed freshwater ecosystems, principal biotic indexes were fixed. The analysis of the dominant structure of the found taxa was presented to the level of the component communities. During 2012, 16 species and 181 specimens of freshwater and passage fish were examined with standard techniques for parasites. Six species of examined fish (Aspius aspius (L., 1758) , Carassius gibelio (Bloch, 1782), Chondrostoma nasus (L., 1758), Zingel zingel (L., 1758), Cyprinus carpio (L., 1758) and Lepomis gibbosus (L., 1758)) were free of parasites. In ten species of fish (Abramis brama (L., 1758), Alburnus alburnus (L., 1758), Alosa pontica (Eichwald, 1838), Ballerus sapa (Pallas, 1811), Barbus barbus (L., 1758), Romanogobio albipinnatus (Lukasch, 1933), Gymnocephalus schraetser (L., 1758), Neogobius fluviatilis Pallas, 1811, Perca fluviatilis L., 1758, Rutilus rutilus (L., 1758)) seven species of parasites (Gyrodactylus elegans Nordmann, 1832, Diplozoon paradoxum Nordmann, 1832, Nicolla skrjabini (Iwanitzky, 1972), Pomphorhynchus tereticollis (Rudilphi, 1809), Eustrongylides excisus (Jgerskild, 1909) larvae, Hysterothylacium aduncum (Rudolphi, 1802), Camallanus truncatus (Rudophi, 1814)) were fixed. New parasite and host records were determined. All fixed parasite species are core for the parasite communities of examined fishes with the exception of N. skrjabini. Bioindicator significance of parasite species was studied.

Key words: *bioindication, fish parasite communities, heavy metals, Lake Srebarna, River Danube.*

INTRODUCTION

The Danube River is the second longest river in Europe (about 2 800 km length), connecting Central and South-Eastern Europe and flows through nine countries (Germany, Austria, Slovakia, Ukraine, Hungary, Croatia, Serbia, Bulgaria, Romania). The richness in habitats, flora and fauna determine Danube River as important ecosystem for protection of biodiversity. The river basin, including tributaries and wetlands, is home to around 2 000 plant and 5 000 animal species, including numerous endangered or nearly extinct species. The Bulgarian part of the river and its wetlands on the Lower River, include Lake Srebarna, have important place in the Bulgarian and Europaean ecological network. While the river and adjacent wetlands are under permanent negative anthropogenic impacts of industrial accidents and wastewaters. As a result, pollutions of the water ecosystems killed a lot of fishes and other freshwater organisms (Literathy and Laszlo, 1995, 1999). Parasites of freshwater fishes and their communities are reliable as sensitive indicators of heavy metals in aquatic ecosystems (Baruš et al., 2007; Djikanovic, 2012; Nachev et al., 2010; Marcogliese and Cone, 1997; Oros and Hanzelová, 2009; Overstreet, 1997; Tielen et al., 2004, etc.). Fish parasite communities, heavy metal content and the state of freshwater ecosystem of the Danube River are studied from different authors (Atanasov, 2012; Gabrashanska et al., 2004; Kakacheva-Avramova, 1977, 1983; Kakacheva, Margaritov, Grupcheva, 1978; Margaritov, 1959, 1966; Michalovic, 1954; Moravec et al., 1997; Nachev, 2010; Nache, Sures, 2009; Nedeva et al., 2003; Ricking and Terytze, 1999; Woitke et al., 2003, etc.) but they are comparatively small from the Srebarna Lake (Hristov, 2010; Margaritov, 1959; Shukerova 2007; Shukerova, Kirin, 2008; Shukerova,

Kirin, Hanzelová, 2009, etc.). This paper presents the results of an examination of heavy metal content in sediments, fish tissues and organs, fish parasites and dominant structure of fish parasite communities from the Bulgarian part of the Lower Danube River (villade of Vetren) and the Danube wetland with international importants, Lake Srebarna.

MATERIALS AND METHODS

During June, 2012 sediments, fish and fish parasites were collect and examined from the Lower Danube River (village of Vetren, Bulgarian part) and Lake Srebarna, Fig. 1:

Figure 1. Danube River and Lake Srebarna

Figure 1. Danube River and Lake Srebarna

The village of Vetren ($44^0133'$N, $27^0033'$E) is situated on the riverside, in the northeastern part of the Danube Valley. About 5 km from the village of Vetren is located Lake Srebarna. It is declared as a Biosphere Reserve (UNESCO), as site of the World Natural Heritage (Ramsar Convention), as an object in the List of Wetlangs of International Importance and Important Bird site (BirdLife International). The Srebarna Lake is situated in Northeastern Bulgaria ($44^07'$N, $27^05'$E) near to village of Srebarna. It is freshwater euthrophic lake connected throught an artificial canal with the Danube River. The lake is distinguished, as well as the Danube River, with significant diversity of highly protected species (Michev et al., 1998; Uzunov et al., 2001; Pehlivanov et al., 2006, etc.).

A total of 10 sediment samples and 181 freshwater and passage fish specimens belonging to 5 families and 16 species were collected and examined in June, 2012. The fishes were caught by nets, by angling and eloctrofishing under a permit issued by the Ministry of Agriculture and food and Ministry of Environment and waters of Bulgaria. The scientific and common names of fish hosts were used according to the FishBase database (Fröse and Pauly, 2012).

Samples of sediments were collected according to the Guidance on sampling of rivers and watercourses-ISO 5667-6:1990, introduced as a Bulgarian standard in 2002. Heavy metal concentration of the water and sediment samples, fish tissues, organs and parasites were carried out according to standard techniques. The samples were analyzed for content of Cd, Cu, Pb and Zn by ICP Spectrometry (Bíreš et al., 1995).

The model of fish species chosen for examination of the heavy metal content in this study were the European perch, *Perca fluviatilis* L. of the Danube River and Lake Srebarna and Common barbel, *Barbus barbus* (L., 1758) of the Danube River.

Helminthological examinations were carried out following recommendations and procedures described by Bykhovskaya-Pavlovskaya (1985), Gusev (1983, 1985), Moravec (1994, 2001), Georgiev et al. (1986), Shigin (1986), Malmberg (1970), etc.

The analysis of the dominant structure of the found fish parasite taxa were presented to the level of the component communities. The ecological terms prevalence, mean intensity are used, based on the terminology of Bush et al. (1997). Analyses of helminth community structure were carried out during the three seasons and in both levels: infracommunity and component community. The infracommunity data were used to calculate the total number of species, mean number of helminths, Brillouns diversity index (HB), etc. (Kennedy, 1993, 1997; Magurran, 1988). Fishes were weighed and measured. Samples of muscles, fat and liver were collected from all individuals. In order to determine the relative accumulation capability of the fish tissues in comparison to the sediments, bioconcentration factor (BCF= [Chost tissues]/ [Csediments]]) were calculated (Sures et al., 1999). The bioconcentration factors were computed to establish the accumulation order and to examine fishes for use as biomonitors of trace metal pollutants in

freshwater environments. The differences in concentration factors were particularly discussed in respect to the bioavailability of trace metals from sediments. A linear correlation coefficient, r_s was used to test associations between the bottom sediments, fish tissues, organs and fish parasites.

RESULTS AND DISCUSSIONS

Fish communities

A total of 55 and 126 fish specimens were collected and examined from the Srebarna Lake and the Danube River, respectively (total 181 specimens). The fish species *Cyprinus carpio* Linnaeus, 1758 and *Lepomis gibbosus* (Linnaeus, 1758) were collected only from the Srebarna Lake. *Ballerussapa* (Pallas, 1811), *Aspius aspius* (Linnaeus, 1758), *Barbus barbus* (Linnaeus, 1758), *Chondrostoma nasus* (Linnaeus, 1758), *Romanogobio albipinnatus* (Lukasch, 1933), *Gymnocephalus schraetser* (Linnaeus, 1758), *Zingel zingel* (Linnaeus, 1758), *Alosa pontica pontica* (Eichwald, 1838) and *Neogobius fluviatilis* Pallas, 1811 were collected and examined only from the Danube River. Common to Srebarna and Danube are the fish species *Abramisbrama* (Linnaeus, 1758), *Carassius gibelio* (Bloch, 1782), *Rutilus rutilus* (Linnaeus, 1758) and *Perca fluviatilis* Linnaeus, 1758. From studied 16 species of fishes, 11 species were estimated as least concern species (LC=Least Concern; IUCN Red List Status), 2 species (*C. carpio*; *A. pontica*) were estimated as vulnerable species (VU=Vulnerable; IUCN Red List Status). Six species (*A. aspius*, *Ch. nasus*, *G. schraetser*, *R. albipinnatus*, Z. *zingel*, *A. pontica*) were included in Appendix 3 of the Bern Convention; 2 species (*Z. zingel*, *A. pontica*) were included in appendices II and V of the Habitats Directive (Directive) and the other two species (*A. aspius*, *R. albipinnatus*) are only included in Appendix II of the Directive. Nine fish species are included in Red Book of Bulgaria (2011), of wich 8 are vulnerable species (VU – *A. aspius*, *B. barbus*, *C. carpio*, *V. melanops*, *R. albipinnatus*, *G. schraetser*, *A. pontica*, *N. fluviatilis*) and one is endangered species (EN – *Z. zingel*). Two species (*G. schraetser*, *A. pontica*) are listen in Anex II and IV of the Biological Diversity Act (BDA, 2002); 2 species (*A. aspius*, *R. albipinnatus*) in Appendix II of the Act; 2 species (*B. barbus*, *Z. zingel*) in Anex IV of the Act. Six species of fish (*Aspius aspius*, *Carassius gibelio*, *Cyprinus carpio*, *Chondrostoma nasus*, *Zingel zingel*

