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GENETICS AND BREEDING

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DETERMINING THE RELATIONSHIPS BETWEEN GENOMIC AND PHENOTYPIC BREEDING VALUES

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Abstract

The main aim of the animal breeding is to increase the yields of economic traits in future generations and increase the obtained production per animal. Genomic selection studies have become applicable as a result of rapid increase of both genetic and computer sciences. In this study, Bayes A, Bayes B, Bayes C, Bayes Cpi which are used in genomic selection and BLUP (best linear unbiased prediction) methods which are used traditional selection will be compared. For this aim genomic breeding value and phenotypic breeding value has been estimated for lactation milk yield of Holstein cows breeding in a private company in USA. Holstein cows (400 individuals) were genotyped with 54k SNPs. The marker input file was coded as -10, 0, and 10 for marker genotypes AA, AB, and BB, respectively. A total of 50,000 iterations were used, with the first 5000 excluded as the burn-in. Bayes A, Bayes B, Bayes C and Bayes Cpi were performed using the software GenSel 4.55 and phenotypic breeding values for lactation milk yield in Holstein cows were estimated by restricted error maximum likelihood (REML), BLUP procedures using an MTDFREML (multiple trait derivative free restricted maximum likelihood) program employing an animal model. Correlations were examined between phenotypic and genomic breeding values with Spearman correlation. As a result, for estimating breeding values, while the highest correlation was found between BLUP with Bayes Cpi, the lowest correlation were found between BLUP with Bayes A.

Key words*: Bayes, BLUP, Genomic Selection, Breeding Value.*

INTRODUCTION

The main aim of the animal breeding is to increase the yields of economic traits in future generations and increase the obtained production per animal. Genetic improvement of breeding stock has been the goal of livestock producers for centuries (Verbyla, 2010).

During the first decade of the 21st century, there has been a rapid development of genomic selection tools. Through the application of genomic selection (Meuwissen et al., 2001), marker information from high-density SNP genotyping can increase prediction accuracies at a young age, shorten generation intervals and improve control of inbreeding (Daetwyler et al., 2007), which should lead to higher genetic gain per year.

Whole genome prediction (WGP) using commercially available medium to high density (50.000) single nucleotide polymorphism (SNP) panels have transformed livestock and plant breeding. Typically, the allelic substitution effects of all SNP markers are jointly estimated in WGP evaluation models assuming additive inheritance and summed to predict breeding values of each individual animal on the basis of its SNP genotypes (Yang and Tempelman, 2012).

The availability of high-density single nucleotide polymorphism (SNP) genotypes across the whole genome has enabled more accurate prediction of breeding values than conventional pedigree-based methods, as well as the mapping of QTL across the genome. Current routine genomic evaluations of cattle populations are performed using selected genotypes that are obtained from the ~54.000 SNPs that are included in the BovineSNP50 or so-called 50 K array. However, high-density Affymetrix (648.874 SNPs) and Illumina (777.962 SNPs, referred to as the 770 K array) genotyping arrays are now available (Hassani et al., 2015).

Another approach is to develop genomic predictions that are accurate in multiple breeds through training on multibreed populations of purebred or crossbred animals. This has the advantage of allowing small populations to be pooled to create reference populations of the

scale required to derive accurate genomic predictions (Weber et al., 2012).

Many simulation studies have shown the benefits of this technology, depending on heritability, number and distribution of effects of QTL, population structure, size of training data set used to estimate SNP effects, and other factors. However, studies on real data are still scarce (Wolc et al., 2011).

In this study, we aimed to compare Bayes A, Bayes B, Bayes C, Bayes Cpi which are used in genomic selection and BLUP (best linear unbiased prediction) methods which are used traditional selection.

MATERIALS AND METHODS

Genomic breeding value and phenotypic breeding value has been estimated for lactation milk yield of Holstein cows breeding in a private company in USA. Holstein cows (400 individuals) were genotyped with 54k SNPs.

The marker input file was coded as -10, 0, and 10 for marker genotypes AA, AB, and BB, respectively. A total of 50.000 iterations were used, with the first 5000 excluded as the burn-in. Bayes A, Bayes B, Bayes C and Bayes Cpi were performed using the software GenSel 4.55 and phenotypic breeding values for lactation milk yield in Holstein cows were estimated by restricted error maximum likelihood (REML), BLUP procedures using an MTDFREML (multiple trait derivative free restricted maximum likelihood) program employing an animal model.

RESULTS AND DISCUSSIONS

Estimated Spearman rank correlation among breeding values obtained from Bayes A, Bayes B, Bayes C, Bayes Cpi and BLUP were given in Table 1.

Table 1. Spearman rank correlations for BV

Specification	BLUP	Bayes A	Bayes B	Bayes C
Bayes A	-0.519			
Bayes B	-0.518	0.999		
Bayes C	-0.613	0.941	0.942	
Bayes Cpi	-0.387	0.642	0.653	0.654

All the obtained correlation coefficients were found statistically significant ($P<0.01$). In analysis 6414 markers was removed because was monomorphic. Estimated parameters were given in Table 2 and Table 3.

Table 2. Genetic parameters estimated from Bayes methods

Specification	Bayes A	Bayes B	Bayes C	Bayes Cpi
Genetic Variance	0.000506953	0.000514429	0.00708704	0.00239004
Residual Variance	0.0254734	0.0254589	0.0190007	0.0238036
Total Variance	0.0259804	0.0259733	0.0260877	0.0261936
h2	0.019	0.019	0.272	0.091
Pi	-	-	-	0.999
Compute Time (sn)	4300	5908	1071	7669

Table 3. Genetic parameters estimated from BLUP method

Variance Components	Value
σ_a^2	264879.6
σ_c^2	643304.2
σ_e^2	1161231.1
σ_p^2	2069414.9
h^2	0.13
r	0.44

Estimated heritability from BLUP was found as expected. Mall heritability values observed from Bayes methods except Bayes C which was relatively high than other methods.

Negative correlations were estimated BLUP versus Bayes methods as expected for less heritable traits such as milk yield (Dekkers, 2007).

CONCLUSIONS

Bayes methods produced similar results for studied data. Negative Spearman rank correlations were observed between BLUP and Bayes methods and the case of these results attributed to less heritable trait of studied milk yield. Further studies should be conducted with larger sample sizes.

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THE HERITABILITY STUDY OF THE CHARACTERS FOR DEVELOPMENT, REPRODUCTION AND MILK PRODUCTION AT THE ACTIVE RSC POPULATION FROM THE BUCHAREST AREA OF MILK SUPPLY

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Abstract

This paper presents RSC characteristics regarding heritability parameter for the development, reproduction and milk production at the Pantelimon and Afumati farms. The milk production is the main target when we follow the goal of milk production exploitation from those two farms. To determine the heritability of morphologic characteristics and capability for the mechanic milking, it is obvious the milk cows from the group of study have a level of genetic determination for waist between 43% at Pantelimon farm and 35% at Afumati. So, they are fitting under variability limits of heritability coefficient. A higher heritability value to the entire population in this study is the height at the croup character. At the Afumati farm it was h^2= 0.49 and at Pantelimon farm h^2 = 0.47. The milking speed represents the most important parameter to reflect the capability of the udder for the mechanic milking, the heritability coefficient being between 28% and 48%, results obtained also by the other authors. The heritability coefficient determined for the Calving interval (CI) indicate a weak genetic determination, so, at the Pantelimon farm h^2 = 0.195 and Afumati farm h^2 = 0.110. The heritability of the between birth and the service period (SP) has lower values, between h^2 = 0.140 at Pantelimon farm and h^2 = 0.100 at Afumati farm. The heritability coefficient of milk production 0.31 - 0.35 and the fat percent was 0.48-0.51. So, at the both farms, we obtained data can be compared with similar data from the profile literature.

Key words: *heritability, calving interval, service period.*

INTRODUCTION

Cattle are in the economy in general and agriculture in particular, an important socio-economic features that result from their main function, food function, as it provides 96% of the world production of milk, 30% of the world production of meat and over 90% of the world production of skins and directly participate in the growth, development and health insurance mankind.

This paper studies the characteristics of the Romanian Spotted Cattle breed, in terms of milk production and economic parameters in Pantelimon and Afumati farms.

MATERIALS AND METHODS

This paper presents characteristics of heritability for the development, reproduction and milk production at the RSC population from Pantelimon and Afumati farms. Milk production is the main objective pursued in milk production operation of the two farms analyzed.

Heritability (h^2) is defined by I. Lush (1941), as the proportion of the total variant which can be assigned to the medium effect of the genes, meaning the fraction of additive genetic variant and the total phenotypic variant.

The total phenotypic variant can be defined as a regression of the amelioration value to the phenotypic value, or as the square of the correlation between amelioration value and the phenotypic value.

Heritability expresses oneself in absolute values with decimals or relative.

The noteworthy and use of the heritability coefficient by E.Negrutiu is:

- The phenotype of a character is always the result of the interact between the genotype and the environment,

- The heritability value is the dependency of the entire variation components,
- The genetic components are influenced by the gene frequencies in a population and, as result, the heritability may be different from a population to another,
- Population of the animal homozygous has more hereditary conservativism, a heritability coefficient higher than heterozygotes one,
- The heritability has a practical signification in the amelioration process,
- The heritability coefficient is one of the components in all relations about prediction in an improvement of the value of cattle – index for selection, genetic progress etc.

RESULTS AND DISCUSSIONS

Heritability of the taxonomy and capability for the mechanic milking – the analysis of the quantitative characters had developed once with the progress to the scientific amelioration and creating the programs of amelioration and optimized plans of selection with the special contribute of Robertson and Rendel (1950) etc.
We are presenting below the value of the heritability coefficient at the both farms, in it ranged, from the character nature point of view (Table 1 and 2):

Table 1. h^2 coefficient values from the main morphological characters and capability for the mechanic milking to the milk cows from Pantelimon farm

Nr.crt.	Character	N	$h^2 \pm e$
1.	Size	175	0.370±0.13
2.	The height at the croup	175	0.470±0.18
3.	The oblique length of the body	175	0.570±0.16
4.	Thoracic perimeter	175	0.370±0.12
5.	The body weight	175	0.380±0.17
6.	The milking speed	175	0.480±0.18

Table 2. h^2 coefficient values from the main morphological characters and capability for the mechanic milking to the milk cows from Afumati farm

Nr.crt.	Character	N	$h^2 \pm e$
1.	Size	150	0.350±0.14
2.	The height at the croup	150	0.490±0.17
3.	The oblique length of the body	150	0.480±0.16
4.	Thoracic perimeter	150	0.350±0.14
5.	The body weight	150	0.450±0.15
6.	The milking speed	150	0.360±0.18

The height at the croup has a higher heritability value to the entire population analyzed. Thus, we calculate to the Afumati farm h^2 =0.49 and to the Pantelimon farm h^2=0.47.
The oblique length of the body present the highest genetic determinism to the Pantelimon farm (57%), the value obtained being superior data from the scientific literature (44% - A. Petre).
The h^2 determined at the body weight is 0.38 l (Pantelimon farm) and 0.45 (Afumati farm).

The milking speed is the most important parameter in the measurements of the udder capability for the mechanic milking, the value of the heritability determined for the population of cows is ranged between 28% and 48%.
The reproduction characters heritability represent an important leverage which assures the safety for determination in amelioration potential of the bulls.

Table 3. The values of the heritability coefficient in main reproductive characters at the milk cows in Pantelimon farm

Nr.crt.	Character	n	h^2±e
1.	Age of first birth	175	0.413± 0.17
2.	Calving interval (CI)	175	0.195±0.12
3.	The period from birth to fecund insemination (SP)	175	0.140±0.14
4.	Dry period	175	0.250±0.15
5.	The lactation period	175	0.225± 0.12

Table 4. The values of the heritability coefficient in main reproductive characters at the milk cows in Afumati farm

Nr.crt.	Character	n	h^2±e
1.	Age of first birth	150	0.310±0.16
2.	Calving interval (CI)	150	0.110±0.17
3.	The period from birth to fecund insemination (SP)	150	0.100±0.14
4.	Dry period	150	0.210±0.18
5.	The lactation period	150	0.200±0.15

The age at the first birth heritability value determined in this study is fitting in the medium to intense characters genetic determinate group, making a fine precision for the selection of this character.

The period heritability between birth and the first fitting fecund (SP) at the milk cows population in this study has small values, ranged between h^2= 0.140 (Pantelimon farm) and h^2 = 0.100 (Afumati farm) with a genetic determinism small grade and impossible to be significant improve through amelioration.

The value of the heritability coefficient for the milking period to the analyzed population is 0.225 to Pantelimon farm and 0.200 to Afumati farm. The value of this indicator is at an inferior level for the middle characters genetically determined, with low chances to be improved through the amelioration action. Regarding the amelioration of this character, we suggest to optimize the breeding and exploitation links of technology in both farms.

For the heritability of the milk production characters, we obtained comparative data with those results in the others authors on different RSC populations.

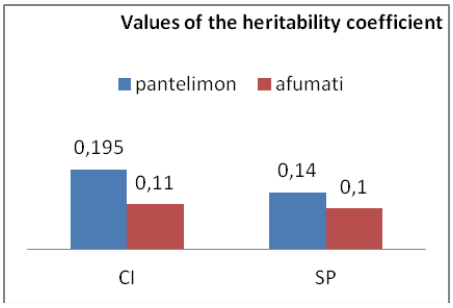

Fig.1. Values of the heritability coefficient at the Pantelimon and Afumati farms

Table 5. The heritability value for the milk production at the cows from Pantelimon and Afumati farms

Nr. crt	Farm	Milk quantity	Fat quantity	Fat %
1.	Pantelimon	0.30±0.15	0.35±0.15	0.51±0.17
2.	Afumati	0.28±0.12	0.31±0.11	0.48±0.16

About the heritability of the fat quantity, we obtained results at the middle of those obtained by others authors.

The heritability of the fat percent has a very high value on both farms, which is characteristic for a pronounced genetic determinism and, at the same time, indicates the efficiency of amelioration factors in the improvement of fat quantity to the RSC population.

Table 6. The comparative range of h^2 milk production characters
On analyzed population by lactations sequence

Nr.crt	Farm	Milk quantity			Fat quantity			Fat %		
		I	II	III	I	II	III	I	II	III
1.	Pantelimon	0.30± 0.17	0.21± 0.11	0.25± 0.15	0.39± 0.16	0.48± 0.10	0.24± 0.17	0.48± 0.14	0.53± 0.15	0.45± 0.12
2.	Afumati	0.31± 0.13	0.28± 0.18	0.26± 0.16	0.35± 0.12	0.30± 0.12	0.30± 0.17	0.48± 0.10	0.52± 0.14	0.46± 0.12

CONCLUSIONS

Analyzing the dynamic heritability of milk production characteristics to the RSC population we found that:

- h^2 of milk quantity has the high value at the first lactation after that is decreasing,
- h^2 of fat quantity has the higher value at the second lactation to the Pantelimon farm,
- at the both farms, h^2 of the fat percent has a slow increasing to the second lactation.

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GROWTH PERFORMANCES OF FEMALE AND MALE HOLSTEIN CALVES FED WITH MILK AND MILK REPLACERS

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Abstract

The objective of this study was to compare growth performances of male and female Holstein calves fed milk and milk replacers. A total of 60 Holstein calves were used in the study. Calves were divided into three equal groups. In each group, there were 10 female and 10 male calves. Calves were offered colostrum for 3 days after birth and were weighed at fourth day for the trial. Initial body weights of calves in dietary treatments were statistically similar. The first, second and third groups were fed milk, milk replacer-I (CP 21% and CF 16.5%) and milk replacer-II (CP 24% and CF 18%), respectively. In addition to milk and milk replacers, calves were supplemented with ad libitum concentrate feed and alfalfa. Dietary treatment was significantly effective (P<0.05) on body weight of calves at 60 days of age. In conclusion, growth performances of calves increased with increasing protein content of milk replacer, also growth performances of calves fed milk and milk replacer containing high-protein had better than those of calves fed milk replacer containing low-protein. Therefore, during the suckling period, in feeding of Holstein calves, milk or milk replacer containing high-protein should be preferred primarily.

Key words: *Holstein calf, milk replacer, milk, growth performance.*

INTRODUCTION

Efficient growth of young dairy calves is important for profitability of the dairy enterprise. Before weaning, intake of nutrients from liquid feeds is usually limited to stimulate early dry feed intake, allow development of ruminal function and early weaning (Bush and Nicholson, 1986). Appropriate and ample supply of nutrients for calves through liquid feed (milk or milk replacer) is essential for performance and welfare. Conventionally, dairy calves are separated from dams within few hours of their birth and receive a restricted amount (typically 10% of BW/day) of milk or milk replacer through a nipple or bucket (Lee et al., 2009).

Milk replacers are generally cheaper and offer an alternative to whole milk feeding to weaning. These practices are common in the management of calves from temperate breeds such as Holstein-Friesians in contemporary dairy systems (Bhatti et al., 2012). Modern milk replacers can be classified by protein source, protein/fat levels and inclusion of medication or additives. Protein and fat levels are both important to consider when choosing a milk replacer (BAMN, 2013). Good quality milk replacers are also very good sources of liquid feed for calves (Grobler, 2008). Feeding of calves with milk replacers is limited. Therefore, further researches are needed on this subject in calves.

The objective of this study was to compare growth performances of male and female Holstein calves fed milk and milk replacers.

MATERIALS AND METHODS

The study was conducted on a commercial dairy farm (DIMES, Kazova Vasfi Diren Agriculture Farm) located in Turhal district of Tokat province, Turkey. A total of 60 Holstein calves were used in the study. Calves were divided into 3 groups (n=20, 10 male and 10 female). Two different milk replacers (MR-I and MR-II) and milk were used for calves feeding. The first group was fed MR-I, the second group was fed MR-II, and the third group was fed milk. Calves received milk and

milk replacers during 60 days. Calves were separated from their mothers within 1 h of birth, and were kept in individual hutches (1.2x1.4x1.3 m) bedded with straw. Calves were offered 4 liters of colostrum for 3 days after birth and were weighed at the fourth day. Body weights of calves in dietary treatments were statistically similar. Milk replacers and milk feeding were started at fourth day.

Calves were weighed at intervals of 15 days until 60th day.

Body weights of calves were determined with a scale (TESS, Yildirim Electronic Weighing Systems, Yenibosna, Istanbul) that is fine-tuned to 50 g. Average daily gains were determined from birth to 60th day. To prepare milk replacers, diluted milk replacers were prepared in buckets by mixing 0.125 kg of milk replacer in 1 liter of warm water (50°C). Diluted milk replacers were then cooled to 38-40°C for feding. Prepared milk replacers and milk were poured to the bucket having nipples, and fed to the calves twice in a day. Daily milk replacers and milk offered were divided equally and fed at 08:00 and 16:00 h. In addition to milk and milk replacers, calves were supplemented with ad libitum concentrate feed and alfalfa between 4-60 days of age. Water intake was ad libitum. Chemical compositions of milk replacers are given in Table 1.

Table 1. Chemical compositions of the milk replacers (MR-I and MR-II)

	MR-I	MR-II
Crude protein (CP) %	21	24
Crude fat (CF) %	16.5	18
Crude cellulose (% max.)	0.5	0.1
Crude ash (% max.)	8	6.9
Ash insoluble in HCL (% max.)	1	0.6
Humidity (% max.)	3.5	3.5
NaCl (% max.)	0.6	0.6
Lactose (%)	25	39
Ca (%)	0.9-1.1	0.9-1.5
P (%)	0.85	1
Vitamin A (IU)	40000	40000
Vitamin D3 (IU)	4000	4000
Vitamin E (mg)	100	-

Feeding programs of Holstein calves fed milk and milk replacers are given in Table 2.

Descriptive statistics for studied variables (characteristics) were presented as mean, standard errors. Two-way analysis of variance was performed to compare means of dietary treatment groups and gender for the body weight scores. Following analyses of variance, the independent samples t-test was used to determine whether differences between the genders.

Table 2. Feeding programs of Holstein calves fed with milk (M) and milk replacers

Colostrum (liter)			
Day-week	Morning	Evening	Total
1-3 days	2	2	4
M, MR-I and MR-II (liter)			
4-7 days	2	2	4
2nd -4th week	2.5	2.5	5
5-6th week	3	3	6
7th week	2.5	2.5	5
8th week	2	2	4
57-60 days	1	1	2
	Starter concentrate	Alfalfa	Water
4-60 days	ad libitum	ad libitum	ad libitum

Descriptive statistics for studied variables (characteristics) were presented as mean, standard errors. Two-way analysis of variance was performed to compare means of dietary treatment groups and gender for the body weight scores. Following analyses of variance, the independent samples t-test was used to determine whether differences between the genders.

Differences between three feeding groups were analyzed by using Duncan multiple comparison test in one-way ANOVA method. Statistical significance levels were considered as 5% and SPSS statistical program was used for all statistical computations (SPSS, 2002).

RESULTS AND DISCUSSIONS

Body weights of female and male Holstein calves fed milk and milk replacers are presented in Table 3. When Table 3 is examined, initial weights for male and female calves in three dietary treatment groups were similar. Body weights of female and male calves at 15 days of age were not affected by dietary treatments. Interactions between dietary treatment and gender during all periods of growth were not statistically significant ($P>0.05$). Body weights of both female and male calves at 30, 45 and 60 days of age were

affected (P<0.05) by dietary treatments. Body weights of calves fed milk and milk replacer-II were heavier than those of calves fed milk replacer-I.

Better body weight gain at weaning in calves fed whole milk compared with those fed milk replacer may be attributed to better bioavailability (digestion and utilization) of nutrients (protein and energy). Availability of ideal protein (casein) and energy-yielding constituents (fat and lactose) along with other known (minerals, vitamins, enzymes, and hormones) and unknown growth factors probably resulted in better body weight gain in whole milk-fed calves compared with those fed milk replacer (Lee et al., 2009). Jaster et al. (1990) reported that increasing fat in milk or milk replacer increased body weight gain during the first months of the calves' life. Eivazi et al. (2013) reported that effects of feeding full milk and milk replacer on average daily gain of Holstein calves were significantly different. Results of the current study was in agreement with the findings reported by Eivazi et al. (2013) for Holstein calves. Bhatti et al. (2012) reported that average daily gain of Sahiwal calves offered milk was higher than those offered milk replacer. Lee et al. (2008) has reported no difference in the performance of Holstein calves fed varying levels of protein in energy in the milk replacer-fed Holstein calves. In a study, Lee et al. (2009) have reported that weaning weights for Holstein calves were lower in milk replacer fed calves than whole milk group, despite similar dry matter intake and gross composition of both milk replacer and whole milk. They described that better weaning weights of calves from whole milk group, was probably because of the better bioavailability of nutrients and some unknown growth factors in milk.

Table 3. Body weights (kg) of female and male Holstein calves fed milk and milk replacers.

Days	Dietary Treatments (DT)	Gender (G)				*Significance of G*	DT x G
		Female		Male			
		Mean	SE	Mean	SE		
0 (Initial)	M	40.40	0.72	41.50	0.56	ns	ns
	MR-I	39.70	0.50	41.60	0.58	ns	
	MR-II	40.00	0.67	41.70	0.65	ns	
	Significance of DT	ns		ns			
15	M	43.90	0.98	46.30	0.63	ns	ns
	MR-I	42.90	0.67	44.90	0.46	ns	
	MR-II	43.30	0.67	45.30	1.33	ns	
	Significance of DT	ns		ns			
30	M	49.50^{bA}	1.09	53.60^{aA}	0.93	*	ns
	MR-I	46.70^{B}	0.97	47.80^{B}	0.71	ns	
	MR-II	50.40^{A}	1.08	51.60^{A}	1.45	ns	
	Significance of DT	*		*			
45	M	59.60^{A}	1.45	62.90^{A}	0.66	ns	ns
	MR-I	56.10^{B}	1.38	54.30^{B}	0.78	ns	
	MR-II	58.30^{A}	1.45	59.80^{A}	2.03	ns	
	Significance of DT	*		*			
60	M	72.80^{A}	1.69	75.80^{A}	1.31	ns	ns
	MR-I	65.40^{B}	1.40	63.90^{B}	1.20	ns	
	MR-II	69.00^{A}	1.78	71.70^{A}	2.01	ns	
	Significance of DT	*		*			

$^{a, b}$:The differences between the means of groups with different superscripts (in the same row) are significant (P<0.05).
$^{A, B}$:The differences between the means of groups with different superscripts (in the same columns) are significant (P<0.05).
ns: Non significant

Average daily gains of female and male Holstein calves fed milk and milk replacers are presented in Table 4. When Table 4 is examined, average daily gains of both female and male calves in the interval day 0-15 and 0-30 were not affected by dietary treatments. But, average daily gains of both female and male calves in the intervals day 0-45 and 0-60 were affected (P<0.05) by dietary treatments. Average daily gains of calves fed milk and milk replacer-II were heavier than those of calves fed milk replacer-I.

In the present study, the average daily gain of Holstein calves fed milk and milk replacer containing high-protein were higher than those of calves fed milk replacer containing low-protein. However, El-jack and Ahmed (2012) reported that the pre-weaning weight gain

obtained by calves fed milk replacer were significantly higher than that obtained by calves fed raw milk. This may be attributed to the highly nutritious value of milk replacer compared to the raw milk. On the other hand, growth performances observed for calves reared on milk replacer in this study were lower than that reported by El-jack and Ahmed (2012). The findings of the current study showed that calves fed milk and milk replacer containing high-protein were grew faster than calves fed milk replacer containing low-protein. Hill et al. (2006) conducted an experiment on effect of feeding a control 20% crude protein, 20% fat milk replacer compared to feeding a 28% crude protein, 20% fat milk replacer (0 to 49 days). They reported that average daily gains of calves in each groups were similar (0.53 and 0.54 kg/day, respectively). However, our study has shown that average daily gains of calves fed with different milk replacers did differ. Growth performances of calves fed certain milk replacers may be increased due to supplementing specific amino acids of the milk replacer (Hill et al., 2007; Hill et al., 2008). Khan et al. (2012) reported effect of milk replacer on weaning weight of different bovine breeds was significant. In the present study, growth performances of calves increased with increasing protein content of MR. This finding is compatible with the finding reported by Blome et al. (2003).

Table 4. Average daily gains (g/day) of female and male Holstein calves fed milk and milk replacers

Days	DT	Gender (G)				*Significance of G*	DT x G
		Female		Male			
		Mean	SE	Mean	SE		
0-15	M	233.33	30.23	320.00	34.14	ns	ns
	MR-I	199.97	24.35	220.00	22.33	ns	
	MR-II	220.00	14.23	240.00	56.39	ns	
	Significance of DT	ns		ns			
0-30	M	303.33^{b}	21.34	403.33^{a}	25.56	*	*
	MR-I	233.33	23.31	206.67	17.78	ns	
	MR-II	346.67	27.76	330.00	32.38	ns	
	Significance of DT	ns		ns			
0-45	M	426.67^{A}	22.90	475.56^{A}	21.00	ns	*
	MR-I	364.44^{aB}	23.00	282.22^{bC}	18.16	*	
	MR-II	406.67^{A}	29.45	402.22^{B}	33.37	ns	
	Significance of DT	*		*			
0-60	M	540.00^{A}	21.11	571.67^{A}	28.00	ns	ns
	MR-I	428.33^{B}	19.88	371.67^{B}	23.05	ns	
	MR-II	483.33^{A}	28.54	500.00^{A}	25.58	ns	
	Significance of DT	*		*			

$^{a, b}$:The differences between the means of groups with different superscripts (in the same rows) are significant ($P<0.05$)

$^{A, B, C}$:The differences between the means of groups with different superscripts (in the same columns) are significant ($P<0.05$).

CONCLUSIONS

Growth performances of calves increased with increasing protein content of milk replacer, also growth performances of calves fed milk and milk replacer containing high-protein had better than those of calves fed milk replacer containing low-protein. Therefore, during the suckling period, in feeding of Holstein calves, milk or milk replacer containing high-protein should be preferred primarily.