Helminth community structure

A total seven species of helminths were fixed in both biotopes. They are belonging to classes Monogenea (2), Trematoda (1), Nematoda (3) and Acanthocephala (1). The freshwater ecosystem of the Danube River showed significantly larger number of taxa (7) in the parasite communities. *Eustrongylides excisus* (Jägerskiöld, 1909), larvae is common parasite species in freshwater ecosystems of the Srebarna Lake and Danube River. The remaining six types of parasites are found only in fishes from the Danube River (*Gyrodactylus elegans* Nordmann, 1832; *Diplozoon paradoxum* Nordmann, 1832; *Nicolla skrjabini* (Iwanitzky, 1928) Slusarski, 1972; *Pomphorhynchus tereticollis* (Rudolphi, 1809); *Hysterothylacium aduncum* (Rudolphi, 1802) and *Camallanus truncatus* (Rudolphi, 1814)). *Gyrodactyluselegans* was reported as parasite species on the gills of *A.brama*, *B. sapa*, *A.ballerus*, *A. brama*, *C.carassius*, *C.carpio*, *G.gobio*, *M.fossilis*, *R.rutilus*, *T.tinca*, *V.vimba* (Moravec, 2001; Kakacheva, Margaritov, Grupcheva, 1978). *Diplozoonparadoxum* was reported as parasite species on the gills of *A.brama*, *A.bjoerkna, A.bipunctatus, A.alburnus, A. aspius, B. barbus, C. carassius, Ct.idella, C. carpio, G. gobio, G.cernuus, L. cephalus, L.leuciscus, L. idus, Ph.phoxinus, Rh.sericeus, R.rutilus, Sc. erythrophthalmus, T.tinca*, *V. vimba* of the Danube River (Gusev, 1985; Margaritov, 1959, 1966; Moravec, 2001; Kakacheva, Margaritov, Grupcheva, 1978). The adult *Nicollaskrjabini* are parasite species of *A.acerina*, *S,glanis, G.cernua*, *G. schraetser*, *A.ruthenus*, *A. brama*, *A. ballerus*, *P.cultratus*, *C.carassius*, *C.carpio*, *C.bulgarica*, *C. balcanica*, *S.lucioperca*, *S. volgense*, *P.fluviatilis*, *Z.zingel*, *Z. streber*, *G.kessleri*, *Pr.marmoratus*, *Bl. bjoerkna*, *A. aspius*, *P. cultratus*, *G. gobio*, *C.bulgarica*, *G. fluviatilis*, *G. cephalarges*, *P.minutus, N.kessleri* (Atanasov, 2012; Kakacheva, Margaritov, Grupcheva, 1978; Moravec, 2001). The first intermediate host are the snails *Lithoglyphus*

naticoides and the secong – *Gammarus* (*Rivulogammarus*) *balcanicus*, *Pontogammarus crassus* and *Dikerogammarus haemobaphes* (Kakacheva-Avramova, 1983; Komarova, 1968; Stenko, 1976). *Pomphorhynchus tereticollis* (Rudolphi, 1809) has intermediate host's amphibians. Definitive hosts are fish species (*Gadus* sp., *Ac.sturio*, *S.fario*, *M.lineata*, *C.regale*, *S.foetens*) (Petrochenko, 1956). The isolated specimens of *Pomphorhynchus* were determined by Špakulová, based on revision of the genus (Špakulová et al., 2011). The specimens *Pomphorhynchus*, established of *A. brama*, *B. sapa*, *B. barbus*, *G. schraetser* and *N. fluviatilis* of the freshwater ecosystem of the Danube (Biotope Vetren) are defined as *P. tereticollis*. *Eustrongylides excisus*, larvae is developed with participation of the first intermediate host oligochets (*Lumbricus variegatus*, *Tubifex tubifex*, *Limnodrilus* sp.) and the second, fish species, amphibians (*R.ridibunda*) and reptiles (*N.tesselata*). The adult nematodes parasitic in the glandular stomach of cormorants (*Ph.carbo* and *Ph. pygmaeus*) (Moravec, 1994). The species was presented of *S. lucioperca* (as paratenic host) and of *Gobius* sp. (as intermediate host) of the Mandra Lake (Margaritov, 1960), of *A. aspius* of the Danube River (Margaritov, 1959); of *P. fluviatilis* (Nedeva, Grupcheva, 1996); of *S.glanis*, *L.lota*, *N.cephalarges*, *N. kessleri*, *P. fluviatilis* of the Danube River (villages of Archar, Dobri dol, Gotomartsi) (Atanasov, 2012), etc. *Hysterothylacium aduncum* was reported of *A.gueldenstaedti*, *A.alosa*, *A. falax*, *A.pontica*, *S.trutta*, *S. salar*, *O.mykiss*, *Oncorhynchus* sp., *C.lavaretus*, *C. nasus*, *O.eperlanus*, *T.tinca*, *Ph.poxinus*, *E.lucius*, *L.lota*, *P.fluviatilis*, *A.anguilla*, *M.quadricornis*, *P.flesus*. Intermediate hosts are pelagic copepods (*Acartia bifilosa*, *Eurytemora affinis*, etc.) (Moravec, 1994). *Camallanus truncatus* was reported of *A. brama*, *A.anguilla*, *A. aspius*, *C.gobio*, *E.lucius*, *G. albipinnatus*, *G.cernuus*, *G. schraetser*, *L.lota*, *P. fluviatilis*, *S.glanis*, *S.lucioperca*, *S. volgense*, *Z. zingel* of the Danube River (Moravec, 2001). Intermediate hosts are cyclops (*Mesocyclops leuckarti*, *Megacyclops viridis*, *Macrocyclops albidus*, *Cyclops strenuus*) (Moravec, 1994).

Six species, parasitic in different fish hosts are generalists and are the most abundant in freshwater ecosystem of the Danube River: two monogenean species (*G. elegans*, *D. paradoxum*), one digenean species (*N. skrjabini*), two nematoda species (*H. aduncum*, *C. truncatus*) and one acanthocephalan species (*P. tereticollis*). *E. excisus*, which use fishes as intermediate hosts represented the allogenic species. In freshwater ecosystem of the Danube River with the highest species diversity were distinguished helminth communities of *A. brama* (3 species). In *A. brama*, the highest prevalence are showed *P. tereticollis* (67%), followed by *G. elegans* and *D. paradoxum* (on 50%). They are core species of the parasite communities of the Danube bream (Biotope Vetren). Helmint communities of the bream are followed by the biodiversity of the helminth communities of the striped ruffe from the same biotope, represented by two helminth species (*N. skrjabini* (7.7%) and *P. tereticollis* (46.2%)). *N. skrjabini* is an accidental species of the helminth communities of the striped ruffe and *P. tereticollis* is a component species for them. All other fish hosts are represented by one species of parasites (Fig. 2, Fig. 3). Each of them is a core species of the studied fish host. From them, with the highest prevalence are distinguished *E. excisus* of the *P. fluviatilis* and *R. rutilus* (on 100%) and *P. tereticollis* of the *B. barbus* (100%), *A. alburnus* (75%) and *B. sapa* (70%). The highest number of specimens for *H. aduncum* (180 specimens) of *A. pontica* and *P. tereticollis* (122 specimens) of *A. alburnus*, followed by number of specimens for *P. tereticollis* of *B. barbus* (32 specimens) are found. All other species range from 1 to 13 specimens. The mean intensity of infection is the highest for *P. tereticollis* (MI=16±2.83) of *B. barbus*, followed by *H. aduncum* (MI=7.5±7.92) of *A. pontica*. With the lowest mean intensity of infection are *C. truncatus* (MI=1±0.70) of *R. albipinnatus* (Fig. 3). *E. excisus* of the parasite communities of *P. fluviatilis* of the Srebarna Lake were distinguished with high prevalence (P=100%), but with low mean intensity (MI=2±1.54). This is a core species of the helminth communities of the perch from the lake freshwater ecosystem. The highest Brillouins biodiversity infex (HB=1.06) are determined for the

helminth communities of *A. brama*, followed by this of *G. schraetser* (HB=0.06).

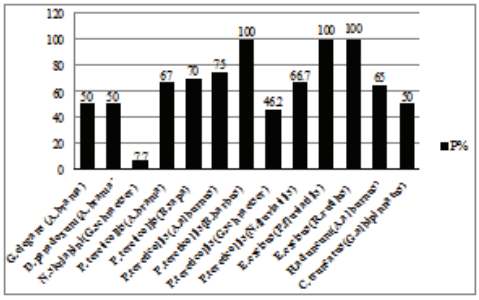

Figure 2. Prevalence (%) of fish parasite species, Danube River, 2012

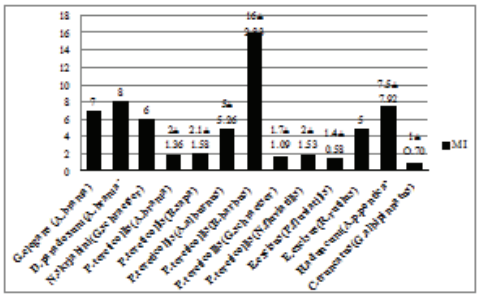

Figure 3. Mean intensity (MI±SD) of fish parasite species, DanubeRiver, 2012

Content of heavy metals insediments, fish and parasites

The result of the chemical analyzes (Pb, Cu and Zn) of 40 samples of muscle, liver, kidneys and bones of *Perca fluviatilis* of the Srebarna Lake; *Perca fluviatilis* and *Barbus barbus* of the Danube River are presented. The content of Pb, Cu and Zn in two parasite species: *Eustrongilides excises* and *Pomphorhynchus tereticollis* were determined. The content of heavy metals in sediments from the two freshwater ecosystems was fixed. Based on the results of chemical analyzes, mean concentrations (mg/kg) in tissues, organs of the fish, parasites and sediments, as well as the bioconcentration factor (BCF= [Chost/parasite tissues]/ [Csediments]) were defined. For the freshwater ecosystem of the Srebarna Lake was found higher content of zinc and lead in *E.excisus*, than in sediments and in the opposite, higher content of copper in the sediments (Table 1).

From the fish tissues and organs the highest content of copper has liver (15.813 mg/kg). The content of lead was the highest in bone (37.221 mg/kg) and that of zinc – in kidney (145.314 mg/kg). $BCF_{E.\ excisus}$ was the highest for lead (4.850), followed by those for zinc and copper compared to content in the sediments from the lake. With regard to fish organs and tissues, BCF_{Cu} is the highest in liver (0.486); BCF_{Zn} – in liver (1.380) and for lead-in kidney (BCF_{Pb}=1.153). $BCF_{E.\ excisus}$ is higher in muscles for the three heavy metals but is the highest for zinc (11.316), followed by this for copper and lead (Table 1).

The content of copper and zinc was higher in sediments of the Danube River than in *E. excisus* and this of lead was higher in *E. excisus* than in the sediments (Table 2). With regard to fish tissues and organs, the highest concentrations of copper were reported in liver (36.522 mg/kg); of lead-in bone (9.121 mg/kg) and of zinc-in the kidneys (51.634 mg/kg). $BCF_{E.\ excisus}$ was the highest for lead (2.525), followed by these for zinc and copper. BCF_{cu} was the highest of the liver (0.433), for lead – of the bones (BCF_{Pb}=0.211) and for zinc – of the kidneys (BCF_{Zn}=0.325). $BAF_{E.\ excisus}$ was the highest for the muscles and the three trace elements, but was with the highest values in relation to the concentration of lead, followed by these of copper and zinc (Table 2).

According to the results of this study, for the first time was presented the data for content of Pb, Cu and Zn of barbel tissues and organs and of their acanthocephalan parasite *P. tereticollis* of the Danube River (Biotope Vetren). *P. tereticollis*, core helminth species of the helminth communities of barbel of the Danube River, was distinguished with higher content of heavy metals than in sediments. With regards to barbel tissues and organs, the highest contents of copper and zinc were fixed from the liver (38.258 mg/kg, 14.281 mg/kg, respectively) and of lead – from the bones (2.352 mg/kg).

$BCF_{P.\ tereticollis}$ was the highest of lead (8.167), followed by these of copper and zinc (Table 3) with no significant differences between them from the rest. BCF_{Cu} and BCF_{Zn} were the highest from the liver and of lead – from the bones. $BAF_{Ptereticollis}$ was the highest of the

muscles for the three trace metals, but was the highest of lead (BCF_{Pb}=2323.993) (Table 3).