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ASSESSMENT OF RAMS KARAKUL BREEDING VALUE AFTER SELECTION COMPLEX INDEX

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Abstract

The aim of the research was to: elaboration a method for estimating the value of Karakul breeding rams through the construction and applying selection complex indexes. The research was conducted on Moldavian Karakul sheep at the flock of National Institute of Animal Livestock and Veterinary Medicine, Maximovca village, Anenii Noi district, Republic of Moldova. In this paper has been examined the methodology of constructing complex index of breeding rams selection of Moldavian Karakul race. It was found that, prior, for construction selection index complex has been reduced the number of characters and appropriation of lambs skin evaluation marks from 29 to 7, and the latter have been synthesized into a single character - Class lamb expressed in points after the decimal system. Have been identified three basic morpho-productive characters (skins quality of descendents, own body weight, milk production of maternal ram) and followed by the selection of breeding rams. It was been determined the economic value (share) of each selection character in the total income of one animal per year. For each selection character in part was been calculated coefficient phenotype aggregate (C_{fa}), which allows expression of the value of each character in unique units of measure, using the following formula:

$$C_{fa} = \frac{P_{ve}}{M_s} \qquad (1)$$

where, C_{fa} *– coefficient phenotype aggregate;*
P_{ve} *– share the economyc value of selection character;*
M_s *– phenotype standard size of selection character*

Having the aggregate phenotype coefficient for each character selection basis, we have built complex selection index of ram, after following formula:

$$I_{cs} = (M_{fp} \cdot C_{fap}) + (M_{fmc} \cdot C_{famc}) + (M_{fpl} \cdot C_{fapl}) \qquad (2)$$

where, I_{cs} *– selection complex index of ram;*
M_{fp} *– phenotype size of skin quality of rams descendents;*
C_{fap} *- coefficient phenotype aggregate of skin character;*
M_{fmc} *– phenotype size of own body weight of ram;*
C_{famc} *- coefficient phenotype aggregate of body weight character;*
M_{fpl} *– phenotype size of mothers ram milk production;*
C_{fapl} *- coefficient phenotype aggregate of milk production.*

Key words*: Complex index, selection, rams, Karakul.*

INTRODUCTION

Determining the targets value, complex breeding of animals of different species of animals in the result of evaluation marks present a actual permanent problem which is of concern to livestock researchers and specialists in the field. Ordinary appreciation of the animals after several characters long applied in livestock. Class summary of the animal itself have been determined after evaluation marks represent, in her essence, an index. However, this ordinary appreciation by summary of accumulated points, can not be considered and a complex one, because it is not reflects the importance of the economic value of each selected character. In summary class the all characters have the same value, while in complex selection index each character, obligatorily find its economic value and breeding. Selection effect after complex indices it is, according to data by 10% (Тарасевич Л.И., 1979; Таинберг Р.Р., 1971), according to others (Гуревнина И.В., 2002) of 2.0 times higher than the method based on selection independent limits of selection characters.

The Moldavian Karakul sheep type has a mixed productivity (combined) of skins, meat, milk, which determines the necessity of the selection of animals after this complex of characters that may be organized through different method: in tandem, by the independents limits or by complex selection index. Latter model is considered the most modern method. Determination the animal selection index represent, in fact, a complex evaluation of its breeding by main selected characters, taking into account the productive performance and economic value.

Application selection indices at the Karakul race, in generally is not widespread. Are known only some publications (Гуревнина И.В., 2002; Карынбаев А.К. , 2009; Карынбаев А.К. et. al., 2014; Юлдашбаев Ю.А. et. al., 2010), relating the selection indices to determine the growth potential of Karakul lambs during the ontogenesis postnatal period. The authors communicate about the application of the selection indices of the harmony of body conformation, but these (indexes) have not a complex character because it reflects only some co reports of some external characters (size) such as: body weight, thorax perimeter and oblique trunk length. Therefore, mentioned selection indices by these authors can not be used to determine the overall value of the animal breeding.

Currently, in determining the general value of breeding (class) Moldavian Karakul sheep, according to the Guidelines of evaluation Karakul sheep principled of improvement in the Republic of Moldova (Buzu I.A. et. al., 1996), are not taken into account some of the most important morph-productive characters selection (such as the milk production and the meat - the body weight). In fact, the main flaw of the actual Guidelines is that production of skins is considered only basic character, expressed by the lamb class, but the body weight and the milk production are considered characters associated (secondary) and are not taken into consideration anymore in determining the breeding value of the animal (class). So between the value of main morpho-productive characters and the value of the animal breeding there is a evident rupture, requiring integrated into a unique complex of phenotypic, genotypic and economic values of animal. From here, appears the pressing need to improve these instructions through the development and inclusion in the methodology of selection some modern methods for estimating the breeding value with application complex indices of selection.

In this context, it was proposed to develop a method for estimating the breeding value of Karakul rams through the construction and applying the complex selection indices.

MATERIALS AND METHODS

The research was conducted on the Moldavian Karakul sheep from the flock of the National Institute of Livestock and Veterinary Medicine from Maximovca village, Anenii Noi district, the Republic of Moldova. In view of the fact that the effectiveness of selection for more characters is inversely proportional to the square root of the number of characters selected ($\frac{1}{\sqrt{n}}$), according to the recommendations by Iliev T.V. (1992), first of all, we have been limited the number of characters selected from appreciating the skin of lambs, from 29 to 7, and the latter have been synthesized into a single character - class lamb expressed in points after the decimal system. Finally, as a first step, for building selection complex indices of Karakul adults rams, have been identified only 3 major morph-productive characters:

- skin quality - the descendents - lambs at the evaluation marks, expressed in points assessment after the decimal system, or value (score) their skin - without assessing after skin qualities of descendents;
- own body weight of the ram, determined annually (in October) at the adults rams, in autumn before the start of the mating company, by weighing at the technical weight with 150 kg capacity (Buzu I., 2014);
- milk production of maternal ram, or the average of daughters milk production - in case of their result lactation, expressed in kilograms and determined according to the methodology perfected by us (Buzu I., 2014).

The second step, carried by us in the way of building indices selection was to determine the

economic value of the three selection characters and establishing its share in total income from a ewe per year (Buzu I. et al., 2014). Systematizing and generalizing the above research results, we have deduced the following shares the economic values of character selection:

- the skin quality - 12 %;
- the body weigth - 28 %;
- the milk production - 60 %.

As mentioned selection characters have different phenotypic measurements and values for building summary complex selection indices, we have proceeded to calculate *coefficients phenotype aggregate*, which allow phenotypic transformation size of the character in the economic value weighted single of the animal selection complex index.

As a reference point for determining the coefficients of phenotype aggregate served the phenotype standard size (Ms) of the selection character, that represents the race standard (level of I class), for each age group and sex of animals in part, developed by us for the type of Moldavian Karakul sheep (Buzu I., 2012).

The coefficient of phenotype aggregate of the ram was calculated for each character individually selected following formula:

$$C_{fa} = \frac{P_{ve}}{M_s} \qquad (1)$$

where,

C_{fa} –coefficient phenotype aggregate;

P_{ve} – share economic value of the character selection;

M_s –phenotypic standard size of character selection.

Having available the coefficients aggregate phenotype for each selected character, we have deduced the complex selection indices of the ram, after following formula:

$$\mathbf{I_{cs}=(M_{fp}\cdot C_{fap})+(M_{fmc}\cdot C_{famc})+(M_{fpl}\cdot C_{fapl})}$$

where,

$\mathbf{I_{cs}}$ – index selection complex of ram;

$\mathbf{M_{fp}}$ – phenotype size of skin quality;

$\mathbf{C_{fap}}$ - coefficient phenotype aggregate of skin character;

$\mathbf{M_{fmc}}$ – phenotype size of rams body weight;

$\mathbf{C_{famc}}$ – coefficient phenotype aggregate of body weight caracter;

$\mathbf{M_{fpl}}$ - phenotype size of milk production;

$\mathbf{C_{fapl}}$ – coefficient phenotype aggregate of milk production.

RESULTS AND DISCUSSIONS

The research results have shown that complex selection index, determined according to the above formula, combine, through coefficients phenotype aggregate, the summary value of the three main selection characters of respectively ram. The numerical value of the index is expressed in numbers without measuring units, in the range of two or three integer digits and one (tenths) or two (hundredths) digits rounded after the comma. In case which the phenotypic size of the three characters selection will coincide exactly with the breed standard, the selection index value will be equal to 100. According to the size of the phenotypic selection characters, the value of the complex selection index can be lower or higher than 100. In principle, the complex selection index indicates the level of animals breeding value compared to the rase standard and, also shows the extent to which it fails, approaching or exceeding this standard. In case than selection index value exceeds 100, we can conclude that value of the animal breeding is greater than the race and, conversely, if the index is below 100, breeding value of the animal does not meet the race standard.

At the Moldavian Karakul sheep we have built complex selection indices of breeding rams, and established the following succession of calculating it.

1. Determination the coefficient phenotype aggregate of selection characters.

1.1. Giving the formula (1), the coefficient aggregate phenotype for the quality of skin was as follows:

$$C_{fap} = \frac{P_{vt}}{M_s} = \frac{12}{6} = 2.0$$

where,

C_{fap} – the coefficient phenotype aggregate for the skin quality;

P_{ve} – share economic value of leather character established by 12%;

M_s – Phenotype standard size of the character skin quality at level of class I = 6 points.
As a result of calculations made, the coefficient aggregate phenotype for the skin quality of character is equal to 2.0.

1.2. The coefficient aggregate phenotype for body weigth was:

$$C_{famc} = \frac{P_{ve}}{M_s} = \frac{28}{75} = 0.373$$

unde:

C_{famc} – the coefficient aggregate phenotype for body weigth;

P_{ve} – share economic value of the body weight = 28;

M_s – Phenotypic standard size for body mass for breeding rams is 75 kg.
As a result of calculations made, the coefficient phenotype aggregate of rams body weight character is equals 0.373.

1.3. The coefficient phenotype aggregate for milk production was:

$$C_{fapl} = \frac{P_{ve}}{M_s} = \frac{60}{70} = 0.857$$

where:

C_{fapl} – the coefficient phenotype aggregate for milk producton;

P_{ve} – share economic value of milk production = 60 kg;

M_s – phenotypic standard size of milk production = 70 kg
As a result of calculations made, the coefficient phenotype aggregate of ram mothers milk production character equals 0.857.

2. Determination the complex indexes of ram selection.
Thus, having coefficients aggregate phenotype of characters selection for breeding race, we built following formula of index complex selection:

$$\mathbf{I_{csb}=(M_{fp}\cdot 2{,}0)+(M_{fmc}\cdot 0{,}373)+(M_{fpl}\cdot 0{,}857)}$$

where,

I_{csb} – the complex selection index of reproducing ram;

M_{fp} – the phenotypic size of the descendants skin quality score expressed in mean value scores, or the value (score) own skin - in without assessing after the skin qualities of descendents;

M_{fmc} - phenotype size of their body weight;

M_{fpl} - phenotype size of mothers ram milk production, or daughters - in case of there of lactation results;

2.0 – the coefficient phenotype aggregate of the quality skin descendents;

0.373 - the coefficient phenotype aggregate of ram own body weight;

0.857 - the coefficient phenotype aggregate of rams mother milk production.

According to this formula, we have calculated complex selection index for breeding-rams from investigated flock (Tab. 1).
Thus, the data presented in table reveals that the largest amount of ram breeding has nr. 5422, which occupies the first rank in the string breeding rams.
Having 96 kg body weight, with the average score of quality skin descent worth 6.14 points and maternal milk production equal to 83 kg, the complex index of selection was:

$$\mathbf{I_{cs5422} = (6{,}14\cdot 2{,}0) + (96\cdot 0{,}373) + (83\cdot 0{,}857) = 12.28+35.81+71.13 = 119.2}$$

Examining the achieved complex index of selection, we can say that the ram no. 5422 has a complex superior breeding value breed standard 19.2% and part of the elite breeding of the flock, whose value exceeds the breed standard. This ram can be involved in the company mating with the best females in the flocks intensively using it to improve productivity, because it exceeds the quality breed standard of the race after descent skin quality with 2.3%, body weight with 28.0% and milk production with 18.6%. After complex index of selection this ram is the most valuable breeding of the flock.

Table 1. Complex selection indexes of breeding-rams from flock

Nr. of rank	Nr. matriculation ram	Mean score skin descendents	Body weight ram, kg	Milk production of mother, kg	Complex indices of selection
1	5422	6.14	96	83	119.2
2	4228	6.54	89	82	116.5
3	7823	6.04	91	78	113.6
4	6356	6.00	82.4	81	112.1
5	0073	6.16	88	75	109.4
6	6502	5.08	92	73	107.0
7	9125	5.02	89	74	105.7
8	1668	5.37	93	68	103.7
9	6218	4.58	81	71	100.2
10	4103	6.10	74.5	70	100.1
11	3982	4.17	78	72	99.1
12	8144	4.94	87	65	98.0

Another obvious example presents the ram no. 4228, with body weight of 89 kg, with the average score of quality skin descents worth 6.54 and the mothers milk production equal to 82 kg, whose complex selection index was:

$$I_{cs4228} = (6,54 \cdot 2,0) + (89 \cdot 0,373) + (82 \cdot 0,857) = 13.08 + 33.2 + 70.27 = 116.5$$

After calculating complex selection index, we can conclude that this getter is also a valuable breeding fairly good compared to the breed standard and the other rams, being part of breeding elite flock, whose value is estimated at high. This ram can be trained, also, mating with the best females in the flock using it intensively for productivity improvements. After the productive performance, this getter exceeds the standard race after quality of descendents skin by 9.0%, own body weight with 18.7%, and mother milk production with 17.1%. After the complex selection index of the ram exceeds standard with 16.5 units.

Another example shows the ram no. 4103 quality skins of 6.10 points, 74.5 kg body weight and the mothers milk production of 70 kg. Complex selection index of this ram is obviously located in the breed standard (class I) and are:

$$I_{cs4103} = (6,10 \cdot 2,0) + (74,5 \cdot 0,375) + (70 \cdot 0,857) = 12.2 + 27.94 + 59.99 = 100.1$$

As we observe this ram is neutral, after the productive performance, does not differ at all from the average breed standard. From this ram descendents cannot wait an amelioration of the morpho-productive performance compared to the breed standard. Use of this ram mating can lead to stabilization of morpho-productive performances in the flock.

If all breeding ram from flock for will be calculated the complex indices of selection, livestock specialist will be have the possibility determining the ranking each of them and revelation the most valuable breeding.

Generalizing the results of building the complexes indices of selection at the breeding rams, we find that the constructive principle of complex selection index is based on the integration of the three selection characters, such as, skin quality, body weight and milk production. The difference between the complexes indices of selection of each ram consists in the diversity of phenotypic values of skin qualities progeny, their body weight and production of breast milk, the last two character being the decisive complex in formula for calculating the selection index.

Having the above formulas, livestock specialist, at the end of the year, calculated the complex indices of selection, and determines the breeding value of each animal. These calculated indices, is entered in the register of complex evaluation marks of sheep. Based on these entries, the specialist determines the rank of animals in the flocks, selecting the most valuable of these in batches of breeding for reproduction.

CONCLUSIONS

1. Assessing the value of breeding of ram by complex selection index, reflects objectively

the actual quality of breeding, combining integrally three important selection characters, such as skin quality, body weight and milk production.

2. Implementing the method for estimating of value breeding animals after complex selection index will be help increase the efficiency of Moldavian Karakul sheep selection.

3. The method for estimating the value of breeding the rams after complex selection indices to be formalized by inclusion of these provisions in the rules (instructions) livestock of evaluation marks and certification of material sheep genetically material of breeding, with their approval in the established manner.

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COMPARATIVE PERFORMANCE AND EGG QUALITY OF LAYING HENS IN ENRICHED CAGES AND FREE-RANGE SYSTEMS

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Abstract

Enriched battery cages and free-range as an alternative raising systems are intended to improve welfare of hens. Comparison of the productivity performance and egg quality of laying hens housed in two different systems regarding the welfare of laying hens was the objective of the study. A totally, eight hundred 19-wk-old Lohmann Brown hens were housed in enriched cages (n=400; 16 cages; 25 hens per cage) and in free-range system (n=400) to 50 wk of age. Hen-day egg production, feed intake, feed efficiency were measured at 30, 40 and 50 wk. In both rearing systems, eggs were recorded for 2-wk intervals between 30 and 50 wk of age to measure egg quality parameters. Hen-egg production was significantly higher in enriched cages than free range system throughout the experiment (P<0.05). Hens raised in free-range system had greater egg weight, egg shell thickness and dirty eggs than in enriched cages (P<0.05). In addition, the feed intake and feed efficiency were higher in the free-range raising system than in the enriched cages at 30 wk. However, the heights and width of egg albumen and yolk were not affected by the raising systems (P>0.05). Based on the results the interior egg quality parameters appear to have similar for hens kept in both rearing systems. However, the higher proportion of dirty eggs for hens raised in free-range system was the greatest problem and still needs to be considered.

Key words*: enriched cages, free-range, egg quality, performance, laying hens.*

INTRODUCTION

After the ban decision on conventional cages in the European Union by 2012 (CEC, 1999) various alternative housing systems such as aviaries, floor husbandry, free-range and enriched (furnished) cages have been approved. Egg production system is probably one of the most important challenges for the egg producing industry in the last decade.

There are various factors including diseases, behavior, nutritional value, genetics and air conditions in house affecting the level of welfare laying hens. Traditional (conventional) battery cages are not sufficiently for allowing hens behaviour, new rearing systems including free cage rearing offer hens a significantly improved level of animal welfare than do conventional battery cage systems (Duncan, 1998; Duncan, 2004).

The use of enriched cages and free-range housing systems have received a considerable attention raises on hens well-being among the other alternative systems.

Battery cages have many disadvantages for welfare including behaviour, but also some benefits such as resulting in a low level aggression and cannibalism (Appleby, 1998) and maintaining a small group size, hygiene and animal health conditions (Rodenburg et al., 2005)

Differences between the alternative raising systems may affect the welfare, health and hygiene and resulting in the performance and egg quality parameters.

The aim of the present study was to evaluate the differences in laying hens performance and internal and external egg quality for laying hens kept in enriched cages and free-range systems.

MATERIALS AND METHODS

A totally eight hundred 19-wk-old Lohmann Brown hens were housed in enriched cages (n=400; 16 cages; 25 hens per cage) and in free-range system (n=400) to 50 wk of age. The enriched cages (120 x 55 x 45; length × width × height) had wire floors and solid metal walls. In fee-range system, hens were housed in a stocking density with six hens per m^2 door and 0.2 hens per m^2 of range area.

Both groups of hens were fed a commercial feed containing 17.5 % CP, 2750 kcal ME/kg, 3.5 % Ca and 0.85% available P. Thought the experiment lights were on a 16L:8D schedule, from 07:00 to 2300 h. Feed intake, feed efficiency were measured at 30, 40 and 50 wk. In both rearing systems, eggs were recorded for 2-wk intervals between 30 and 50 wk of age to measure egg quality parameters. Body weight and feed intake and feed efficiency were determined each week during the all period of experiment. Egg production per group, per-cage-hen-day production and quality parameters were 30, 40 and 50 weeks of age on the random sample of 30 eggs per treatment.

Statistical analysis was performed using the mixed model and *t*-test procedure of SPSS 15.0. Tukey's test was used to separate group means. A significant difference was at $P < 0.05$.

RESULTS AND DISCUSSIONS

Housing system has an important influence on the performance (Anderson and Adams, 1994; Moorthy et al., 2000), welfare (Stojcic et al., 2012) and for the productive performance of laying hens (Mugnai et al. 2009).

Egg production, feed intake and feed efficiency results were presented in Table 1. Feed efficiency was lower in the hens kept in the enriched cages compared with those reared in the free-range system at 30 wk of age ($P<0.05$). Previous studies showed that housing system of hens had significant effect on hen egg production (Flock et al., 2002; Stojcic et al., 2012). In present study, hen-egg production was significantly higher in enriched cages than free range system throughout the experiment ($P<0.05$).On the other hand, Roll et al. (2009) showed no difference in egg production between laying hens kept in conventional cages and in floor pens. Rearing system did not affect the feed consumption of laying hens at 30 and 40 wk of age ($P>0.05$). However, hens in enriched cage system consumed significantly lower feed than hens in free-range system at 50 wk of age ($P<0.05$)

Hens raised in free-range system had greater egg weight than in enriched cages ($P<0.05$) (Table 2) in the third period (30, 40 and 50 wk of age). Besides, egg weight increased over time at 30, 40 and 50 wk of age in both rearing systems. Similarly, Singh et al (2009) also found greater egg weights in floor pens than in conventional cages. In contrast to our findings, Yakabu et al. (2007) reported that eggs from floor pens were lighter than those from conventional cages.

Significant influence of rearing system was obtained on egg shape index at 30 wk of age, on egg shell thickness throughout the experiment ($P<0.05$). However, the shape index did not differ between two rearing systems at 40 and 50 wk of age ($P>0.05$). The thickest egg shell was recorded in eggs from hens reared in free-range at 40 and 50 wk of age ($P<0.05$).

Mortality is a main indicator of poor welfare, management and other housing conditions. Tauson and Abrahamsson (1999) reported that a greater mortality of hens kept floor pens than cages. Contrary, in our study mortality during the rearing period in enriched cages was higher than free-range housing system (7.8 % for enriched cages and 5.4 % for free range).

Egg quality is important for the economic success of a producer and also consumer appeal (Singh et al. 2009). Egg quality may be influenced by several factors including housing regimen and nutritional values. The overall egg internal quality parameters (albumen height, width and yolk height, width) were not significantly ($P>0.05$) different between the 2 rearing systems (Table 3).

Table 1. Effect of rearing (enriched cage and free-range) systems on egg production and performance of laying hens

Period	Hen-egg production (%)		Feed consumption (g/hen per d)		Feed efficiency (g of feed/g of egg)	
	Enriched	Free-range	Enriched	Free-range	Enriched	Free-range
Wk 30	91.6^{a}±1.12	87.4^{b}±1.22	103.7±0.47	107.6±0.44	2.15^{b}±0.06	2.20^{a}±0.09
Wk 40	94.0^{a}±1.36	90.3^{b}±1.10	115.2±0.38	118.4±0.46	2.08±0.03	2.10±0.06
Wk 50	91.1^{a}±1.22	88.6^{b}±1.31	118.6^{b}±0.40	124.7^{a}±0.39	2.12±0.04	2.14±0.05

^{a,b}Means± SE within each period with different superscript letters are significantly different ($P < 0.05$).

Table 2. Weight, shape index, shell weight and shell thickness of eggs of laying hens in enriched cages and free-range systems

Period	Egg weight (g)		Shape index		Shell weight (g)		Shell thickness (mm)	
	Enriched	Free-range	Enriched	Free-range	Enriched	Free-range	Enriched	Free-range
Wk 30	56.2^{b}±0.31	59.8^{a}±0.23	76.0^{b}±1.11	77.5^{a}±0.22	6.3±0.09	7.5a±0.09	0.30±0.005	0.33±0.004
Wk 40	60.1^{b}±0.47	62.8^{a}±1.03	77.3±0.24	76.9±0.35	6.7±0.09	7.0±0.09	0.27^{b}±0.004	0.31^{a}±0.003
Wk 50	62.1^{b}±0.32	64.0^{a}±0.47	77.2±0.27	76.7±0.38	7.1±0.08	7.4±0.11	0.26^{b}±0.003	0.29^{a}±0.004

^{a,b}Means± SE within each period with different superscript letters are significantly different ($P < 0.05$).

Table 3. Albumen height and width, yolk height and width of eggs of laying hens in enriched cages and free-range systems

Period	Albumen height (mm)		Albumen width (cm)		Yolk height (mm)		Yolk width (mm)	
	Enriched	Free-range	Enriched	Free-range	Enriched	Free-range	Enriched	Free-range
Wk 30	8.2±0.14	8.0±0.16	7.6±0.13	7.4±0.12	18.4±0.09	18.4±0.12	41.0±0.24	42.2±0.22
Wk 40	8.0±0.18	8.2±0.14	7.6±0.22	7.4±0.19	18.6±0.11	18.5±0.09	42.1±0.13	41.3±0.15
Wk 50	8.4±0.22	8.5±0.18	7.3±0.08	7.2±0.09	18.6±0.10	18.7±0.10	40.9±0.22	40.3±0.23

^{a,b}Means± SE within each period with different superscript letters are significantly different ($P < 0.05$).

The influence of rearing systems differed dirty eggs and cracked eggs (Table 4).

Table 4. Cracked and dirty eggs of laying hens in enriched cages and free-range systems

Period	Cracked eggs (%)		Dirty eggs (%)	
	Enriched	Free-range	Enriched	Free-range
Wk 30	0.35±0.02	0.40±0.03	2.59^{b}±0.11	5.33^{a}±0.18
Wk 40	0.54^{a}±0.04	0.78^{a}±0.06	2.04^{b}±0.13	6.72^{a}±0.17
Wk 50	1.12^{a}±0.09	0.86^{b}±0.06	1.88^{b}±0.09	8.41^{a}±0.22

In our study proportions of dirty eggs were significantly higher in the free-range system than enriched cage system (P<0.05). However, no significant differences were found in interior egg quality traits between keeping systems (P>0.05). A similar housing effect was found by Abrahamsson and Tauson, (2005). Besides, the percentage of cracked eggs was influenced by rearing system and increased with age (P<0.05). The highest percentage of cracked eggs was observed in free-range systems (P<0.05).

CONCLUSIONS

Based on the results the interior egg quality parameters appear to have similar for hens kept in both rearing systems. However, the higher proportion of dirty eggs for hens raised in free-range system was the greatest problem and still needs to be considered.

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EFFECTS OF CALVING AGE AND SEASON ON SOME MILK YIELD TRAITS IN ANATOLIAN BUFFALOES

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Abstract

The objective of the study was to investigate the non-genetic factors affecting the some milk yield traits of Anatolian buffalo raised at public hand in Amasya province, Turkey. A total of 239 buffalo calved in 2014 year were constituted the research material. Calving age and calving season were assessed as affecting non-genetic factors on daily milk yield (DMY), lactation milk yield (LMY) and lactation length (LL). The overall means of DMY, LMY and LL were 2.76±0.051 kg, 470.91±9.784 kg and 171.8±1.66 day, respectively. Calving age had a significant (P<0.05) effect on DMY and LMY, but its effect on LL was not significant. The effects of calving season on DMY, LMY and LL were not significant. DMY and LMY increase progressively until 8th calving age, and the highest milk yield found in the 8thage, then decline gradually in the 9th and 10th ages. The current results show that good selection programme and improvement management including for calving age could improve milk yield traits.

Key words*: Anatolian buffalo, calving season, daily milk yield, lactation milk yield, lactation length.*

INRODUCTION

Buffalo farming has been an important production source for Turkey. However, Turkey's buffalo population and its amount of production have declined dramatically during the last 40 years because of increasing demand for cattle production rather than buffalo production (Soysal, 2014).

Turkish water buffalo, which is called as Anatolian buffalo are practically classified as a river water buffalo of Mediterranean water buffaloes group (Cicek et al., 2009; Soysal, 2014). Buffaloes are having high capacity to face adverse environmental conditions and a remarkable longevity. Anatolian Buffalo breeding which is a traditional production model has great importance in the rural household economy with small holding of Turkey (Pawar et al., 2012). They are mostly bred in North, Middle, West, East, and Southeast Anatolia in Turkey (Atasever and Erdem, 2008). Anatolian buffaloes are a considerably preferred due to their resistance to diseases and lower feed consumption (Şahin et al., 2014). Most important reasons for rearing Anatolian water buffalos are their milk and meat (Soysal et al., 2015).

Lactation milk yield (LMY) and lactation length (LL)are important parameters of dairy buffaloes (Chaudhry, 1992). Milk yield in buffaloes are depended upon genetic and non-genetic factors. The non-genetic or environmental factors such as management, amount and quality of feed and season (Afzal et al., 2007; Pawar et al., 2012) are also closely interacted with animal's health and productivity (Kamble et al., 2014). The milk yield traits in buffaloes are influenced by numerous environmental factors (Zakariyya et al., 1995), for example calving age and calving season (Raza et al., 1999; Khosroshahi et al., 2011; Şahin and Ulutaş, 2015). In order to enhance productivity of a dairy buffalo cow, it is necessary to develop and understanding of the factors effecting its milk production (Afzal et al., 2007; Pawar et al., 2012). Only a few reports about these factors on milk yield and lactation length for Anatolian buffaloes. Thus, further studies are needed to determine on milk yield traits of Anatolian buffaloes. The objective of this investigation was to determine

the effects of non-genetic factors on some milk yield traits in Anatolian buffaloes.

MATERIALS AND METHODS

Data were obtained from the scope of the project of improvement of Anatolian buffalo in public hand supported by General Directorate of Agricultural Research and Policies in Amasya province.

Milk records collected a total of 239 Anatolian Buffalo cows calved 2014 year in Amasya province was used for the present study.

Milk records were obtained from individual farms. Abnormal records and lactations affected by disease were excluded from the study.

The milk production was calculated on monthly record. Buffaloes are milked one a day by hand and machine in the morning. They were fed *ad libitum* in shelter.

The lactating buffaloes grazed outside between the months of April to December, while being kept and fed indoors through the winter. The buffaloes were fed a total mixed ration all year round.

The data was classified according to eight calving ages (from 3 to 10) and four calving seasons' groups (autumn, winter, spring and summer).

The environmental factors were evaluated included calving age and birth season.

The statistical model assumed for the evaluation of environmental factors on daily milk yield (DMY), lactation milk yield (LMY) and lactation length (LL) were as fallows;

$\gamma_{ijk}=\mu + a_i + b_j + e_{ijk}$

γ_{ijk} = The k^{th}observation in the i^{th}calving age and j^{th}calving season

μ = overall mean,

a_i= effect of i^{th} calving age (*i: 3 to 10*)

b_j= effect of j^{th}calving season (*j: autumn, winter, spring and summer*)

e_{ijk} = random error.

Analyses were performed by the general linear model technique in SPSS for Windows statistical package programme (SPSS, 13.00). Duncan's multiple range test was used to find out difference between means within the same statistical package programme.