Table 1. Bioconcentration factor (BCF= [Chost/parasite tissues]/ [Csediments]) of P. fluviatilis and E. excisus of the Srebarna Lake

Perca fluviatilis	Srebarna		
BCF	Cu	Pb	Zn
$C_{E.excisus}/C_{Sediments}$	0.600	4.850	3.75
$C_{Liver}/C_{Sediments}$	0.486	1.212	1.380
$C_{E.excisus}/C_{Liver}$	1.234	4.000	2.719
$C_{Kidney}/C_{Sediments}$	0.298	1.153	0.595
$C_{E.excisus}/C_{Kidney}$	2.012	4.205	6.303
$C_{Bones}/C_{Sediments}$	0.105	0.962	0.343
$C_{E.excisus}/C_{Bones}$	5.725	5.041	10.916
$C_{Muscles}/C_{Sediments}$	0.083	0.763	0.332
$C_{E.excisus}/C_{Muscles}$	7.198	6.358	11.316
Sediments mg/kg	32.5	30.7	105.3

Table 2. Bioconcentration factor (BCF= [Chost/parasite tissues]/ [Csediments]) of P. fluviatilis and E. excisus of the Danube River

Perca fluviatilis	Danube		
BCF	Cu	Pb	Zn
$C_{E.excisus}/C_{Sediments}$	0.346	2.525	0.566
$C_{Liver}/C_{Sediments}$	0.433	0.095	0.277
$C_{E.excisus}/C_{Liver}$	0.800	26.430	2.045
$C_{Kidney}/C_{Sediments}$	0.040	0.142	0.325
$C_{E.excisus}/C_{Kidney}$	8.633	17.821	1.738
$C_{Bones}/C_{Sediments}$	0.082	0.211	0.260
$C_{E.excisus}/C_{Bones}$	4.239	11.973	2.176
$C_{Muscles}/C_{Sediments}$	0.020	0.0009	0.039
$C_{E.excisus}/C_{Muscles}$	17.303	265.073	14.356
Sediments mg/kg	84.332	43.251	158.612

Table 3. Bioconcentration factor (BCF= [Chost/parasite tissues]/ [Csediments]) of B. barbus and P. tereticollis of the Danube River

B. barbus	Danube		
BCF	Cu	Pb	Zn
$C_{P.\ tereticollis}/C_{Sediments}$	1.603	8.167	1.512
$C_{Liver}/C_{Sediments}$	0.454	0.031	0.090^{s}
$C_{P.\ tereticollis}/C_{Liver}$	3.535	263.224	16.790
$C_{Kidney}/C_{Sediments}$	0.131	0.046^{ns}	0.062
$C_{P.\ tereticollis}/C_{Kidney}$	12.282	60.944	24.243
$C_{Bones}/C_{Sediments}$	0.026	0.054	0.076
$C_{P.\ tereticollis}/C_{Bones}$	60.944	173.927	19.924
$C_{Muscles}/C_{Sediments}$	0.015	0.0004	0.037
$C_{P.\ tereticollis}/C_{Muscles}$	107.585	2323.993	41.342
Sediments mg/kg	84.332	43.251	158.612

A linear correlation coefficient, (r_s, Spearman correlation coefficient) are determined to test associations between the bottom sediments, fish tissues, organs and fish parasites. Very significant correlation ($p< 0.001$) are fixed for relationship between $Sediments_{Pb}$-$E.excisus_{Pb}$ of the Danube River and Lake Srebarna. Very significant correlation ($p< 0.001$) were fixed between sediments for the three trace elements and *P. tereticollis*, parasite from helminth communities of *B. barbus*, river Danube ($Sediments_{Cu}$-*P. tereticollis*; $Sediments_{Pb}$-*P. tereticollis*; $Sediments_{Zn}$-*P. tereticollis*; $p< 0.001$).

The major negative anthropogenic impact from Bulgaria of the Danube River and Srebarna Lake ecosystems ar from farm activities (fertilizers, pesticides; wastewater from livestock, etc.) (Dimitrov, 2009; Dimitrov et al., 2012; Stefanov, Dimitrov, 1986). Danube River and Srebarna Lake are included in the National monitoring program (Regulation 1/2011).

The obtained values for the content of Pb, Zn and Cu in sediments, freshwater fish organs and tissues and their parasites from the Danube River and Lake Srebarna are slightly higher than those reported by other authors for the same ecosystem, but for another biotopes of the Danube River (Bulgarian part of the river) (Atanassov, 2012; Hristov, 2010; Nachev, 2010).

CONCLUSIONS

As a result of this examination new species a new host records were found for the freshwater ecosystem of the Danube River. *G. elegans* and *D. paradoxum* are presented for the first time for the parasite communities of *A. brama*. *N. skrjabini* was found for the first time in Biotope Vetren. *P. tereticollis* are reported for the first time for helminth communities of *A. brama*, *B. sapa*, *A. alburnus*, *B. barbus*, *G. schraetser* and *N. fluviatilis*. *E. excisus* are reported for the first time for helminth communities of *R. rutilus*. *H. aduncum* are reported for the first time for freshwater ecosystems of the Danube River and helminth communities of *A. pontica*, as well as for the first time for the helminth communities of the fish in Bulgaria *C. truncatus* was found for the first time for the freshwater ecosystem of the Danube River. *B. barbus* is a new host record for this helminth species in Bulgaria.

New data for heavy metal contents in sediments, fish tissues and organs and their

parasites from the Danube River and Lake Srebarna are present. From the tissues and organs of the studied species (*P. fluviatilis* and *B. barbus*), the lowest concentrations of Pb, Cu and Zn were found in the muscles. In general, the liver and kidneys of both fish species from both studied ecosystems were found in higher content of heavy metals than the bones and muscles. The high significant correlations determined *E.excisus* as sensitive bioindicator for Pb and *P.tereticollis* as a sensitive indicator of Pb, Cu and Zn.

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BIODIVERSITY AND ECOLOGICAL APPRAISAL OF THE FRESHWATER ECOSYSTEM OF THE RIVER ARDA, BULGARIA

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Abstract

Study of biodiversity of freshwater fish and parasite communities from the River Arda, southern Bulgaria characterized by heavy metal pollution was carried out. Investigation of the actual state of the water pollution from an aspect of loading environment with heavy metals and their impact on fish, fish parasites and biodiversity of water organisms in general was accomplished. Assessment of fish parasites as potential bioindicators of water pollution was made. For examinations were used standard methods and techniques. The studies, belonging to 65 specimens of fishes representing 8 species during the three seasons were presented. The dominant structures of helminth communities are characterized. The model of fish species chosen for examination of the heavy metal content in this study were the European perch, Perca fluviatilis (L., 1758) and Macedonian vimba, Vimba melanops (Heckel, 1837) of the Arda River. The contents of heavy metals (Pb, Zn, Mn and Cu) (mean concentration, bioconcentration factors) in the tissues and organs of fish species and fish parasites (Eustrongylides excisus (Jägerskiöld, 1909) and Caryophyllaeus sp.) were discussed. The studies carried out could be used in various monitoring systems for screening the pollution on the water environment and the organisms inhabiting the anthropogenous ecosystems.

Key words: *biodiversity, freshwater fishes, helminth communities, heavy metal pollution, River Arda.*

INTRODUCTION

The Arda River is related to the Aegean water collecting region. The river forming picturesque defiles and gorges and giving exclusive attractiveness of the south-east parts of the Rhodopa Mountain, Southern Bulgaria. The valley of the river from the sources to the State border with Greece is indicated as a region with middle and high degree of importance in respect to the date about the species richness, the endemic and rare taxa The variety of habitats – oak forests, Mediterranean bushes, rock massifs, at the upper parts - preserved beech forests, have determined the existence of an extraordinary biological variety. (Assyov, 2012; BirdLife International, 2013).

Basic ecological problems in the region are caused from the negative impact and damages of the environmental components from the performed (Government decree No. 140, Official Newspaper 101/2000) mining-extractive, ore-dressing and metallurgy activity (mining of lead and zinc ores and flotation), leading to a high pollution and aciding of large surfaces with deposited mine masses (Kirin et al., 2004; Koev, 1998; Koev et al., 1998; Yorova et al., 1992).

Arda River is included in the National monitoring program (Regulation 1/2011).

Endoparasite species are particularly interesting as indicators of the ecological status of the freshwater ecosystems because the completion of their life cycle requires interactions with several host vertebrates and invertebrates, and the effects on each of the hosts differ according to the pollution level of the habitat in question (Baruš et al., 2007; Cone et al., 1993; Gelnar et al., 1997; Kennedy, 1997; MacKenzie et al., 1995; Marcogliese and Cone, 1997; Oros, Hanzelová, 2009; Overstreet, 1997; Sures, Siddall, 1999; Thielen et al., 2004; Tieri et al., 2006, etc.).

Fish parasite communities, heavy metal content and the state of freshwater ecosystem of the Arda River are studied from different authors (Kirin, 2002a,b; Kirin, 2003; Kirin, 2005;

Kirin, Turcekova, Shukerova, Pehlivanov, 2010; Marinova et al., 2007, etc.)
This paper presents the results from an examination of heavy metal content in sediments, fish tissues and organs, fish parasites and dominant structure of fish parasite communities from the Bulgarian border part of the Arda River (town of Majarovo).

MATERIALS AND METHODS

During June, 2012 sediments, fish and fish parasites were collect and examined from the Arda River (town of Majarovo) (Fig. 1).

Figure 1. Aegean Water Basin and Arda River

Fig. 1. Aegean Water Basin and Arda River
The town of Majarovo ($41^0$38.25′N, $25^0$54.12′E) is situated on the riverside, about 30 km far away from the Studen kladenec Reservoir and about 10 km before the Ivaylovgrad Reservoir. It is distinguished with a less depth and slow running water, with rapids at some places. The waterside vegetation is represented mainly by *Salix* sp. and *Alnus glutinosa*, and the water vegetation – by the species *Fontinalis antipyretica* and *Drepanocladus aduncus.* The riverbed is considerably mote slanting with good formed sandy bottom. The region of the town and the riverside are distinguished with significant diversity of highly protected species and territories declared as protected with national and international nature protective status (Assyov, 2012; Kirin et al., 2004).