RESULTS AND DISCUSSIONS

Means of milk yield traits and standard error of means, and effective factors are given in Table 1. The overall means of DMY, LMY and LL were 2.76±0.051 kg, 470.91±9.784 kg and 171.8±1.66 day, respectively. Similar results were observed by Şekerden et al. (1999) for DMY, and Şahin and Ulutaş (2014) for LMY and LL in Anatolian buffaloes. The mean of DMY, LMY and LL were generally lower than the findings of Özenç et al. (2008), Soysal et al. (2015), Kücükkebapçı et al. (2015) and Ugurlu et al. (2015) for Anatolian buffalo. The difference may be due to various management and environmental conditions, herd and farm size (Afzal et al., 2007), variations in feed and fodder availability, sire used for breeding and their genetic potential (Jamuna et al., 2015).

Effect of calving age on DMY in present study was significantly important ($P<0.05$). DMY was the highest in buffaloes which calving in the 8th age, butthe lowest in the 3^{th} and 10^{th} age. Generally, DMY was increase progressive with age, especially between 4^{th} to 8^{th}ages, then reduce in the 9^{th} and 10^{th} age. The result of this study was closely in agreement with the results of Şahin and Ulutaş (2015) and Eskandari and Karimpour (2012), who found that effect of calving age on DMY was significantly important in Anatolian Buffalo and Iranian Khuzestan Buffalos, respectively. Similar to present study, Khosroshahi et al. (2011) reported that the first parity was significantly different from the others ($P<0.05$). In addition, Khosroshahi et al. (2011) also found that the lowest milk production was in the first calving ($P<0.05$).

In present study, DMY was not significantly affected by calving season. Similar results were obtained by other researches (Dutt and Yadav, 1986; Ghaffar et al., 1991; Jamuna et al., 2015), who season of calving had non-significant on DMY. It can be explained that climate stress factors may be minimize and overcome through better feeding and management (Afzal et al., 2007). The results of present study were not in line with the findings of Zaman et al. (2007), Khosroshahi et al. (2011); Şahin and Ulutaş (2015), who reported that the season of calving had a significant effect on DMY. Khosroshahi et al. (2011) stressed that Buffaloes calving in

the spring had the highest DMY, but lowest in the summer. Hassan Razaet al. (1999) showed that the highest milk production in Nili Ravi buffaloes was in autumn.

Table 1. Means of milk yield traits in Anatolian Buffaloes (Mean±SE)

Factors	Classes of the factors	Number	DMY (kg)	LMY (kg)	LL (day)
Calving age	3	54	2.60±0.107[b]	443.44±19.553[ab]	173.1±3.56
	4	37	2.79±0.111[ab]	470.53±21.607[ab]	168.5±3.46
	5	45	2.74±0.085[ab]	468.23±16.677[ab]	172.1±3.85
	6	33	2.86±0.133[ab]	490.85±28.692[ab]	170.6±4.27
	7	26	2.92±0.278[ab]	506.48±46.430[ab]	175.8±3.79
	8	12	3.14±0.169[a]	534.97±36.941[a]	170.6±7.82
	9	17	2.62±0.165[ab]	467.72±33.901[ab]	178.6±6.59
	10	15	2.61±0.106[b]	425.57±34.353[b]	162.9±10.52
Calving Season	Autumn	25	3.00±0.164	522.80±31.346	174.5±3.28
	Winter	30	2.69±0.090	474.05±20.730	176.5±4.47
	Spring	105	2.66±0.086	454.28±15.373	172.8±2.68
	Summer	79	2.84±0.084	475.39±17.104	167.8±2.89
	Overall	239	2.76±0.051	470.91±9.784	171.8±1.66

DMY: Daily Milk Yield, LMY: Lactation Milk Yield, LL: Lactation Length
[ab]: Means in the same column with no common superscripts differ (P<0.05)

Differences between this research results may be largely due to different management methods, environmental condition employed and different breed (Eskandari and Karimpour, 2012).

Calving age had a significant effect on LMY (P<0.05). The highest LMY determined in the 8th age, but lowest in the 10th age. LMY was increase progressively until 8th calving age, then decline gradually (Table 1). This is in agreement with the previous researches (Khosroshahi et al., 2011; Şahin and Ulutaş, 2015) reported that effect of calving season on LMY was significantly important. Afzal et al. (2007) determined that effect of parity on milk yield was statically important and milk yield did not differ between 2nd to 7th lactations. Swain and Bhatnagar (1983) found that mean lactation yield for 2nd to 9th parities were similar in Murrah buffaloes. Increased milk production in subsequent lactations is explained by maturation and continued to grow and mammary gland (Afzal et al., 2007; Pawar et al., 2012). Bashir et al. (2015) stressed that age may be more precise factor to be incorporated into models for lactation milk yield. Because, the culling of animals with lower production and reproduction contribute toward better lactation yield of herd in subsequent lactations (Khan, 1997). Conversely, Pawar et al. (2012) reported that effect of parity on LMY was not important.

In this study, LMY was not affected by calving season. Similarly, Ghaffaret al. (1991) reported a non-significant effect of season of calving was found on milk production in Nili-Ravi buffaloes. Conflicting reports on effect of season on milk production indicated that these stress factors might be overcome through better feeding and management (Afzal et al., 2007). However, the findings of Chaudhry (1992), Eskandari and Karimpour (2012), Pawar et al. (2012) and Bashir et al. (2015) did not confirm the findings of present study and they determined that season of calving had a significant effect on LMY. Patel and Tripathi (1998) also reported maximum milk yield in the winter calving and minimum in the autumn calving. In Italian buffaloes, milk yield was also maximum in winter calving and minimum in summer calving (Catillo et al., 2002). Afzal et al. (2007) founded that the buffaloes calving in spring showed the highest and those calving in summer showed the lowest milk yield.

LL is defined as number of days in which an animal has milk. In this study, effects of calving age and calving season on LL were not statistically significant. This finding is close to the finding of Chaudhry (1992) in Nili-Ravi buffaloes, which calving month on LL was not important. The controversy results were reported by Şahin and Ulutaş (2015) and they found that effects of calving age and calving season on LL were statistically important.

Bashir et al. (2015) found that LL influenced by season of calving. Khalil et al. (1992), who observed that spring calving had the longest LL in Egyptian buffaloes. Dhar and Deshpande (1995) noted that the means of LMY in summer calving were significantly higher than the other seasons. Differences in feed resources and environmental conditions were major determinants of variation among herds (Bashir et al., 2015).

CONCLUSIONS

The DMY and LMY of Anatolian buffaloes were significantly affected by calving age. However, the effect of calving age on LL was not important. The effect of the calving season on all milk yield traits was not important. Generally, DMY and LMY were increased with progressing of age, but decrease after 8th calving age. To conclude, calving age plays a major role on milk yield, good selection programme and improvement management including calving age could improve milk yield traits.

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REPRODUCTION AND PRODUCTION PERFORMANCES IN LARGE WHITE SOWS AND IN LANDRAS x LARGE WHITE CROSSBRED SOWS

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Abstract

The aim of research was to study, for a period of 24 months, sows' reproduction and production performances. In total 120 Large White sows and Landrace x Large White crossbred sows reared in the same conditions, were used in the study. Monitored and registered parameters were: the duration of the reproductive cycle; the birth rate and mortality; production efficiency of the sow etc. The crossbred sows (Landrace x Large White) showed: the lowest number of unproductive days, versus Large White sows, respectively 6.24 vs 9.52 and the lowest percentage of mortality respectively 4.31 vs 4.96. The crossbred sows had also the highest number of piglets born alive/sow/year, than pure breed sows, respectively 25.59 vs 24.89, highest number of weaned piglets/sow/year, respectively 10.33 vs 9.38, and highest weight of weaned piglets, reapectively 6.65 vs 6.42. Independently from the genetic type, piglets born from fifth delivery sows, resulted the heaviest at birth and weaning time. Growing photoperiod reduced calving interval, and neonatal deaths.

Key words*: sow, crossbred, reproductive cycle, birth rate, weaning piglets.*

INTRODUCTION

The process of reproduction is complicated and involves many highly specific biological functions. The external environment (diet, housing, social surroundings, temperature, disease, etc.) has a far greater influence on reproductive performance than on any other biological process because the newborn of any species require special protection from environmental extremes. The size of the first litter was the best indicator of reproductive potential if 9.5 or more pigs were born (Thompson, 2010). The quality and quantity of meat production depends on the mode of treatment and management of sows. To get maximal production of sows is important to do the optimization of all phases of the reproductive cycle. To do so we must control all the parameters that expressing reproductive effectiveness, and the different stages of growth (Seren and Mattioli, 1998; Tarocco, 1994, Tarocco, 1998; Kim et al., 2000; Scheepens and Rozzen, 2008).

MATERIALS AND METHODS

The aim of research was to study, for a period of 24 months, sows' reproduction and production performances. In total 120 Large White sows and Landras x Large White crossbred sows reared in the same conditions, were used in the study.

Monitored and registered parameters were: the duration of the reproductive cycle, the birth rate and mortality, production efficiency of the sow, etc. During the test, piglet weaning age was 28 days. Their diet was based on starter feed. To evaluate the effect of photoperiod the data of reproductive cycle, were analysed for the growing and reduced photoperiod. To evaluate the effect of genetic type, effect of "the born alignment'' and effect of photoperiod the data were analysed according the general linear model (GLM, STATGRAF Centurion XVI.), as follow:

$$Y_{ijkln} = \mu + a_i + b_j + yk + e_{ijkln}$$

where:

Y_{ijkln} - reproductive and productive characteristic

μ - theoretical average

a_i - effect of genetic type (1.2)

b_j - effect of "the born alignment'' (1...6)

yk - effect of photoperiod (1.2

e_{ijkln} - residual effect

RESULTS AND DISCUSSIONS

The genetic type

The analysis of variance proved difference between two groups of the sows with different genetic types. The ANOVA results, for reproductive characteristics, are presented in Table 1.

Table 1. The average values of reproductive characteristics

The genetic type	pregnant days	No. of unproductive days	calving interval	repeat breeder sows	weaned piglets
Large White sows	113.51a	9.52A	151.78A	27.03A	28.72
Landrace X Large White crossbred sows	113.64b	6.24B	149.02B	23.88B	28.52
Means	113.57	7.88	150.4	25.48	

ab = P≤ 0.05; AB = P≤ 0.01

The crossbred sows (Landrace x Large White) showed: the lowest number of unproductive days, and repeat breeder sows versus Large White sows, respectively 6.24 vs 9.52 and 23.88 vs 27.03; the highest calving interval than pure breed sows 149.02 vs 151.78. (P≤0.01).

Results of some authors to this problem are the same (Freschi et al., 1999), but other authors report for a reduced calving interval (Thomson, 2010; Kim et al., 2000). The average values of reproductive and productive characteristics are presented in Table 2.

The data of Table 2 showed that the crossbred sows had also the highest number of piglets born alive/sow/year, and number of weaned piglets/sow/year than pure breed sows, respectively 25.59 vs 24.89, & 10.33 vs 9.38. The difference is evident for the weight at 28 days in favour of crossbred sows. respectively 6.65 vs 6.42 and the lowest percentage of mortality, respectively 4.31 v.s 4.96. (Deckert and Sciopioni, 1996) (P≤ 0.01).

Table 2. The average values of reproductive and productive characteristics

Parameters	Large White sows	Crossbred sows	Means
Percentage of calving	94.78	95.33	95.05
Percentage of mortality	4.96	4.31	4.63
No. of piglets born alive/sow/year	24.89	25.59	25.24
No. of piglets bor alive /calving /sow/year	10.37	11.18	10.77
No. of weaned piglets/sow/year	9.38	10.33	9.85
The mummified piglets	2.01	1.92	1.96
Weight of born	0.81	0.83	0.82
Weight of weaned piglets (28 days)	6.42	6.65	6.53

P≤ 0.01

The born alignment

Independently from the genetic type, the effect of the born aligment is significant for reproductive and productive characteristics of sows. The average values of this effect were estimadet from analysis of variance and are presented in Table 3.

As seen in Table no. 3, the effect of factor "born alignment" is significant regarding the calving interval, the number of unproductive days, the number of piglets born alive calving/sow/year, the repeat breeder sows, the number of piglets born alive/sow/year, the weight of born, the weight at 28 days.

The sows to the first, second and three parturition show; the highest calving interval compared with sows to the fifth parturition, respectively 155.7 d; 151.70 d, 149.33 d. vs 146.72 d, and the number of unproductive days 12.62d; 5.78, 9.95d vs 4.65d.

Table 3. The average values of reproductive and productive characteristics

The 'born alignment'	pregnant days	No.of unproduc tive days	calving interval	repeat breeder sows	No. of piglets born alive / calving /sow/year	litter size /sows /year	No. of piglets born alive /sow /year	No. of weaned piglets/ sow/ year	Weight of born	weight of weaning time
1	113.57	12.62	155.7	53.97	9	2.3	20.7	9.8	0.74	6.46
2	113.62	5.78	149.33	35.76	9.7	2.25	21.83	7.32	0.77	6.82
3	113.33	9.95	151.70	15.77	10.22	2.33	23.81	11.3	0.79	6.88
4	113.24	5.86	147.70	21.57	10.87	2.3	25	12.98	0.83	6.89
5	113.55	4.65	146.72	19.26	10.94	2.3	25.16	11.74	0.9	7.7
6	113.43	5.16	149.13	7.13	10.94	2.35	25.71	11.54	0.77	7.5

(P≤ 0.05) (P≤ 0.01) (P≤ 0.001)

Statistically proven difference (P≤ 0.001) were observed for number of piglets born alive/calving/sow/year which grows progressively from the first parturition (9 heads) to the fifth and six parturition (10.94 heads). Piglets born from fifth delivery sows, resulted the heaviest at birth (0.9 kg) and weaning time (7.7 kg) (Figure.1)

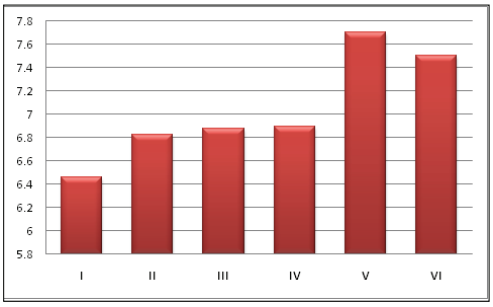

Figure 1. Live weight at weaned piglets

The Photoperiod

Effect of photoperiod on the reproductive activity of farm animals is studied by various authors (Tarocco, 1994; 1998; Barbari et al., 1995; Seren and Mattioli, 1989; Frechi et al. 1999). In Table 4 included data for the effect of fotoperiodes in sows performance.

Table 4. Effect of fotoperiodes in sows performance

Fotoperiodes	pregnant days	No. of unproduc tive days	Calving Interval (d)	No. of piglets born alive/sow/year	neonatal deaths.
Growing photoperiod	113.45a	7.87	150.68	11.00	0.74
The reduced	113.64b	9.33	152.03	10.69	0.84

ab=(P≤ 0.05)

As seen in Table 4 growing photoperiod reduced calving interval, from 152.03 days to the 150.68 days and neonatal deaths from 0.84 to the 0.74.

The positive result of growing photoperiod is clearly visible for the lowest number of unproductive days 7.87d vs 9.33 and the highest number of piglets born alive/sow/year 11.00 heads vs 10.69 heads.

CONCLUSIONS

Management and selection must be used to create an environment that enables swine to express their reproductive potential, and we must develop new means of determining reproductive potential in order to propagate the more prolific lines. Improving of the evaluation methods of the sows reproductive efficiency are very necessary to increase production. The sows that will be bred to produce potential replacement gilts should be selected according to the number of pigs farrowed and weaned in their first litters, for soundness of feet and legs, and for teat number and proper functioning. The current genetic progress in litter size at birth increases the need for a broader breeding program which includes piglet survival and growth genetic makeup of their daughters

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CONSTRUCTION OF RECOMBINANT YEAST (*Saccharomyces cerevisiae)* PRODUCING β(1.3) GLUCANASE AS A FISH FEED ADDITIVES

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Abstract
The main goal of the feed industry is to manufacture the high quality food economically by increasing the biological value of the feed proteins. An addition to that, it is to provide higher quality animal products in sufficient amounts while keeping the input costs at optimum levels. Keeping these goals in our mind, in this study, the pRS416G recombinant vector carrying the β(1.3) glucanase enzyme, which has indirecting effects on the immune system significantly was transferred to Saccharomyces cerevisiae using the electroporation method. PCR reaction where Saccharomyces cerevisiae/pRS416G DNA is used as a template was examined on %0.8 agarose gel yielding approximately 1.9 kbç DNA band carrying the β(1.3) glucanase gene was visualized. After a zymogram analysis, the yellow zone expressed by β(1.3) glucanase gene was observed in the recombinant yeast extracts. This study will be pioneer work for the development of recombinant single cell proteins, thereby including immune stimulant of the β(1.3) glucanase as fish feed additives.

Key words: *Electroporation, Cloning, pRS416G, Saccharomyces cerevisiae, β(1.3) glucanase.*

INTRODUCTION

Increasing world population and standard of living necessitate the consumption of higher amounts and quality of food derived from animal products. Therefore, the proper feeding of the animals and improvement of animal genetic structure application of feed additives, such as antibiotics, enzymes, growth agents that mimic the effects of hormones, to improve the condition and productivity of livestock have an important role in fulfilling these requirements (Kaya et al., 1997).

It is well known that the use of antibiotics as a feed additive has positive effects on growth and on conversion ratio feed. However, antibiotic residue might occur due to excessive antibiotic use as a feed additive (Keser and Bilal, 2008). This residue may cause food poisoning in humans. For example, in poultry, pathogenic microorganisms like *Salmonella* and *Campylobacter* might develop multiple antibiotic resistances on animal bodies over the time. If animal products contaminated with these resistant pathogenic microorganisms are consumed by humans, food poison will be threated public health. As a result, the use of antibiotic growth factors was completely banned in the European Union after January 1st, 2006 (Keser and Bilal, 2008). Following this ban, studies to develop alternative feed additives for maintaining the digestive system ecology in balance and controlling enteric the bacterial diseases by reinforcing animal immune systems.

In recent years, enzymes as alternatives to antibiotics have increased. Xylanase, β-glucanase, pectinases, cellulases, proteases, lipase, phytase, galactosidase, β-mannanase and similar enzymes are used in the feed industry alone or in combinations with each other. The use of enzymes increases the digestion rates of the feed as well as yielding some immune stimulants on the feed (Karademir and Karademir, 2003).

In livestock farming, the main input that determines productivity and cost is the feed. In feed, feed stock with high protein content is heavily used. In fish feed, fish flour is an indispensable source of protein due to its high protein content, balanced aminoacid composition and its attractiveness for fish. However, due to reduced fish stock in recent years and its increasing use in human food, the manufacturing of fish flour has decreased, and feed manufacturers have started importing fish flour. As a result, the price of fish flour has increased feed costs and the use of plant based

sources came into consideration. In this context, various studies are being performed by fish feeders to reduce feed costs and to discover alternative protein sources and their usage conditions to replace fish flour Akyima et al., 1995; Webster et al., 1992; Wu et al., 1995).

It was determined that enzymes are used in various countries in recent years to increase digestion and as a result providing better live weight gain and feed benefit rate in feed manufactured using plant based stock (Karademir and Karademir, 2003).

Among these enzyme products, β(1.3) glucanes with indirect immunostimulant property for culture fisheries may be useful. It was reported that the fish became more resistant to various bacterial, viral and parasite related diseases, the mortality rates due to opportunistic pathogens in the larval stage has been decreased, increases were observed in the effectiveness of antimicrobial materials and growth rates and the negative effects of stress was reduced (Raa, 2000).

In fishes, the increase in the growth performance related to β(1.3) glucane is mostly related to maintenance of the health the feeding duration, environmental temperature and the studied species. It is reported that to achieve an increase in growth performance, feeding strategies must be developed for each fish species with regards to the dose and administration duration of β-glucane (Tonheim et al., 2008). As animals are exposed to stress factors every day, their immune systems weaken and this makes them sensitive against infections and other diseases. Animals fed with β(1.3) glucane containing feed have higher serum immunoglobulin levels and they become more resistant to infections and other diseases as a result. However, the addition of this enzyme to fish feed externally is very expensive, and it increases feed costs.

Insufficiency of feedstock that form the basis of the manufactured feed, and the decreasing amounts and increasing prices are the problem that must be overcome in culture fisheries (Arıman and Aras, 2002). In this regard, the addition of some enzymes or enzyme products to the feed imposes a very low cost. The prices of commercial enzymes are around 3-4 Euro/kg (9-12 TL/kg). Since in feed enzymes are used in very low ratios, around, 1-2 units per thousand, adding enzymes imposes a very small cost, around 8TL per 1 metric tonne of feed. Today, commercial enzymes for use in poultry feed are available on the market, but especially enzymes intended for use with fish feed have only recently appeared (Yiğit and Koca, 2011).

The goal of this study is to develop a recombinant *S. cerevisiae* strain that produces the bacterial β(1.3) glucanase enzyme as a source of single cell protein as well as feed addictive. This will allow the recombinant yeast to both provide single cell proteins (SCP) for growth source of the β(1.3) glucanase enzyme yielding β(1.3) glucan from yeast cell will in digestive tract of the fish to strength immune system.

MATERIALS AND METHODS

Yeast, Plasmid and Growth Environments

The pRS416G vector developed by Mazı et al. (2012) (Figure 1), the *Saccharomyces cerevisiae* yeast and the equipment used in this study are available in the Animal Biotechnology and Genetic Engineering Laboratory, Zootechnology Department, Faculty of Agriculture, Çukurova University. Chemicals were obtained from various companies. *Saccharomyces cerevisiae* was left to reproduce in YPD liquid medium overnight at 30˚C (Johston, 1994). The next day, the breeding yeast were planted into a solid medium containing antibiotics (ampicillin 50 µg/ml) against bacterial contamination with a sterile needle and this was left in YPD to incubate for another night at 30°C to colonise.

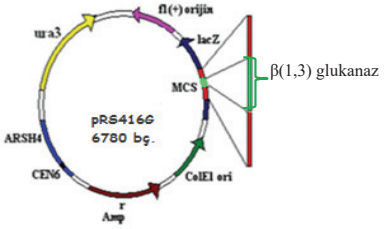

Figure 1. Map of pRS416G plasmid

Electroporation to Saccharomyces cerevisiae yeast

After breeding in the liquid medium, the yeast, after reaching the OD_{600}=1.3-1.5 value, was

made competent and electroporation was applied. Afterwards, the yeast was spread over minimal culture plates and left to incubate at 30ºC overnight to form colonies (Figure 2) (Özcan, 1992; Özcan, 2001).

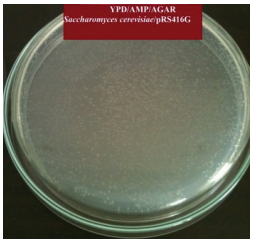

Figure 2. Yeast of *Saccharomyces cerevisiae*/pRS416G growth on YPD/Amp/Agar plates

DNA Isolation and PCR Reaction

pRS416G DNA was isolated from *Saccharomyces cerevisiae* (Harju et al., 2004). The primary string of the β(1.3) glucanase gene that is to be used for replication is based on 5'-AGAGCTCGTGGCACTGCACTCGTTCGAG TCT-3' (forward) and 5'-AGAGCTCGACGGGCGCGGTCA GAGCGTCCAG-3' (backward) gene strings (Shen et al., 1991). To detect the presence of the β(1,3) glucanase gene in the isolated DNA; a PCR reaction where pRS416G DNA is used as a template was prepared. The components of the prepared PCR reaction are as follows; 5 µL reaction buffer, 1 µL $dNTP_{mix}$ (200 µM for each), 1 µL each primary forward and backwards (20 pmol each primer), 0.5 µL Pfu DNA polymerase (2.5 U/µL), 1 µL DMSO 50% w/v (final concentration 1% w/v) and the total volume was adjusted to be 50 µL. The PCR program used was: at first 94ºC 2 minutes, afterwards 98ºC 30 seconds and 68ºC 5 minutes 30 cycles, afterwards 72ºC 5 minutes and kept at +4ºC. The result of the reaction was examined in 0.8% agarose gel with the help of marker DNA (Figure 3) (Özcan, 2001,).

SDS-PAGE experiments were performed (Laemmli, 1970). The bacteria culture was planted into 25 ml LB liquid medium with a sterile needle and left to incubate at 37ºC for 48 hours. At the end of this time *Saccharomyces cerevisiae* was drawn into 10 ml centrifuge tubes and precipitated by spinning it for 10 minutes at 4500 RPM. 5 ml from the upper phase were taken into clean centrifuge tubes; 1 volume 20% w/v TCA was added and homogenized with a pipette.

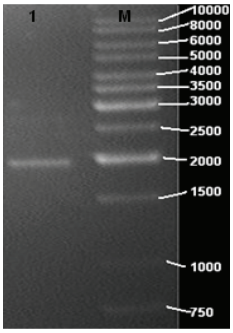

Figure 3. SDS-PAGE, Zymogram Analysis and Obtaining Extracellular Proteins through TCA Treatment

The mixture thus prepared (supernatant+TCA) was incubated at room temperature for 24 hours, and at the end of this time, the samples were put in a centrifuge for 10 minutes at 4500 RPM for the proteins to form pallets. The upper liquid part was poured away and the protein pellets were dried and then dissolved in 1 M Tris (pH 8). Zymogram analyses were modified based on Özcan (1992).

Display of Bands Responsible for 3.2. β(1.3) glucanase enzymes in SDS-Laminarin-PAGE

The gel was carefully removed from between two glass plates, transferred to the renaturation solution (80% v/v 50 mM Na-phospate solution (pH 7.2), 20% v/v isopropanol) and incubated in this solution for 1 hour. This way SDS was removed from the gel and proteins were renatured. The gel was removed from this solution and placed in 50 mM Na-phospate solution (pH 7.2) and left to incubate for 1 hour at room temperature. Afterwards the gel was transferred to 50 mM Na-phospate solution +5 mM β-mercaptoethanol + 1 mM EDTA solution and left to incubate overnight at +4ºC. The next day the gel was placed in sodium phospate solution and incubated for one hour at +4ºC; afterwards, the gel was removed from the sodium phospate solution, wrapped in cling wrap to prevent fluid loss and left to incubate at 30ºC for 4-5 hours. At the end of incubation the gel was dyed by the Congo-red dye for 1 hour. The gel was removed from the dye solution and washed in 1 M NaCl and 5 mM NaOH to

remove the excess dye, revealing the band responsible for the laminarinase enzyme that appears as a yellow zone (Özcan, 1992; Teather and Wood, 1982).

RESULTS AND DISCUSSIONS

Gene Expression results of Saccharomyces cerevisiae/pRS416G

Total proteins belonging to *Saccharomyces cerevisiae* were demonstrated in SDS-PAGE with Coomassie blue dye (Figure 4a). Protein samples were loaded into 12% SDS-Laminarin-PAGE (0.2% w/v laminarin) gel for the proteins to progress in the gel. After the protein progression in the gel is completed, the gel was treated according to the protocol given in 3.2 to display the band responsible for β(1,3) glucanase enzyme. The activity band belonging to the enzyme is shown by Congo-red dye in SDS-Laminarin-PAGE (Figure 4b) (Özcan, 1992).