A total of 5 sediment samples and 65 freshwater fish specimens belonging to 2 families and 8 species were collected and examined in June, 2012. The fish were caught by nets, by angling and eloctrofishing under a permit issued by the Ministry of Agriculture and food and Ministry of Environment and waters of Bulgaria. The scientific and common names of fish hosts were used according to the FishBase database (Fröse and Pauly, 2012).
Samples of sediments were collected according to the Guidance on sampling of rivers and watercourses - ISO 5667-6:1990, introduced as a Bulgarian standard in 2002. Heavy metal concentration of the water and sediment samples, fish tissues, organs and parasites were carried out according to standard techniques. The samples were analyzed for content of Cd, Cu, Pb and Zn by ICP Spectrometry (Bíreš et al., 1995).
The model of fish species chosen for examination of the heavy metal content in this study were the European perch, *Perca fluviatilis* (L., 1758) and Macedonian vimba, *Vimba melanops* (Heckel, 1837) of the Arda River.
Helminthological examinations were carried out following recommendations and procedures described by Bykhovskaya-Pavlovskaya (1985), Dubinina (1987), Georgiev et al. (1986), Gusev (1985), Hotenovskij (1985), Kulakowskaya (1961), Moravec (1994, 2001), Scholz and Hanzelova (1998), Scholz et al. (1998) etc.
The analysis of the dominant structure of the found fish parasite taxa were presented to the level of the component communities. The ecological terms prevalence, mean intensity are used, based on the terminology of Bush et al. (1997). Analyses of helminth community structure were carried out during the three seasons and in both levels: infracommunity and component community. The infracommunity data were used to calculate the total number of species, mean number of helminths, etc. (Kennedy, 1993, 1997; Magurran, 1988). Fish were weighed and measured. Samples of muscles, fat and liver were collected from all individuals. In order to determine the relative accumulation capability of the fish tissues in comparison to the sediments, bioconcentration factor (BCF=[Chost tissues]/[Csediments]])

were calculated (Sures et al., 1999). The bio-concentration factors were computed to establish the accumulation order and to examine fish for use as biomonitors of trace metal pollutants in freshwater environments. The differences in concentration factors were particularly discussed in respect to the bioavailability of trace metals from sediments. A linear correlation coefficient, r_s was used to test associations between the bottom sediments, fish tissues, organs and fish parasites.

RESULTS AND DISCUSSIONS

Fish communities

A total of 65 fish specimens from 8 species were collected and examined from the Arda River: *Alburnus alburnus*(Linnaeus, 1758), *Barbus plebejus* Bonaparte 1839, *Carassius gibelio* (Bloch, 1782), *Rutilus rutilus* (Linnaeus, 1758), *Scardinius erythrophthalmus* (Linnaeus, 1758), *Squalius orpheus* Kottelat & Economidis, 2006, *Vimba melanops* (Heckel, 1837) and *Perca fluviatilis* Linnaeus, 1758. From studied 8 species of fishes, 6 species were estimated as least concern species (LC=Least Concern; IUCN Red List Status) and for one species are not enough data (*V. melanops*, DD=Data Deficient; IUCN Red List Status). One fish species is included in Red Data Book of the Republic of Bulgaria (Golemanski (Ed.), 2011) (*V. melanops*, VU=Vulnerable). *A. alburnus*, *B. plebejus*, *R. rutilus* and *Sc. erythrophthalmus* are freshwater brackish, benthopelagic fish species. *V. malanops* is demersal fish species and *P. fluviatilis* is brackish, demersal introduced fish species. *Sq. cepalus* is pelagic fish species. Five species of fish (*A. alburnus*, *B. plebejus*, *R. rutilus*, *Sc. erythrophthalmus* and *Sq. cepalus*) are free of parasites.

Helminth community structure

A total two taxa of helminths were fixed (*Eustrongylides excisus* (Jägerskiöld, 1909) and *Caryophyllaeus* sp.). They are belonging to classes Nematoda (1) and Acanthocephala (1). *Eustrongylides excisus* (Jägerskiöld, 1909), larvae is developed with participation of the first intermediate host oligochets (blackworm *Lumbricusvariegatus* Linnaeus, 1758, sludge worm *Tubifextubifex* (Müller, 1774), *Limnodrilus* sp.) and the second fish species, amphibians (Marsh frog, *Pelophylax ridibundus* (Pallas, 1771) (=*Ranaridibunda* Pallas, 1771) and reptiles (Dice snake, *Natrixtesselata* (Laurenti, 1768)). The adult nematodes parasitic in the glandular stomach of cormorants (Great Black Cormorant *Phalacrocorax carbo* (Linnaeus, 1758) and Pygmy Cormorant *Microcarbo pygmeus* (Pallas, 1773) (=*Ph. pygmaeus* Pallas, 1773)) (Moravec, 1994). In Bulgaria, the species is presented of *Sander lucioperca* (Linnaeus, 1758) (=*Lucioperca lucioperca* Linnaeus, 1758) (as paratenic host) and of *Gobius* sp. (as intermediate host) of *Aspius aspius* (Linnaeus, 1758) from the Danube River (Kakacheva, Margaritov, Grupcheva, 1978; Margaritov, 1959); of *P. fluviatilis* from the Zhrebchevo Reservoir (Nedeva, Grupcheva, 1996) and from the Srebarna Lake (Hristov, 2010; Shukerova, Kirin, 2007; Shukerova et al., 2010); of *Silurus glanis* (Linnaeus, 1758); *Lotalota* (Linnaeus, 1758), *Neogobius melanostomus* (Pallas, 1814) (=*Neogobiuscephalarges* Pallas, 1814), *N.kessleri* (Günther, 1861), *P. fluviatilis* from the Danube River (Atanasov, 2012), etc. Caryophyllidean (Platyhelminthes:Eucestoda) parasites represent a widely distributed group of intestinal helminthes of Cyprinidae and Siluridae fishes occurring in all zoogeographical regions except the Neotropics. Some cariophyllideans may be pathogenic for their fish hosts (Mackiewicz, 1994; Oros et al., 2010; Scholz and Hanzelová, 1998). *Caryophyllaeus brachycollis* Janiszewska, 1951 is developed with participarion of the first intermediate host *Limnodrilus hoffmeisteri* Claparède, 1862 and *T. tubifex* and the second different fish secies. *C. laticeps* (Pallas, 1781) is developed with first intermediate host *Limnodrilus claparedeanus* Ratzel, 1868*, T. tubifex andT. barbatus* (Grube, 1861). The supposition intermediate host of *Caryophyllaeides fennica* (Schneider, 1902) is the oligohaete *Stylaria lacustris* (Linnaeus, 1767) (Kakacheva-Avramova, 1983). In Bulgaria caryophyllidean tapeworms were presented from different fish species and freshwater ecosystems: as *C. laticeps* (Pallas, 1781) - of *Barbus barbus* (Linnaeus, 1758) (Margaritov, 1959; 1966) from the Danube River; of *B. cyclolepis* Heckel, 1837 (Margaritov, 1959) from the Iskar River; of *A.*

alburnus (Margaritov, 1959) of the Tunja River; of *B. barbus*, *Vimba carinata* (Pallas, 1814), *Abramis brama* (Linnaeus, 1758) and *Ballerus sapa* (Pallas, 1811) (=*A. sapa* Pallas, 1811) (Kakacheva, Margaritov, Grupcheva, 1978) from the Danube River; of *Leuciscus cephalus* (Linnaeus, 1758) (Cakic et al., 2004) from the Danube River; as *C. fennica* (Schneider, 1902) – of *B. barbus* (Margaritov, 1959; 1966) from the rivers Iskar and Tunja; of *B. cyclolepis* and *L. cephalus* (Margaritov, 1959) from the Iskar River; of *L. cephalus*, *V. melanops* and *B. cyclolepis* (Margaritov, 1963/64) from the rivers Maritsa and Topolnitsa; of *B. cyclolepis*, *L. cephalus*, *V. melanops* (Kakacheva, 1965) from the rivers Asenitsa, Harmanlijska, Topolnitsa, Syuyutlijka, Sushenitsa and Bedechka; of *B. barbus* and *S. lucioperca* (Margaritov, 1966) from the Danube River; of *B. petenyi* (Kakakcheva, 1969) from the rivers Nishava, Ogosta, Vodomerka, Buchinska, Vrabnishka, Barsiya, Chuprenska, Iskrecka, Botunya, Bebresh; of *L. cephalus* and *R. rutilus* (Margaritov, 1977) from the Shiposhnitsa River and Reservoir Iskar; of *V. carinata*, *A. brama*, *B. sapa*, *Ballerus ballerus* (Linnaeus, 1758) (=*Abramis ballerus*), *Blicca bjoerkna* (Linnaeus, 1758), *A. alburnus*, *B. barbus*, *S. lucioperca*, *Sc. erythrophthalmus* and *Pelecus cultratus* (Linnaeus, 1758) (Kakacheva, Margaritov, Grupcheva, 1978) from the Danube River; of *L. cephalus* and *R. rutilus* (Kakacheva, Menkova, 1978) from the Palakariya River; of *B. barbus* (Kakacheva, Menkova, 1981) from the Struma River; of *B. cyclolepis*, *A. alburnus*, *Sq. orpheus*(=*L. cephalus*) (Kirin, 2002b, 2003) from the Arda River; of *L. cephalus* (Cakic et al., 2004) from the Danube River; as *C. brachycollis* Janiszweska, 1953 - of *B. cyclolepis* and *L. cephalus* (Kakacheva, 1965) from the rivers Asenitsa, Sjujutlijka, Chepinska, Bedechka and Topolnitsa; of *L. cephalus*, *V. melanops*, *A. alburnus*, *B. cyclolepis*, *R. rutilus* (Margaritov, 63/64) from the rivers Maritsa, Vacha, Chepinska, Topolnitsa, Bistritsa; of *L. cephalus*, *Barbus petenyi* Heckel, 1852 and *B. barbus* (Kakacheva, 1969) from the rivers Vrabnishka, Nishava, Mirkowska, Botunya, Ogosta, Malak iskar; of *L. cephalus* (Kakacheva, Menkova, 1978) from the Palakariya River; of *B. petenyi*, *L. cephalus* (Kakacheva, Menkova, 1978) from the rivers Devinska, Sarneshka and Vacha; of *B. petenyi*, *B. barbus*, *L. cephalus* (Kakacheva, Menkova, 1981) from the rivers Blagoevgradska Bistritsa, Struma, Zheleznitsa and Gradevska; of *P. fluviatilis* (Nedeva, Grupcheva, 1996) from the Zhrebchevo reservoir; of *B. cyclolepis*, *A. alburnus*, *Sq. orpheus*(=*L. cephalus*) (Kirin, 2002b, 2003) from the Arda River; and of *L. cephalus* (Cakic et al., 2004) from the Danube River; as *Caryophyllaeus* sp. – of *L. cephalus* and *A. alburnus* (Kakacheva, 1965) from the rivers Maritsa, Syuyutlijka and Harmanlijska; of *Cyprinus carpio* Linnaeus, 1758 (Margaritov, 1975, 1976) from the Fish Farming–Yambol; of *C. carpio* (Kakacheva, Menkova, 1981) from the Fish Farming–Blagoevgrad; of *V. melanops* (Kakacheva, 1965) from the Harmanlijska River; of *Cobitis bulgarica* (Drensky, 1928) (Margaritov, 1966) from the Danube River; as as *Caryophyllaeus* sp. juv. - of *Gobio gobio* (Linnaeus, 1758), *B. cyclolepis*, *V. melanops* (Kakacheva, 1965) from the river Maritsa, Chepinska and Harmanlijska; of *B. petenyi* (Kakacheva, Menkova, 1978) from the Palakariya River; of *C. carpio* (Margaritov, 1992) from the Fish farms-Yambol, Blagoevgrad, etc.

Caryophyllaeus sp., parasitic in *V. melanips* is generalist and *E. excisus*, which use fish as intermediate hosts represented the allogenic species for the helminth communities of the examined freshwater fish species of the Arda River ecosystem. *Caryophyllaeus* sp. of *V. melanops* and *E. excisus* of the parasite communities of *P. fluviatilis* of the Arda River were distinguished with high values of prevalence (P=52.17% and P=54.54%, respectively) but with lower value of mean intensity for *E. excisus* (MI=6±5.12, 1-14, SE Mean 1.48, C.V. 85.28; MI=1.6±1.52, 1-2, SE Mean 0.21, C.V. 30.98, respectively). The two helminth species are core species of the helminth communities of the perch and vimba, respectively.

Content of heavy metals in sediments, fishes and parasites

The result of the chemical analyzes (Pb, Cu and Zn) of 40 samples of muscle, liver, kidneys and bones of *Vimba melanops* and *Perca fluviatilis*

of the Arda River were presented (Table 1 and 2). The content of Pb, Cu and Zn in two parasite species: *Caryophyllaeus sp.* and *Eustrongilides excisus* were determined. The content of heavy metals in sediments from the two freshwater ecosystems was fixed. Based on the results of chemical analyzes, mean concentrations (mg/kg) in tissues, organs of the fish, parasites and sediments, as well as the bioconcentration factor (BCF=[Chost/parasite tissues]/[Csediments]) were defined (Table 3 and 4).

Table 1. Content of heavy metals (Cmg/kg±SD) of P. fluviatilis and E. excisus

Perca fluviatilis	Arda River		
	Cu	Pb	Zn
$C_{E.excisus}$	12.94± 0.02	51.65± 0.29	321.22± 0.11
C_{Liver}	11.58± 0.33	1.95± 0.02	71.48± 0.25
C_{Kidney}	1.02± 0.09	3.06± 0.06	259.92± 0.50
C_{Bones}	2.19± 0.12	4.39± 0.16	72.04± 0.08
$C_{Muscles}$	0.55± 0.03	0.18± 0.04	25.89± 0.16
Sediments mg/kg	25,51± 1.02	19,98± 0.45	682,56± 1.45

Table 2. Content of heavy metals (Cmg/kg±SD) of V. melanops and Caryophyllaeus sp.

Vimba melanops	Arda River		
	Cu	Pb	Zn
$C_{Caryophyllaeus\ sp.}$	9.97± 0.84	33.35± 0.67	279.69± 0.35
C_{Liver}	12.98± 0.49	1.98± 0.24	43.32± o.20
C_{Kidney}	1.55± 0.04	4.51± 0.26	148.44± 0.22
C_{Bones}	2.49± 0.24	2.18± 0.14	79.35± 0.67
$C_{Muscles}$	0.52± 0.02	0.95± 0.07	23.72± 0.60
Sediments mg/kg	25,51± 0.75	19,98± 0.55	682,56± 1.25

The highest mean content of Cu showed the sediment samples of river (25.5 mg/кg), followed by those of the parasite species *E. excisus* (12.938 mg/кg). From fish tissues and organs, with the highest content of Cu are distinguished the liver (11.587 mg/кg).

Table 3. . Bioconcentration factor (BCF=[Chost/parasite tissues]/[Csediments]) of P. fluviatilis and E. Excisus

Perca fluviatilis BCF	Arda River		
	Cu	Pb	Zn
$C_{E.excisus}/C_{Sediments}$	0.507	2.595	0.471
$C_{Liver}/C_{Sediments}$	0.454	0.097	0.105
$C_{E.excisus}/C_{Liver}$	1.116	26.525	4.493
$C_{Kidney}/C_{Sediments}$	0.04	0.154	0.380
$C_{E.excisus}/C_{Kidney}$	12.684	16.872	1.235
$C_{Bones}/C_{Sediments}$	0.086	0.221	0.105
$C_{E.excisus}/C_{Bones}$	5.892	11.759	4.459
$C_{Muscles}/C_{Sediments}$	0.021	0.0009	0.037
$C_{E.excisus}/C_{Muscles}$	23.652	283.769	12.407
Sediments mg/kg	25,51	19,98	682,56

Table 4. Bioconcentration factor of V. melanops and Caryophyllaeus sp.

Vimba melanops BCF	Arda River		
	Cu	Pb	Zn
$C_{Caryoph.sp.}/C_{Sediments}$	0.391	1.676	0.409
$C_{Liver}/C_{Sediments}$	0.508	0.099	0.063
$C_{Caryoph.sp.}/C_{Liver}$	0.768	16.819	6.456
$C_{Kidney}/C_{Sediments}$	0.060	0.226	0.217
$C_{Caryoph.sp.}/C_{Kidney}$	6.423	7.393	1.884
$C_{Bones}/C_{Sediments}$	0.097	0.109	0.116
$C_{Caryoph.sp.}/C_{Bones}$	3.996	15.313	3.525
$C_{Muscles}/C_{Sediments}$	0.020	0.047	0.035
$C_{Caryoph.sp.}/C_{Muscles}$	19.147	35.035	11.794
Sediments mg/kg	25,51	19,98	682,56

The highest mean content of Pb are defined in *E. excisus* (51.646 mg/kg), followed by those in the sediments (9.19 mg/kg). Of tissues and organs, higher concentrations were obtained for the content of Pb in bones and kidneys (4.392 and 3.061 mg/kg, respectively). The mean content of Zn showed higher values in the sediments (682.5 mg/kg) than of *E. excisus* (321,221 mg/kg). Of tissues and organs, the highest concentrations were differed of Zn. The highest content of this trace heavy metal was detected for kidneys (C_{kidney}=259,918 mg/kg), followed by those for bones and liver (C_{bones}=72,035; C_{liver}=71,481 mg/kg, respectively). The lowest values of Zn are detected in

the muscles of examined perch (C_{musles}=25,889 mg/kg) (Table 1).

BCF of *E. excisus*, parasite species of *P. fluviatilis* of the Arda River was the highest for Pb (BCF $C_{E.excisus}/C_{SedimentsPb}$=2.595), followed by those for Cu (BCF $C_{E.excisus}/C_{SedimentsCu}$=0.504) and Zn (BCF $C_{E.excisus}/C_{SedimentsZn}$=0.471) (Table 3). With regard to the examined fish tissues and organs, BCF was the highest for Cu in liver ($BCF_{liver/sedimentsCu}$=0.454), followed by those for Zn in kidneys ($BCF_{kidneys/sedimentsZn}$=0.380) and for Pb in bones ($BCF_{bones/sedimentsPb}$=0.221). BCF was with the lowest values for the tree trace heavy metals for perch muscles. Accumulation of heavy metals in *E. excises* to their content in the fish organs and tissues was the highest of Pb from the muscles ($BCF_{E.excisus/musclesPb}$=283.769), followed by those of Pb for liver ($BCF_{E.excisus/liverPb}$=26.525), of Pb for kidneys and of Pb for bones ($BCF_{E.excisus/kidneysPb}$=16.872; $CF_{E.excisus/bonesPb}$ =11.759). Generally, the accumulation of the tree trace heavy metals were the highest of fish parasite species *E. excisus*, compared to their contents in muscles. As a result of this study (Table 2), the content of Cu and Zn was the highest in the sediments of the Arda River (C_{Cu}=25.5 and C_{Zn}=682.5 mg/кg, respectively) and the content of Pb was the highest in *Caryophyllaeus* sp. (33.353 mg/кg). With regard to organs and tissues, the content was the highest for copper in the liver ($C_{liverCu}$=12.977 mg/кg) and for lead and zink it was the highest in the kidneys ($C_{kidneysPb}$=4.511 and $C_{kidneysZn}$=148.441 mg/кg, respectively). The highest values of bioconcentration factor for liver and cooper and also for *Caryophyllaeus* sp., lead and zink ($BCF_{liver/Cu}$=0.508; $BCF_{Caryophyllaeussp./Pb}$=1.676; BCF $BCF_{Caryophyllaeussp./Zn}$=0.409) were established. The highest values of the bioacculation for lead were fixed (BCFmusclesPb=35.035; BCFliverPb=16.819; BCFbonesPb=15.313 and BCFkidneysPb=7.393).

Generally, the accumulation of the tree trace heavy metals were the highest of fish parasite species *Caryophyllaeus* sp., compared to their contents in muscles (Table 4). A linear correlation coefficient, (r_s, Spearman correlation coefficient) were determined to test associations between the bottom sediments, fish tissues, organs and fish parasites. Very significant correlation ($p<0.001$) were fixed for relationship between $Sediments_{Pb}$-$E.excisus_{Pb}$ and between $Sediments_{Pb}$-*Caryophyllaeus* $sp._{Pb}$ of the Arda River. The obtained values for the content of Pb, Zn and Cu in sediments, freshwater fish organs and tissues and their parasites from the Arda River are higher than those reported by other authors for the same ecosystem, but for another fish species (*Sq. cephalus*, *Sc. erythrophthalmus*) and fish parasites (*P. cuticola*, *Ac. anguillae* and *L. intestinalis*) but they are lower for *P. fluviatilis* (Kirin et al., 2010).

CONCLUSIONS

As a result of this examination a total of 65 fish specimens from 8 species were collected and examined from the Arda River. *Caryophyllaeus* sp., parasitic in *V. melanips* is generalist and *E. excisus*, parasitic in P. fluviatilis is allogenic species.

The received data for heavy metal contents in sediments, fish tissues and organs and fish parasites from the Arda River were presented for the first time for *P. fluviatilis*, *V. melanops* and their parasites *E. excisus* and *Caryophyllaeus* sp., respectively. The highest mean content of Pb are defined in *E. excisus* and *Caryophyllaeus* sp. (51.646 mg/kg, 33.353 mg/кg, respectively), followed by those in the sediments (19.98 mg/kg). Of tissues and organs, higher concentrations were obtained for the content of lead in bones and kidneys of the perch and for the content of lead in liver of the vimba. Generally, the accumulation of the tree trace heavy metals were the highest of fish parasite species *E. excisus* and taxa *Caryophyllaeus* sp., compared to their contents in muscles of the two fish species, respectively.

The high values of the bioconcentration factors and of the significant correlations determined *E.excisus* and *Caryophyllaeus* sp. as sensitive bioindicators for lead.

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DAPHNIA TEST – A SHORTCUT FOR HUMAN HEALTH PROTECTION ASSESSMENT

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Abstract

Daphnia magna toxicity testing was evaluated as a method for estimating the potential trace metals hazard to the environment. Electroplating whole effluent evaluation was used as case study. D. magna proved to be sensitive enough to Zn, Cr and to a certain extent to Ni to serve as a quick but reliable method for assessing possible human health hazard by bioconcentrated trace metals via freshwater fish consumption.

Key words: *Daphnia magna, metals, toxicity testing, effluent, human health.*

INTRODUCTION

Even today's toxicity tests on *Daphnia magna* are the only universal tests with freshwater invertebrates that are formally accepted as standard ones by all the most important internatiomal organizations: EC, OECD, ISO, US EPA (Mark and Solbe, 1998). As such, they are included in the obligatory monitoring of waste and recipient waters, but also as a re-liable and quick method for evaluation of the impact of certain hazardous materials on life environment (Adams, 1995). A few compre-hensive studies (Dierickx and Bradael-Rozen, 1996; Lilius and Isoma, 1955 – quot. Marks and Solbe, 1998; Nelson and Roline, 1998), as well as the data from AQUIRE database (US EPA, 2000a) show that *D. magna* is one of the most sensitive freshwater organisms to inorganic pollution, especially to heavy metals. For that reason data on lethal concentrations of most metals have served as a basis for establishing environmental standards and criteria (US EPA, 1986).