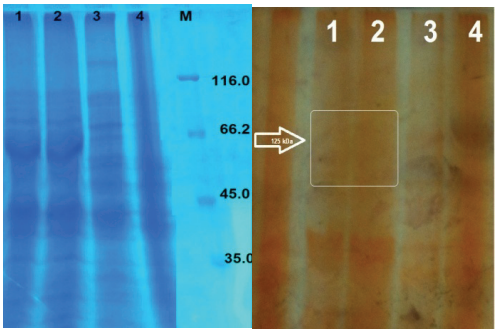

Figure 4. SDS-PAGE (a) and SDS-Laminarin-PAGE (b) analysis of total protein yeast of carrying the gene β (1.3) glucanase (M: Marker 1-2-3: Rekombinant yeast of carrying the gene β(1.3) glucanase4:Original yeast)

Today, the developments in biotechnology significantly benefit the primary goals of livestock farming, increasing productivity in amount and quality, thus increasing profitability. Among the biotechnological products that are presented to livestock farmers, enzymes, organic acids and probiotics are the most important alternatives to some problematic feed additives, with their natural sources, and their safety with regards to animal and human health. More studies and development on these products that can be used both for animal health and to improve productivity, and encouragement of their more widespread use, would make it possible for the livestock industry to reach larger profits (Karademir and Karademir, 2003). For this reason, in this study the pRS416G vector containing β(1.3) glucanase enzyme that has a significant place in the immune system was transferred to *Saccharomyces cerevisiae* using the electroporation method. Afterwards, through Zymogram analysis, it was determined that the molecular weight of the laminarinase (β(1.3) glucanase) enzyme was approximately 125 kDa (Figure 4b). Similar to the findings obtained, it was determined that the molecular weight of β(1.3) glucanase enzyme is 125 kDa (Fuchs et al., 2003). It was determined that the Endo-β(1.3-1.4) glucanase gene was the DNA part that carries the 1.4 kb *PvuI-ClaI* cutting enzyme recognition string during the cloning analysis of the Gram+ bacteria *B. subtilis*. To produce the plasmid that is formed as pEHB9 this gene was first cloned at the yeast's LEU2 area with the pJDB207 vector. Afterwards pEHB9 was transferred to *S. cerevisiae* and it was shown that the yeast synthesized endo β(1.3-1.4) glucanase. However, it was shown that the β(1.3-1.4) glucanase activity in pEHB9 was very low and only detectable (Hinchliffe and Box, 1984). Similarly the EXG1 gene that codes *S. cerevisiae* exo β(1.3) glucanase was cloned and expressed in yeast (Van et al., 1997). By appending the 2.7 kb DNA fragment that codes the *B. subtilis* endo β(1.3-1.4) glucanase gene from the *E. coli* plasmid pFG1 to the *E. coli*/yeast shuttle vector the hybrid plasmid YCSH was created. By transferring the hybrid plasmid to *S. cerevisiae* the *bgl S* gene was expressed. The expression level change of the *bgl S* gene in *S. cerevisiae* was 2.3 times and this is related to the 2.7 kb DNA fragment orientation. The enzyme substrate specificity and the pH optimum was determined to be similar to *B. subtilis* endo β(1.3-1.4) glucanase enzyme and it was noted that the *bgl S* gene expression level in *S. cerevisiae* was lower compared to *E. coli* (Chen et al., 2005). β(1.3) glucanes have an important niche as a feed additive. There are various studies that demonstrate that this enzyme is effective on the immune system of fish. Researchers have observed that there were changes in the fish performance when β(1.3) glucanase was used as a feed additive for large yellow croaker

(*Pseudosciaena crocea*) (Ai et al., 2007), *Pagrusauratus* species of bass (Cook et al., 2003), Nile bream (Whittington et al., 2005), *Oncorhynchus mykiss* (Sealey et al., 2008), carp (*C. carpio*) (Bogut et al., 1995). In another study, for 3 groups of broilers, in order, additive-free ration (control), antibiotic added ration and yeast β(1.3) glucane added ration were given. During the trial period, no significant difference was observed between the groups with regards to feed consumption and feed benefit. As a result, these researchers have stated that the lack of difference between the antibiotic group and the β(1.3) glucane group with regards to performance parameters can be considered an indication that β(1.3) glucane can be used as an alternative to antibiotics (Rathgeber et al., 2007).

CONCLUSIONS

This study aims to form β(1.3) glucanes that are very expensive to add to the feed from outside sources and that reinforce the non-specific immune system directly in the fish digestive system through *Saccharomyces cerevisiae*/pRS416G (Yeast cell wall + β(1,3) glucanase enzyme=β(1.3) glucane) for the purposes of reinforcing the fish immune system, improving resistance to various bacterial, viral and parasite-related diseases, preventing larval deaths due to opportunistic proteins and to reduce the negative effects of stress without using antibiotics. Also with the β(1,3) glucanase producing *Saccharomyces cerevisiae* strain, a new recombinant probiotic and/or single cell protein was developed.

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ESTIMATING EFFECTIVE POPULATION SIZE USING PEDIGREE DATA

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Abstract

Determining the sample size is the most important factor to effect of reliability of all scientific studies. The reliability of genetic parameter estimations obtained from phenotypic data with using pedigree depends on sufficiency of sample size. In this study was aimed to determine the effective population size for cattle, sheep and goat breeds. For this aim, methods depend on sex ratio, variance of progeny size and inbreeding rate were compared. Results indicated that the methods depends on variance of progeny size was suggestible for interested breeds.

Key words*: effective population size, sample size, breeding.*

INTRODUCTION

Easy coverage of the animal-derived proteins share in protein consumption is closely related to the supply of cheap animal protein source. Hence the importance of enhancing the country food of animal origin are included in the strategic planning (Inci et al., 2015; Isik and Ipcak, 2015).

Animal breeding is the effort to increase the proportion of the animals which are economic in appropriate environment in the population in next generation. The main purpose of animal breeding is to raise the yield obtained from animals to economic level (Duzgunes et al., 2012; Onder et al., 2015).

Achieving the aims on animal breeding is depends on the reliability of estimating the genetic parameters such as heritability and breeding value. Adequacy of sampling size is the basic rule of statistics for reliability (Sahin et al., 2003; Yamane, 1967).

In this study we aimed to compare the methods (sex ratio, variance of progeny size and inbreeding rate between two successive generations) to estimate the effective population size for cattle, sheep and goat species (Leroy et al., 2013).

MATERIALS AND METHODS

The data were obtained from private farms for Holstein Frisian and Jersey breeds for cattle, Saaenen and Turkish Hair Goat breeds for goat and Dorper sheep breed. Data of Karayaka sheep breeds was obtained from Ondokuz Mayis University.

Methods used to estimate effective population size Ne

Method based on sex ratio: Nes

Wright's model for estimating Nes is based on sex ratio. This very simple method is supposed to reflect the increased effects of both inbreeding and variance of progeny size under several assumptions, including random mating, no selection and random variation of progeny size across parents. Computation of Nes only requires the estimated numbers of breeding males (M) and females (F) in the reference population and follows equation (1):

$$N_{es} = \frac{4MF}{M+F} \quad (1)$$

Method based on the variance of progeny size: Nev

This method is more sophisticated than the previous one since it directly takes into account the observed variance of progeny size. Parents of the reference population are considered as a group of useful offspring. In each pathway (mm = sire-sire, mf = sire-dam, fm = dam-sire

or ff = dam-dam), observed variance (σ2) and covariance (σ) of progeny size are computed considering those individuals and their own parents (i.e. the grandparents of the reference population). Nev is then computed using equation (2) in which Mr and Fr are the numbers of new male and female parents beginning to reproduce each year averaged over the 10 years before the reference year:

$$\frac{1}{N_{ev}} = \frac{1}{16M_rT}\left[2 + \sigma^2_{mm} + 2\frac{M_r}{F_r}\sigma_{mm,mf} + \left(\frac{M_r}{F_r}\right)^2\sigma^2_{mf}\right] + \frac{1}{16F_rT}\left[2 + \sigma^2_{ff} + 2\frac{F_r}{M_r}\sigma_{fm,ff} + \left(\frac{F_r}{M_r}\right)^2\sigma^2_{fm}\right]. \quad (2)$$

Method based on inbreeding rate between two successive generations: NeFt

Considering two successive generations t and t-1, inbreeding rate (ΔFt) can be computed using equation (3), in which Ft+1 is the average coefficient of inbreeding of the reference population, and Ft the average coefficient of inbreeding of their parents:

$$\Delta F_t = \frac{F_{t+1} - F_t}{1 - F_t} \quad (3)$$

The effective population size can then be computed using the formula $N_{eFt} = 1/2\Delta F_t$.

RESULTS AND DISCUSSIONS

The estimated effective population size for the methods based on sex ratio, variance of progeny size and inbreeding rate between two successive generations were given in Table 1.

Table 1. Estimated effective population sizes

	NeFt	Nes	Nev
Holstein Frizian	697	445	56
Jersey	894	564	102
Saanen	347	26	75
Turkish Hair Goat	497	60	178
Dorper	1035	66	204
Karayaka	894	68	185

Spearman rank correlations estimated among the methods were given in Table 2.

Table 2. Estimated Spearman rank correlations

	NeFt	Nes
Nes	0.740	-
Nev	0.890	0.434

To compare the methods regression analysis was used. Estimated population size was used as response variable and breed was used as explanatory variable. Obtained comparison criteria from regression models were given in Table 3.

Table 3. Comparison criteria obtained from regression

	R^2_d	MSE	Sig.
NeFt	0.566	2.2E7	<0.001
Nes	0.204	7.9E6	<0.001
Nev	0.523	9.1E5	<0.001

When the criteria examined, the method based on inbreeding rate between two successive generations (NeFt) was more reliable than the others because its determination coefficient was the highest and mean square error was the lowest. However, all the methods were found statistically significant so, all three methods can be used to estimate effective population sizes.

CONCLUSIONS

The three methods, sex ratio, variance of progeny size and inbreeding rate between two successive generations, can be successfully used to estimate effective population sizes for cattle, sheep and goat species. The method based on inbreeding rate between two successive generations was found as the best method for this aim.

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THE IMPACT OF GENETICALLY MODIFIED ORGANISMS SPREADING TO AGRICULTURAL ECONOMY

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Abstract

The paper represents a review of vegetal production obtained using GMOs technology targeted to animal feeding or human consumption. It consists of the definition of term, why are GMOs produced and which food contains GMOs. Harvesting, testing, authorizing and GMOs risk assessment are described in case of Romania and EU countries as well. The benefits and debates related to GMOs are shown in details using examples. The work also presents health concerns related to GMOs usage.

Key words: *GMOs, risk assessment, improved cultivation, human consumption, health concerns.*

INTRODUCTION

Genetically modified organisms (GMOs) are organisms in which the genetic material (DNA) has been modified, in a way that does not occur through the natural mechanisms ("technologies", "gene technology", "technology of recombinant DNA," "genetically engineered"). Individual genes allows selected are transferred from one organism to another within the species or a species.
It follows the creation of genetically modified plants whose seeds are then used to drill (GM crops).

WHY GMOs ARE PRODUCED?

GMOs produces and sells to benefit producers and consumers of food derived from GMOs. Advantages means: lower prices, greater benefits for sustainability and / or nutritional value.
The initial objective was to protect crops by creating resistance against plant diseases and pests (insects and viruses), or by creating a better tolerant to herbicides used in agriculture:
- **Insect resistance** was obtained by incorporating into the plant used as raw material for food, a gene that induces the resistance production of a toxin gene taken from a microorganism (Bacillus thuringiensis - BT). This toxin is used as an insecticide long conventional agriculture, it is non-toxic for human consumption. GM crops that permanently produce this toxin have been shown to need much smaller quantities of insecticides used for specific situations, when large populations of pest pressure is high.
- **Resistance against viruses** is achieved by introducing genes from certain viruses that cause plant diseases. Increased resistance against viruses make plants less vulnerable to diseases caused by them, thus increasing productivity.
- **Tolerance to herbicides** through the introduction of a gene is obtained from a bacterium which express resistance to certain herbicides. In situations in which the imperative use of herbicides, herbicide requisite amounts were much lower.

GMO FOOD PRODUCTS

- Bread and pastry - Bread, Biscuits, Snacks
- flour from soy
- Oil from soybeans, cotton and canola GM
- cornmeal and corn from GM maize
- Sweets - chocolate, sweets and ice cream
- Lecithin from soy
- Glucose or glucose syrup from GM maize

- Sugar from GM sugar beets
- Ingredients and additives maize GM
- Soy milk

The production of meat and meat products - soy successfully used as an additive and substitute.

CULTIVATION

The evolution of cultivated areas worldwide:

In 2014 global biotech crop surface continued to grow for the 19th consecutive year of trading; 18 million farmers in 28 countries planted more than 181 million hectares in 2014, up from 175 million in 27 countries in 2013.

Notably, Bangladesh, a small poor country approved Bt brinjal/eggplant for the first time on 30 October 2013, and in record time less than 100 days after approval small farmers commercialized Bt brinjal on 22 January 2014.

Innate™ potato, another food crop, was approved in the US in November 2014. It has lower levels of acrylamide, a potential carcinogen in humans, and suffers less wastage from bruising; potato is the fourth most important food staple in the world.

A safer product and decreased wastage in a vegetative propagated and perishable crop, can contribute to higher productivity and food security. Also in November 2014, a new biotech alfalfa (event KK179) with up to 22% less lignin, which leads to higher digestibility and productivity, was approved for planting in the US.

The first biotech drought tolerant maize, planted in the US in 2013 on 50.000 hectares increased over 5 fold to 275.000 hectares in 2014 reflecting high acceptance by US farmers.

Importantly, a new 2014 comprehensive global meta-analysis, on 147 published biotech crop studies over the last 20 years worldwide confirmed the significant and multiple benefits that biotech crops have generated over the past 20 years, 1995 to 2014; on average GM technology adoption has reduced chemical pesticide use by 37%, increased crop yields by 22%, and increased farmer profits by 68%.

These findings corroborate earlier and consistent results from other annual global studies which estimated increases in crop productivity valued at US$133.3 billion for the period 1996-2013 (James, 2015)

Table 1. Surfaces cultivated with GM crops

Rank	Country	Area (million hectares)	Biotech Crops
1	USA*	73.1	Maize, soybean, cotton, canola, sugarbeet, alfalfa, papaya, squash
2	Brazil*	42.2	Soybean, maize, cotton
3	Argentina*	24.3	Soybean, maize, cotton
4	India*	11.6	Cotton
5	Canada*	11.6	Canola, maize, soybean, sugar beet
6	China*	3.9	Cotton, papaya, poplar, tomato, sweet pepper
7	Paraguay*	3.9	Soybean, maize, cotton
8	Pakistan*	2.9	Cotton
9	South Africa *	2.7	Maize, soybean, cotton
10	Uruguay*	1.6	Soybean, maize
11	Bolivia*	1.0	Soybean
12	Philippines*	0.8	Maize
13	Australia*	0.5	Cotton, canola
14	Burkina Faso*	0.5	Cotton
15	Myanmar*	0.3	Cotton
16	Mexico*	0.2	Cotton, soybean
17	Spain *	0.1	Maize
18	Colombia*	0.1	Cotton, maize
19	Sudan*	0.1	Cotton
20	Honduras	<0.05	Maize
21	Chile	<0.05	Maize, soybean, canola
22	Portugal	<0.05	Maize
23	Cuba	<0.05	Maize
24	Czech Republic	<0.05	Maize
25	Romania	<0.05	Maize
26	Slovakia	<0.05	Maize
27	Costa Rica	<0.05	Cotton, soybean
28	Bangladesh	<0.05	Brinjal/Eggplant
	Total	**181.5**	

* 19 biotech mega-countries growing 50,000 hectares, or more, of biotech crops

** rounded off to the nearest hundred thousand (Source: James, 2015).

TESTING

In the EU the field testing it is not allowed without the consent testing of a competent authority of the Member State concerned. Since 2002, all applications for authorization must be notified to the European Commission.

The purpose of the field test is examining insect resistance, herbicide tolerance, modified composition, resistance to viruses.

Testing in the EU (1992 - 2012): 2589 events

- 85 species tested
- Most tests in descending order were made on: corn, rapeseed, potato, tomato, cotton, soybeans.

Testing in Romania

Electronic registry managed by the National Environmental Protection Agency contains:

- operator data;
- no. authorization; validity; no. European;
- information on the event tested (species identification code, characteristics);
- the purpose of the release into the environment; authorized area, the distance protection;
- import Agreement; results

So far have been tested in field several transformation events for soybeans, corn and plum genetically modified to assess tolerance to herbicides glyphosate and resistance to attack by lepidopteran insect pests and environmental effects or resistance to plum pox virus if plum.