By the processes of bioaccumulation and bio-concentration, metals from the water column get into the tissues of freshwater fishes, and, at elevated ambiental concentrations, even the nutritional consumption of the musculature may present a possible risk to human health (Teodorovic, 1999). The goal of this paper was, on the example of an electric equipment factory, to examine whether the relative sensibility of *D. magna* to metals may be used to serve as a quick, simple, but also reliable method for evaluation of the possible threats to human health by the toxic effects of Zn, Ni and Cr released into the recipient (Tikvesh reservoir).

MATERIALS AND METHODS

The laboratory culture of Daphnia magna was grown in standard conditions (US EPA, 1993): glass vessels with population concen-tration up to 100 units, hard synthetic stan-dard water (NaHCO3 192 mg/l, KCl 8mg/l and MgSO4×7H2O 245 mg/l dissolved in deionized water EC< 20? S/m2 and aerated for 24 h; added CaSO4 95mg/l separately dis-solved in deionized water), with no aeration, laboratory lighting, photo period 16 h of light/ 8 h of darkness, temperature 25±°C, feed – 3 times a week YCT combination (fish pellets, wheat and beer malt). One-off (instant) sample of recipient water for dilution was taken on the left bank of the Crna River, before one Kavadarci town sewerage outlet. The basic physico-chemical parameters were determined in standard and Tikvesh reservoir waters (Table 1).

Hardness and alkalinity were determined titrationally (APHA, 1995), whereas electro-conductivity, pH and O_2 electrochemically. The waste water sample from the electric equipment

factory was taken as a daily composite, and a physico-chemical characterization was performed following standard method (APHA, 1995) (Table 2). Acute toxicity of the waste water was estimated using static test in duration of 96 h, on the neonatals of *D. magna* aged 24 h. The test was set with 5 units per test in a vessel of 50 ml volume, with 30 ml test solution in two runs. The dilutions were made parallely − with standard and with recipient water, in volume concentrations: 6.25, 12.5, 25, 50 and 100%, together with double control (recipient and standard water). The effect under observation was immobilization, or the units′ mortality. The condition for acceptance of the acute test was 90% control survival (US EPA, 1993). The results were processed using Dunnett test with variance analysis (ANOVA) (US EPA, 1991). LC/EC_{50} and $_{10}$ were determined by standard methods: Probit method with X^2 for hete-rogeneity (EPA Probit Calculation Program Version 1.5, US EPA, 1993) and Spearman Karber/Trimmed Spearman Karber method (EPA Trimmed Spearman Karber Version 1.5, US EPA, 1993).

Table 1. Values of basic parameters: standard and Tikvesh waters

Parameter	Standard Water	Tikvesh Water
Temperature (°C)	25	8
pH	8	7.8
Hardness (mg $CaCO_3$/l)	320	230
Alkalinity (mg $CaCO_3$/l)	245	220
Dissolved oxygen mg O_2/l	9	12
Saturation O_2%	85	97
- mS/m^2	700	500

RESULTS AND DISCUSSIONS

By physico-chemical characterization of the waste water (Table 2) the presence of Zn, Ni and Cr was established, which is expected, having in mind that it is a matter of effluents from the electrical equipment factory which, in its production process, has a line for gal-vanic processing of the metals. There were no other potentially hazardous materials, and the other examined parameters (all of them far beneath the MAC, Official Gazette of R. M., 2005) indicate waste water of low organic load.

Waste water toxicity was tested on *D. magna* in 6.25, 12.5, 25, 50 and 100% dilutions made with standard synthetic water as well as with the recipient (Tikvesh reservoir) water. The survival in both controls was 100%, which was expected regarding the favorable properties of the water (Table 2).

Table 2. Physico-chemical characteristics of the waste water

Parameter	Measuring unit	Value	Parameter	Measuring Unit	Value
Air temperature	°C	16	glowed residuum	mg/l	633
Water temperature	°C	16	loss by glowing	mg/l	201
$KMnO_4$	mg O_2/l	20.33	suspended material	mg/l	87
pH		8.4	greases and oils	mg/l	0.23
Draff materials	mg/l	1	surface active mat.	mg/l	0.86
HPK	mg O_2/l	180	Zn	mg/l	0.41
BPK_5	mg O_2/l	33	Ni	mg/l	3.6
Dry residuum at 105°C	mg/l	834	Cr (total)	mg/l	0.6

In the toxicity test where the Tikvesh reservoir water was used as a diluent (Table 3), 100% mortality was found at the 50% and 100% dilutions, which is also a statistically significant different survival compared to the control (one-way Dunnett test; $P< 0.05$). Since the mortality percentages did not monotonously grow together with the concentration of the effluent, LC_{50} was calculated using Trimmed Spearman-Karber′s method: Spearman-Karber estimate 96-h LC_{50} 22.11% (95% trust interval: 15.21-32.14

In the test where standard synthetic water was used as a diluent (Table 4), after 96 h 100% mortality was noticed only in 100% waste water. However, statistically significant different survival compared to the control was noted on the dilutions 50% and 100% (one-way Dunnett test;α =0.05).

The existence of partial mortality and the statistically important X2 test of heterogeneity (X2 calculated: 4.945; X2 – tabular value at 0.05: 7.815) prerequisited the use of Probit method for estimating LC/EC50 and 10:-LC/EC10: 11.181% (95% interval of trust: 14.288-17.319)-LC/EC50: 32.032% (95% interval of trust: 21.744-48.859)

Table 3. Results of the acute (96-h) effluent's toxicity test (diluent – Tikvesh reservoir)

Dilution (%)	Nr. of orga-nisms per testvessel	Nr. of runs	Total nr. of organisms	Mean survival value	sd	cv (%)
Control	5	2	10	1.00	0	0.0
6.25%	5	2	10	0.90	0.1414	15.7
12.5%	5	2	10	0.70	0.4243	60.6
25%	5	2	10	0.60	0.0	0.0
50%*	5	2	10	0.0	0.0	0.0
100%*	5	2	10	0.0	0.0	0.0

statistically significantly different than the control – one-way Dunnett test (α =0.05)

Table 4. Results of the acute (96-h) effluent's toxicity test (diluent – standard water)

Dilution (%)	Number of tested units	Number of dead	Proportion of dead	Probit calculation of deaths
control	10	0	0	0
6.25	10	1	0.1	0.0233
12.5	10	1	0.1	0.1259
25	10	2	0.2	0.3814
50*	10	7	0.7	0.7062
100*	10	10	1	0.9172

statistically significantly different than the control – one-way Dunnett test (α=0.05)

According to the results obtained, 96-h LC_{50} in Tikvesh water as a dilution was 22.11%, which means that the additive effect of Zn (0.09 mg/l), Ni (0.79 mg/l) and Cr (0.13 mg/l) was lethal for 50% of test organisms. But, in the tests with standard water as a dilution, 96-h LC_{50} was only 32.032%, or the mortality of 50% test units arouse as a response to the cumulative effect of 0.13 mg Zn/l, 1.1 mg Ni/l and 0.19 mg Cr/l. In the same test conditions, 96-h LC_{10} was 11.18% of the effluent, or 90% of the test units survived in the effluent with concentrations of 0.046 mg Zn/l, 0.4 mg Ni/l and 0.07 mg Cr/l. Obtained differences in the LC_{50} values in the tests with recipient and standard waters don't surprise. It has been proven that the twovalent cations' chemical form and bioavailability depends on pH, alkalinity and water hardness, or the metals' toxicity and bioaccumulation drops with the increase of hardness and alkalinity (Leland & Kuwabara, 1985). Therefore the explanation for the reduced toxicity of the examined effluent in the standard water ought to be looked for in the water's significantly greater hardness and alkalinity (320 and 245 mg $CaCO_3$/l) compared to the recipient water (230 and 220 mg $CaCO_3$/l) (Table 1).

The results obtained by these investigations agree with the literature data on toxicity of Zn, Cr and Ni on *D. magna.* Analyzing an effluent of the chemical industry, Tišler and Zagorc-Koncan (1994) identify the Zn as a direct cause of the recipient's high toxicity downstream from the outlet and find that 48-h LC_{50} for *D. magna* is 0.8 mg Zn/l. The acute toxicity of the Zn 948-h LC_{50} for *D. magna* varies from 0.04 mg/l at the hardness of 50 mg $CaCO_3$/l, up to 5.5 mg Zn/l at 250 mg $CaCO_3$/l. The first signs of chronical tixicity were registered at 0.07 mg Zn/l (US EPA, 2000b). The acute toxicity of Cr (VI) for *D. magna* in soft water is 0.02 and in hard water 0.04 mg Cr (VI) /l, whereas the data on the toxicity of Cr (III) vary from 0.044 – 0.066 mg Cr (III) /l, depending on water's hardness (US EPA, 1998). Based on available data (Kszos *et al.,* 1992), the toxic effects of Ni on *D. magna* in moderately hard water (100mg $CaCO_3$/l) appear not under 0.16 – 0.3mg Ni/l concentrations.

Because of the proven dependence of the toxicity and bioavailability of Zn, Ni and Cr on water's hardness, at the establishing am-biental criterions for protection of the aquatic species and human health from the toxic effects of some metals, US EPA (1986) gives models following which MACs for individual hydroecosystems of different water hardness ought to be calculated.

Regarding Zn, freshwater organisms are considered protected if 24-h average never exceeds (0.83 [ln (water hardness as CaCO30] + 1.95), i.e. in our conditions 320 – 570 mg Zn/l (US EPA, 1986). Macedonian MACs (Official Gazette of RM, 2005) for I/II and III/IV classes

of water are 0.2 or 1 mg Zn/l respectively, which is drastically above the lethal doses of Zn for D. magna, but at the same time is enough for protection of the human health. That′s why D. magna can be considered a suitable test organism for estimating a hydro-system′s pollution and the possible threat the latter poses to the people. US EPA (1986) prescribes a stricter ambiental concentration that safeguards human health′s protection from the toxic effects of Zn entered via eating fish: 47?g Zn/l. However, tests with D. magna can respond even to such strict demands, for LC10 calculated in this work is exactly 0.046 mg Zn/l.

Regarding Ni, freshwater organisms are considered protected if 24-h average (in μg Ni/l) does not exceeded (0.76 [ln (water hardness as CaCO3)] + 1.06) (US EPA, 1986), which in our conditions means 96 – 160 μg Ni/l (right on the limit MK MAC, Official Gazette of RM, 2005) and if the concentration (in μg Ni/l) never exceeds e (0.76 [ln (water hardness as CaCO3)] + 4.02), i.e. 1.8 – 3.1 mg Ni/l. And here as well D. magna may be called a suitable test organism, since according to MK MAC for III/IV water classes human health is not endangered by the toxic effect of Ni that enters the human organism by eating fish. According to US EPA, human health′s protection from the toxic effects of Ni entered via eating fish is secured at the ambiental concentration of 13.4 μg Ni/l. As a test organism D. magna is not able to meet such strict requirements since it isn′t sensitive enough to Ni.

Regarding sixvalent chromium, EPA (1998) thinks that freshwater organisms are protected if 4-days′ average does not exceed 11 μg Cr (VI) /l more often than once in three years and if 1-hour′s concentrations′ average does not exceed 16 g Cr (VI) /l more often than once in three years. The concentration of 50 μg Cr (VI) /l provides protection of human health from the toxic effects of Cr (VI) entered the human organism via eating fish (US EPA, 1998). D. magna is not able to meet such strict criterions, but it is a sufficiently sensitive test organism to MK MACs which presents no risks to human health via eating fish.

Regarding trivalent chromium, freshwater organisms are considered protected if 4-days′ average (in μg CR (III) /l) does not exceeded (0.8190 [ln (water hardness as CaCO3) + 1.561) more often than once in three years (US EPA, 1998), which in our conditions means 210 – 370g Cr (III) /l; and if 1-hour′s concentrations′ average (in g Cr (III) /l) does not exceeded (0.8190 [ln (water hardness as CaCO3) + 3.688) more often than once in three years (US EPA, 1998), or 1700 – 3100 μg Cr (III) /l. It is considered that ambiental concentration of 170 μg Cr (III) /l provides human health′s protection from the toxic effects of Cr (III) entered via eating fish. Based on the results of these investigations and on literature sources, with its sensitivity to Cr D. magna is a suitable test organism for estimating the threat this metal poses to human health.

CONCLUSIONS

Based on all this, a conclusion can be made that the toxicity test on *Daphnia magna* may enable quick and reliable insight in the hydroeco-system′s pollution with metals, but also give a relevant estimation of the possible threat to human health posed by metals entered via eating freshwater fish.

In this particular examined case, *Daphnia magna′s* high mortality at high dilutions indicates that, even within the zone of total mixing of the waste and the recipient waters, the concentrations of Zn, Ni and Cr in the water can present danger to human health because by the processes of bioconcentration they are able to accumulate within certain fish tissues in significant a-mounts.

For this reason it would be necessary, from time to time, to check the contents of Ni, Cr and Zn in the muscles of the fish caught in that sector if in the vicinity of the examined waste water there is some sewerage system outlet or some outlet of any other organically loaded effluent (that attracts fish).

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EXAMINATION OF CONTENT OF HEAVY METALS AND PESTICIDES IN FISH IN THE ACCUMULATION STREZEVO IN THE REPUBLIC OF MACEDONIA

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Abstract

In the period from 2007 to 2009 th year, the heavy metal concentration (Pb, Cd,? s? Hg) in carp, barbell, catfish and silver carp's muscular tissue, bones and internal organs was examined on three locations in the accumulation Strezevo in the Republic of Macedonia

According to Rulebook of maximum allowed quantities of pesticides, metals and metalloid and other toxic substances in the Republic of Macedonia, in our examined samples the concentration of all examined toxic elements was under maximum allowed limits, except lead and cadmium in the following samples: (carp's bones, silver carp's internal organs) on location 3 in accumulation Strezevo in the Republic of Macedonia where lead concentration was 1.07 and 1.81.

Cadmium concentration was also increased on the same location in the samples of carp's, silver carp's and barbel's bone tissue as in silver carp's internal organs.

Also the concentration of examined insecticides in all samples were significantly under maximum allowed quantities.

The results from our examinations have shown that the above mentioned locations in accumulation Strezevo in the Republic of Macedonia are relatively unpolluted. However, for more realistic ecological picture the number of analised samples should be increased.

Key words: *accumulation, heavy metals, pesticides.*

INTRODUCTION

Environment′s pollution with noxious materials is a consequence of industrial development, imple-mentation of certain agrotechnical measures in agriculture, urbanization and so on.

The contents of heavy metals in natural waters (Bojcic, 1982) are generally very low, the reason for this being their very small presence in Earth′s crust. However, due to the disharmony between the industrial development and the corresponding measures of environmental protection it came, among else, to water pollution with heavy metals (lead, cadmium, arsenic and mercury), as well as with organochloric pesticides′ residuums (chlorinated carbohydrates, organophosphates, carbamates etc.).

Fish are one of the best bioindicators of pollution with these elements (Baltic and Todorovic, 1997), therefore our investigations are oriented in that direction.

Different chemical forms of the same ecosystem may have multiple biological effect. When some po-tentially toxic substance is present in the water, many chemical processes may take place and much later the aquatic organisms to manifest their response. This substance, present in the water, can start interacting with other constituents of the water, so, in example, pH has impact on dissociation of acid and base, while humite acids form complexes with certain heavy metals. Cognition and determination of such processes is very important in understanding the inluence of toxic matters on hydrobionts. The amount of toxicants and their arrangement within the organism are closely related with the physiological processes such as: absorption through gills, bowels and skin, transport and distribtion through vascular system, metabolic transformation, accumulation in various tissues and organs, and excretion as well.

In their work Sorensen (1991) explained in details the emergence of interaction between different physiological processes which can occur if an organism is exposed to two or more toxic substances. Absorbed and accumulated chemicals bond with plasmatic proteins,

influence receptor sites so that the organisms give their response expressed through growth, behaviour, creation of offsprings and advent of death.

Rivers have important role in the transport of substances dissolved or associated with suspended solid particles and as recipients collect waste materials and metals before confluence into the lakes (Mason, 1981). Geologic substrate and erosion regime have the biggest impact on the chemical composition of rivers and the concentrations of heavy metals in them. However, in many areas the influence of atmos-pheric falls, industry and agrotechnical measures very often having prevalent effect on river water′s com-position, heavy metals including as well, prevails. From the aspect of anthropogenic pollution, the impact of big mining, industrial and urban centers is the most significant.

The total flora and fauna have central place in investigating and monitoring the heavy metals and other inorganic toxic substances within the ecosystem. Microorganisms, plants and animals play special role in determining the chemical form transport, also in the transfer to the storage locations of metals. The pos-sibility of potential toxic matters′ transfer through water or food to the people and the effects on people′s health causes great concerns. Heavy metal contamination in rivers and other water ecosystems depends on the type and the amount of waste materials arriving in them, on the vicinity of the confluence point into the recipient and its design and operational efficiency, and on the hydrology and the climate of the region (Harrison et all., 1991). The contaminants arrive into water ecosystems by washing the upper earth layers, filtrating surface waters, and sometimes in the form of vapors that reach water surface. Sewerage system′s waste water is the biggest source of heavy metals distributed in life environment, through effluents or faecal silt that usually contains larger concentrations of copper, lead, zinc, cadmium and silver (Forstner and Wittman, 1983). The inflow of melted snow from urban places often contains relatively high heavy metal concentrations.

Heavy metals in aquatic environment are present in three forms: as dissolved, colloidally dispersed and suspended. The form they appear in is of great importance to the behaviour, toxicity and bioavail-ability. They can also be classified based on the factors which primarily define their behaviour in fluvial environment:

- heavy metals in the largest portion controlled by biological processes – iron and manganese are good examples, whose forms of interaction directly depend on the aquatic system′s redox po-tential, and participate directly in the microbiological and photo-chemical reactions.
- heavy metals which in the largest portion are controlled by geochemical processes: absorption and complexing processes with suspended and colloidally dispersed materials (Cu, Pb, Zn and Cd).

In this paper four heavy metals were analyzed-the ones most often appearing in hydrobionts (Pb, Cd, As and Hg).

MATERIALS AND METHODS

During 2007-2009, the contents of heavy metals and residuums of organochloric pesticides in the muscle tissues, bones and internal organs of carp, tolstolobik, seathfish and babushka were being ex-amined, from five locations in Strezevo accumulation (26 carp samples, 7 barbell samples, 2 catfish samples and 20 silver carp samples).

For determination of the amount of toxic elements (Prpic-Majkic, 1985), the samples were prepared using ″wet combustion″ procedure (warming the sample with addition of Mg $(NO_3)_2$, concentrated nitric acid and hydrogen peroxide). After glowing at the temperature of 5.400°C, resulting ash was dissolved in 0.1 M hydrochloric acid and diluted with demineralized water to the suitable volume (25 ml).

The amounts of lead and cadmium were determined using the method of absorption spectrophoto-metry, on the instrument Varitan Spectar AA-10. For determining mercury (Mesaric, 1974) the technique of flameless (″Cold Vapor″) atomic absorption was used, whereas arsenic was determined spectrophoto-metrically. Organochloric insecticides′ residuums were determined using the method of gas chromato-graphy on VARIAN 3400 gas chromatograph, together with an EC detector with Sc ″3″ foil. Residuums′ extraction was

done using n-hexane together with extract′s purification using concentrated sulfate acid (Vojinovic-Miloradov & al., 1992).

RESULTS AND DISCUSSIONS

The contents of heavy metals in samples of muscle tissue, bones and internal organs of carp, barbell, catfish and silver carp were examined (Table 1).

According to the Regulations on the amounts of pesticides, metals, metaloids and other toxic sub-stances (Sl. list RM, 5/92), all the toxic elements′ concentrations in our samples were below maximum allowed limits, except for lead and cadmium in the samples (carp bones, barbell internal organs) from location 5 where lead concentrations were 1.07 and 1.81 respectively.

On the same location cadmium concentration was also increased in the samples of carp, barbell and silver carp bone tissue, as well as in the internal organs of barbell (Table 1).

Table 1. Contents of toxic elements in examined fish samples

Samples examined	Pb (mg/kg)	Cd (mg/kg)	As (mg/kg)	Hg (mg/kg)
muscle tissue carp – location 1	0.223	< 0.01	< 0.050	< 0.03
muscle tissue carp – location 2	0.215	< 0.01	< 0.050	< 0.03
summary sample internal organs (1+2)	0.420	0.043	< 0.050	0.11
muscle tissue carp – location 3	0.090	< 0.01	< 0.050	< 0.03
internal organs carp – location 3	0.138	0.014	< 0.05	0.08
muscle tissue catfish – location 4	0.253	< 0.01	< 0.050	< 0.03
muscle tissue carp – location 5	0.23	< 0.01	< 0.01	< 0.05
Bones carp – location 5	1.07	0.39	< 0.01	< 0.05
internal organs carp – location 5	0.23	0.07	0.05	< 0.05
muscle tissue silver carp – location 5	0.59	< 0.01	< 0.01	< 0.05
Bones silver carp – location 5	0.45	0.37	< 0.01	< 0.05
internal organs siver carp – location 5	0.31	0.03	0.05	< 0.05
muscle tissue barbell – location 5	0.29	0.04	< 0.01	< 0.05
Bones barbell – location 5	0.39	0.30	< 0.01	< 0.05
internal organs barbell – location 5	1.81	0.14	0.08	< 0.05

Allowed amounts of metals, non-metals and some specific contaminents, expressed in mg/kg according to the Regulations on the amounts of pesticides, metals and metaloids (Sl. list RM 5/92).

Pb 1 mg/kg
Cd 0.1 mg/kg
As 2 mg/kg
Hg 0.5 mg/kg

Table 2. Contents of organochloric insecticides in examined fish samples*

Examined samples	**Aldrine and dialdrine (mg/kg)**	**DDT & deriva-tives (mg/kg)**	**Endrine (mg/kg)**	**Total HCH (mg/kg)**	**Lindane (mg/kg)**	**Hepta-chlorine (mg/kg)**
muscle tissue carp – location 1	n.a.	0.05	n.a.	0.001	0.001	n.a.
muscle tissue carp – location 2	″	″	″	″	″	″
summary sample carp internal organs (1+2)	″	″	″	″	″	″
muscle tissue carp – location 3	″	″	″	″	″	″
internal organs carp – location 3	″	″	″	″	″	″
muscle tissue catfish – location 4	″	″	″	″	″	″
muscle tissue carp – location 5	″	″	″	″	″	″
Bones carp – location 5	″	″	″	″	″	″
internal organs carp – location 5	″	″	″	″	″	″
muscle tissue silver carp – location 5	″	″	″	″	″	″
Bones silver carp – location 5	″	″	″	″	″	″
internal organs silver carp – location 5	″	″	″	″	″	″
muscle tissue barbell – location 5	″	″	″	″	″	″
Bones barbell – location 5	″	″	″	″	″	″
internal organs barbell – location 5	″	″	″	″	″	″

Maximum allowed concentrations of these organochloric insecticides expressed in mg/kg according to the Regulations on the amounts of pesticides, metals and metaloids (Sl. list RM 5/92) are such:

Aldrine and Dialdrine 0.2 mg/kg
DDT & derivatives 1.0 mg/kg
Endrine 0.1 mg/kg
HCH 0.1 mg/kg

CONCLUSIONS

The results obtained indicate that the examined fishing locations are relatively unpolluted, where in larger number of samples certainly should be analyzed so we could get a more realistic ecological picture. Nevertheless, the most important thing is that the muscle tissue of fish from these locations, from the aspect of examined contaminants′ contents, is a health-friendly high-value food of animal origin.

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GENETIC EVALUATION IN A POPULATION OF FRASINET CYPRINIDS USED FOR SELECTION TO MAXIMIZE MEAT PRODUCTION

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Abstract

Genetic improvement of animals is defined as a process of directed change in the productive potential of the hereditary characteristics and the genetic resources of livestock populations in the desired direction by man. Achieving genetic improvement of the population is made by selecting the current generation, animals with the highest value of improvement that real genetic value for bio and ecoeconomic important characters. Breeding value cannot be measured directly in animals, but it can be deduced from phenotypic values of animals. Depending on breeding value, assessed individual is retained or dismissed from reproduction.
This study was conducted on a sample of 215 individuals Frasinet crap that came from 9 mother families. Frasinet carp, is part, of the morphological point of view, in a constitutional and productive category of carp breeds, with high degree of improved. This breed is characterized by high body profile, curved back and small caudal peduncle, head and fins. Individuals were reared in intensive system, in the same environmental conditions, from the juvenile stage by 2.5 ages. Each descendant was measured for three morphological characters: body weight (W), maximum body height (H) and body length (l) at the end of the first three summers of growth. Of the total of 215 candidates were retained for breeding 50% at end of each summer of growth. For the phenotypic characterization of the population have been used classical statistic methods and breeding value for each character was calculated based on individual animal model (B.L.U.P.-Animal Model). After selection of candidates, at the end of summer growth, annual genetic progress for body weight was 3.51%; 1.69%; 1.27%; for maximum body height was 1.35%; 0.85%, 0.43% and for body length was 1.10%; 0.80%, 0.44%. Expressed in the unit of character, annual genetic progress for body weight at the end of the third summer selection is higher than that of the first two years of selection. This is due to increase muscle mass and gonad development, both are processes specific to installation of sexual maturity.

Key words: *breeding value, carp, morphological characters.*

INTRODUCTION

In the context of sustainable animal production, the way which should be followed is to increase the animal production based on improving the genetic potential, together with the improvement of the operating conditions (Grosu, Oltenacu et. al., 2005). The followed goals in the growth of valuable species of fish, carp in our case, consist of transforming some bioeconomic and ecoeconomic features, in order to be useful for humans (Nicolae, 2012a).
The aim is to obtain species with:
- High growth rate;
- A certain external morphological aspect, according to biological and economic considerations;
- Increased precocity and prolificity;
- Resistance to diseases and unfavorable environment factors.

In practice, these objectives are reflected in the transformation of some characters of individuals belonging to the population with which the work is done.
Also, at fish, they can be considered domestic animals, whose genetic evolution is under long human intervention, and that any negligence or mistake in the management of the genetic fund, may have most serious consequences, until the disappearance of some races or local populations (Nicolae, 2004).

MATERIALS AND METHODS

In our country, the carp, which as a wild species was, decades ago, the basic fishery production in the lower Danube basin, was, is

and it will remain, at least as goal, the main fish species in Romania, regardless the economic circumstances.

This study was conducted on a sample of 215 individuals Frasinet crap breed that came from 9 mother families. Frasinet carp is a breed with high level of amelioration and is characterized by a high meet production. This breed is characterized by high body profile, curved back and small caudal peduncle, head and fins. Individuals were reared in intensive system, in the same environmental conditions, from the juvenile stage by 2.5 ages. Each descendant was measured for three morphological characters: body weight (W), maximum body height (H) and body length (l) at the end of the first three summers of growth.

The body weight (W) has been determined by weighing with a scale for small weights.

The maximum body height (H) has been measured in the highest region of the body, at the level of the first radiating from the dorsal fin, with the help of a graduated ruler.

The body length (l) has been measured on the midline of the body, from the top scaly snout to the end cover to the caudal fin, with the help of a graduated ruler.

Of the total of 215 candidates were retained for breeding 50% at end of each summer of growth. For the phenotypic characterization of the population have been used classical statistic methods and breeding value for each character was calculated by B.L.U.P. methodology (Best Linear Unbiased Prediction) based on individual animal model (B.L.U.P.-Animal Model) (Grosu, Oltenacu et. al., 2005).

RESULTS AND DISCUSSIONS

After estimating the breeding value of the 215 individuals of first summer, it was selected the first 108 individuals (50%) to be evaluated next summer, based on morphological characters studied (Nicolae, 2012b).

The value of global breeding value in Frasinet candidates carp breeding population, after first summer of growth, ranged from 2.095 to-0.812, while the retained candidates to selection varied between 2.095 and-0.565.

To estimate genetic progress were calculated by phenotypic parameters of the 108 individuals selected (Nicolae, 2012b). Based by phenotypic parameters of the candidates of selection and the retained candidates was estimated effect of selection (Table 1).

Table 1. The effect of selection in Frasinet carp breeds, after the first summer of growth

Specification	Characters		
	Body weight (W), g	Maximum body height (H), mm	Body length (l), mm
Selection differential	27.25	8.10	16.17
Heritability (h^2)	0.25	0.24	0.24
Genetic progress per generation	6.84	1.98	3.88
Generation interval (years)	3	3	3
Annual genetic progress	2.28 g	0.66 mm	1.29 mm
Annual genetic progress (%)	3.51	1.35	1.10

According to the results, in the next generation will most likely be an increase in body weight with 6.84 g, in maximum body height with 1.98 mm and in body length with 3.88 mm. After selection in first summer of growth, in terms of annual genetic progress, the average population changes by 2.28 g or 3.51% by body weight, 0.66 mm or 1.35% at the maximum body height and 1, 29 mm or 1.10% of the body length.

At the age of two summers, individual performance measures fall within the specific growth data of Frasinet breed. Body size index (l/H) is 2.37, lower than the first summer, which leads to increased meat production due to curvature of the line of back.

After estimating the breeding value of the 108 individuals of the second summer of growth, were selected first 54 individuals (50%) to be evaluated in the third summer, based on morphological characters studied (Nicolae, 2012b).

The value of global breeding value in Frasinet candidates carp breeding population, after the second summer of growth and selection, ranged from 1.865 to 0.899, while the retained candidates to selection varied between 1.865 and 1.278. It notes that the global breeding

value of retained candidates is positive. To estimate genetic progress were calculated phenotypic parameters of the 54 individuals selected (Nicolae, 2012b). Based by phenotypic parameters of the candidates of selection and the retained candidates was estimated effect of selection (Table 2).

Table 2. The effect of selection in Frasinet carp breeds, after the second summer of growth

Specification	Characters		
	Body weight (W), g	Maximum body height (H), mm	Body length (l), mm
Selection differential	100.79	7.02	17.83
Heritability (h^2)	0.34	0.43	0.38
Genetic progress per generation	33.82	2.99	6.73
Generation interval (years)	3	3	3
Annual genetic progress	11.28 g	1,00 mm	2.24 mm
Annual genetic progress (%)	1.69	0.85	0.80

According to the results, in the next generation will most likely be an increase in body weight of 33.82 g, maximum body height of 2.99 mm and a body length of 6.73 mm. As regards annual genetic progress after selection in the second summer of growth, the average population changes by 11.28 g or 1.69% by body weight, 1.00 mm or 0.85% at the maximum body height and 2,24 mm or 0.80% of the body length (Nicolae, 2012b).

At the age of three summers, individual performance measures fall within the specific growth data Frasinet breed. Body size index (l/H) is 2.25, lower than the second summer of growth.

After estimating the breeding value of 54 individuals, after the third summer of growth, were selected the first 27 individuals (50%) to participate in breeding the following year.

The value of global breeding value in Frasinet candidates carp breeding population, after the third summer of growth and selection, ranged from 2.120 to 0.280, while the retained candidates to selection varied between 2.120 and 1.219. It notes that the global breeding value becomes positive compared to previous ages. Also, due to selection, global breeding values are the highest values at the age of three summers.

To estimate genetic progress were calculated phenotypic parameters of the 27 individuals selected (Nicolae, 2012b). Based by phenotypic parameters of the candidates of selection and the retained candidates was estimated effect of selection (Table 3).

According to the results, in the next generation will most likely be an increase in body weight of 76.45 g, maximum body height of 2.17 mm and a body length of 4.96 mm.

As regards annual genetic progress after selection in the third summer of growth, the average population will increase by 1.27% or 25.48 g in body weight, 2.17 mm and 0.43% for maximum body height and 4, 96 mm or 0.44% of the body length (Nicolae, 2012b).

Table 3. The effect of selection in Frasinet carp breeds, after the third summer of growth

Specification	Characters		
	Body weight (W), g	Maximum body height (H), mm	Body length (l), mm
Selection differential	181.20	5.56	10.52
Heritability (h^2)	0.42	0.39	0.47
Genetic progress per generation	76.45	2.17	4.96
Generation interval (years)	3	3	3
Annual genetic progress	25.48 g	2.17 mm	4.96 mm
Annual genetic progress (%)	1.27	0.43	0.44

CONCLUSIONS

As a consequence of the study in the population of Frasinet carp breed, in what regards the genetic evaluation used for selection to maximize meat production, the following has been observed:

1. Selection to maximize meat production in Frasinet carp breed population can be made based on body weight character.

2. In a Frasinet carp population studied, annual genetic progress for body weight at the end of the third summer selection, is higher than that of the first two years of selection. This is due to increase muscle mass and gonad development, both are processes specific to installation of sexual maturity.

3. The values of the analyzed characters refer only to the study of the analyzed population and to the environmental conditions in which it has developed.

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