REGULATORY, CONTROL AND LICENSING

Marketing of food and feed containing, consisting of or produced from GMOs is regulated by EU Regulations no. 1829/2003 / EC and 1830/2003/EC. Regulation no. 1829/2003 / EC establishes European procedure for authorizing GM food and feed, proceeding directly involving the 27 Member States, the European Food Safety Authority (EFSA), the European Commission and Council. Regulation 1830/2003 / EC lays down the principles of traceability and labeling to be respected by food business operators when using a GMO activity. The legal framework for the implementation of these regulations into national Romanian law was adopted in March 2006 Government Decision no. 256/2006 on genetically modified food and feed and the Government Decision no. 173/2006 on traceability and labeling of GMOs and the traceability of food and feed produced from GMOs.

Regarding the deliberate release into the environment for testing EU legislation is Directive 2001/18 / EC of the European Parliament and of the Council on the deliberate release into the environment of genetically modified organisms.

At European Union level, in the authorization process, it has a special role to the European Food Safety Authority (EFSA) - the independent scientific institution with primary role in the risk assessment of food on human health, animal health and environmental protection across the European Union. EFSA's scientific opinions and policy are the basis for European legislation on food safety - a GMO is not subject to Community authorization procedure if you do not enjoy a favorable scientific opinion of the EFSA. According to a basic principle of food safety introduced by the European Parliament and Council Regulation no. 178/2002 / EC on the safety of food and feed, confidence in food should be linked to confidence in science, which is the foundation that led to the establishment of EFSA.

In the European Union (April 2016) are authorized for use as food and feed a number of 55 transformation events as follows:

- 28 types of genetically modified maize
- 12 types of genetically modified soybean
- 10 kinds of genetically modified cotton
- 4 types of genetically modified oilseed rape
- One type of genetically modified sugar beet

National level

The establishments subjected to supervision and official control at the level of 2014 were deposits of soy, corn and canola plants, feed factories, soybean oil factories, corn germ oil or rapeseed processing units soy, corn and canola, to obtain food other than those for the production of oil, soybean trading units / maize to products which contain or are constituted derived from soy / maize. Also, the desk review for compliance with European legislation has been conducted for other products that may contain genetically modified organisms authorized or rapeseed, cotton, sugar beets, etc., products authorized at European level and in the Register Community Genetically modified food and feed. In accordance with Commission

Decision 289/2008 on emergency measures relating to genetically modified organism "Bt63" in rice products was paid special attention to the types of controls on imports rice from China. There were imports of products subject to that decision, all of which are subject to control and sampling. Following checks analytical in the Laboratory of Molecular Biology and GM of the Institute for Diagnosis and Animal Health (National Reference Laboratory for Food and Feed GMO), there were no positive results that highlight the presence of unauthorized GMO Bt63 in rice and rice products.

Results of official controls

Following the official control for traceability and labeling of genetically modified food and feed, Program supervision and control food safety for 2014 were achieved controls and sampling, as follows in Tables 2 and 3.

Table 2. Control activities for soy

Establishment type	No. Carried out controls	No. Taken samples
Storage house	115	42
Fodder	130	13
Soya oil establishment	6	0
Other food processing	225	89
Trading	402	111
TOTAL	**878**	**255**

Table 3. Control activities for maize

Establishment type	No. Carried out controls	No. Taken samples
Storage house	156	75
Fodder	148	68
Establishment of corn germ oil	0	0
Other food processing	78	37
Trading	281	112
TOTAL	**663**	**292**

Actions taken to ensure the effectiveness of control activities

The National Reference Laboratory for Genetically Modified Food and Feed continued modernization and optimization procedures and working methods was developed discernible annual counties for sending samples to the national network of GMO laboratories of molecular biology. Also it involved in a number of official control laboratories 8 DSVSA for the detection of genetically modified soya and 2 for detection of genetically modified maize.

RISK ASSESSMENT OF FOOD FROM OMG GMOs AND HUMAN HEALTH

GMOs risk assessment at EU level is a complex process involving most institutions with scientific role in biotechnology and is coordinated by EFSA.

By evaluating the safety of foods derived from GMOs are envisaged:

(A) direct health effects (toxicity)

(B) tendencies to provoke allergic reaction;

(C) specific components thought to have nutritional or toxic properties;

(D) the stability of the inserted gene;

(E) nutritional effects associated with genetic modification;

(F) any unintended effects which could result from the gene. Thus although Romania's involvement in the GMO risk assessment at the European level is limited, there is scientific bodies holding this role - the Scientific Council and the Commission of Biosafety ANSVSA Ministry of Environment who provided scientific advice and opinions to GMOs.

HEALTH CONCERNS RELATED TO GMOs USAGE

- **Allergic reaction**: there was no evidence of allergic effects related to GM food sold so far.

- **The transfer of genes from GM foods into the human body** or to bacteria found in the human gut: if possible, and if so, the genetic material transferred can affect people's health? This issue is particularly important for the genes which induce resistance to chemicals, in this case, antibiotic, if the transfer of genes as possible. Although the likelihood of this transfer is very small, experts -FAO Fund Food and Agriculture Organization and WHO experts recommend not using the processes of gene transfer of antibiotic resistance to new GMOs.

- **Natural transfer of mixing cultures and seeds from natural crops**, with the gene transfer would affect food safety. This risk is real and has been shown when traces of rice approved to be used only for feed, was found in rice products for human consumption, obtained from genetically modified crops that had not

been voluntary in the US. They have adopted national strategies to reduce mixing, separating clearly the perimeters of crops (GMOs and conventional crops).
Now put in place worldwide for details: monitoring after the marketing of GMOs and GM food new, continuous monitoring of food safety derived from GMOs.

OMGs-BENEFITS AND CONTROVERSY
BENEFITS
Crops
- Improvement of taste and quality;
- Reducing of vegetal growing;
- The growth of nutrient elements, of harvest and stress tolerance;
- Improve resistance to diseases, pests and herbicides;
- New products and growing techniques.

Animals
- Increased resistance, productivity and efficiency of feed conversion
- Improve efficiency in terms of meat, milk or eggs
- Improving diagnostic methods

Environment
- Bioherbicides and bioinsectycides friendly to environment
- Soil, water and energy preserving
- A better management of natural wastes
- Efficient processing

Social
- Increasing food security for growing populations

CONTROVERSY
Safety
- Potential impact on human health - allergens, transfer of antibiotic resistance genes, unknown effects
- Potential environmental impact: unintended transfer of genes through cross-pollination, unknown effects on other organisms, affecting biodiversity

Intellectual property
- The dominance of few companies on global food production
- Increased Independence developing countries of the industrialized

Ethics
- Damage to natural organisms values
- Interference with nature by "mixing" of genes between species
- Objections regarding the consumption of animal genes in plants and vice versa

Labeling
- It is not mandatory in some states

CONCLUSIONS

GMOs can represent an alternative to conventional food & feed in the context of permanent growing of world population and the rapid developments in genetic engineering with appliance in agriculture and medicine.
We can underline here that the US and EU feed industry (98% of GMO imports are used for feed) rely more and more on GMOs, fact that leads to a certain dependency on GMOs in this industry.
Although the food & feed safety concerns are more and more scientifically assessed and no real evidence of harmful effects is found, the issues of preserving the environment and biodiversity is an important aspect that comes along with the debate on the coexistence possibility.

Annex*

INFORMATION ON THE MARKET genetically modified products

1. SOYBEAN

Soybean is one of the agricultural crops of greatest importance for human nutrition and animal nutrition industry. Soybean seeds contain 30% protein and 17-25% oil substances. By processing the soybeans are obtained:
- Soybean meal, used in food industry (milk, cheese, chips, coffee, chocolate, pasta, biscuits, meat substitute etc.)
- Soybean oil (used in the manufacture of margarine, soap, lecithins, etc.
- Soy grits (resulting from oil processing) used in animal feed

Professional organizations on product chain
- Industry Employers' Association of vegetable oils and fats (Ulprod)
- National Union of Agricultural Producers in Romania (Unpar)
- Farmers Association of Romania (AFR)
- National Association of Manufacturers Employers fodder - (Nutricomb)
- Employers' Federation of Food Industry (Romalimenta)

In Romania about 11.000 operators are involved in this industry including both units of sale to the consumer of soy and soy products, and processing technology.
According to ANSVSA statistics and specific activities, processors are using soy:
- Obtaining oil which may have the domestic or export destination;

- Combined feed factories that use soy as a feedstock for the production of animal feed. Thus processed soybean meal as animal feed can be used in livestock farms or households own;
- Soybean processing plants for the production of foodstuffs, other than for oil production (meat processing plants that use soy products mills, sugar confectionery, biscuits, etc.).

Soybeans through properties and texture can be a substitute for meat and meat processors use this product in the process.

The products of soy food chain to reach the following consumer storage networks, distribution and sales in certain forms:

- Soybean oil;
- Soya granules, schnitzel or cubes for household use;
- Soy incorporated into meat products.

To those listed above, soy may be present in chocolate (soy lecithin), ice cream, biscuits etc.

Medical uses:

Some research suggests that if consumed regularly, soy lowers cholesterol, relieves hot flashes, prevent prostate and breast cancer, helps in weight loss and prevents osteoporosis. These effects may be due to a characteristic of soybeans, namely increased isoflavone concentration, a type of estrogen produced by the plant. This research led food manufacturers to develop various types of soy market that is growing.

2. MAIZE

Corn (*Zea mays* L.) is one of the most important crops with multiple uses in human food industry, feed. (According to FAO statistics, distribution, consumption is 21% human food, animal feed 72%, 7% industry). Corn is used in the starch industry, the spirits, glucose and dextrin; the seeds are used for extracting oil, used in foods diet; Corn is used in animal feed as fodder concentrate (grain), corn, green grass (silage), stems (stalks) mixed with urea and molasses and silage (fodder juicy).

Professional organizations on product chain

- Farmers Association of Romania (AFR)
- National Association of Milling and Baking Romania (Anamob)
- Romanian Employers Milling Industry, Bakery and Flour Products (Rompan)
- League of Associations of Agricultural Producers in Romania (Lapar)
- National Union of Agricultural Producers in Romania (Unpar)
- National Association of Manufacturers Employers fodder - (Nutricomb)
- Employers' Federation of Food Industry (Romalimenta)

In Romania around 1000 operators are involved in this industry, consumer marketing units excluding maize and maize products.

3. COTTON

The main uses of cotton refers to the textile industry, but the seeds of *Gossypium*, the source of the cotton-containing oil, vitamins, minerals, salt and food protein, and the pressing are used in the manufacture of cooking oil, soap, margarine, cosmetics, bitumen and production of wax and can be used as animal feed. Cotton is also widely used in medicine, for example refined oil seed *Gossypium* can be used during breastfeeding as a preventive method for arteriosclerosis.

4. RAPE

The main uses of rape refer to the manufacture of biodiesel, a fuel that is used instead of diesel cars, which is cheaper and less polluting. Also it produced by processing rapeseed cakes with high forage value containing 38-42% protein, carbohydrates and minerals. Rapeseed oil is widely used in industry and nutrition. It can also be used in medicine in the treatment of digestive or dermatological disorder.

5. SUGAR BEET

Worldwide, it is the second source beet sugar after sugar cane indispensable product for human nutrition due to high energy value (4000 cal/kg meat and bread to 1500, respectively 2200 cal/kg). Beets provide about 40-45% of world production of sugar.

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Retracted article: THE EFFECT OF SOME ENVIRONMENTAL FACTORS ON MILK COMPOSITION OF ANATOLIAN BUFFALOES

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GROWTH TRAITS OF GUINEA FOWL IN DIFFERENT PRODUCTION SYSTEMS

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Abstract

Two hundred day old guinea fowl keets were reared in free-range intensive system to determine the effect of production system on growth traits. All birds kept indoor until 7 weeks, and half of them had access to outdoor until 18 weeks of age. All birds were individually weighed with two weeks intervals. Feed consumption was determined and feed conversion ratio was calculated. Production system had a significant effect on live weight until 14 weeks of age (P<0.05) and intensively reared birds had higher live weights. The difference between the weights of free-range and intensive system was not significant between the ages of 14-18 weeks. Mean body weight of guinea fowls in free-range system was 1196.61 g, whereas 1203.8 g in intensive system. Male guinea fowls had significantly higher weights than females at all ages in both production systems (P<0.05). Male guinea fowls reached 1241.67 g at 18 weeks and females had a mean body weight of 1158.74 g at the same period. Birds reared in free-range system had significantly higher feed consumption at all ages. Production system had significant effect on feed conversion ratio (FCR), and intensively reared guinea fowls had better FCR.FCR of free-range guinea fowls was found as 6.43 and 5.80 in intensive system.

Key words: *guinea fowl, production system, free-range, live weight, feed conversion ratio.*

INTRODUCTION

Guinea Fowl are raised mainly for meat and egg production and hobby purposes (Joubert, 1980). In Africa, guinea fowl production has cultural significance as a traditional activity (Konlan et al., 2011), and guinea fowl meat and eggs are second to chicken eggs and meat in terms of poultry product consumption (Bernacki et al., 2013). The relatively high resistance of guinea fowl to poultry diseases has made them attractive to farmers in some parts of the world (Joubert, 1980), and the higher protein (23% vs 21%) and lower fat content (4% vs 7%) of guinea fowl meat in comparison to chicken (Nsoso et al., 2003) has spurred the production of guinea fowl as an alternative poultry enterprise, not only in developing countries (Nahashon et al., 2006), but in a number of European countries as well, particularly in France, Belgium and Scandinavia (Baeza et al., 2001).

In commercial production, guinea fowl are raised in confinement with management practices similar to those of chickens. But, they are mainly reared under extensive or semi-intensive systems (Karacay and Sarica, 2004), which, when compared to intensive systems, offer a number of advantages to producers, especially in developing countries. Their adaptability to different environmental conditions makes them attractive to farmers looking to raise small flocks in their yards under free-range production systems characterized by very low inputs, albeit low productivity as well.

The majority of studies related to guinea fowl focus on growth performance, meat quality and egg production, with only limited research investigating effects of different production systems on growth parameters. Therefore, this study assessed live weight, feed consumption and feed conversion ratios for guinea fowls

reared indoor and free-range systems until 18 weeks of age.

MATERIALS AND METHODS

This trial was conducted at the Ondokuz Mayis University Agricultural Faculty's Research Farm from May to August 2015. 200 day-old keets were randomly selected for use in the experiment.

Keets were randomly allocated to pens belonging to either an indoor or outdoor-access ('free range') production system that were interspersed within windowed houses, with 4 pens per system and 25 keets per pen. Pens (3.5 x 3.5 m) were separated and covered by 0.5x0.5 cm wire mesh to prevent birds from flying between pens. Each pen contained 1 round feeder and 1 round drinker. The indoor pen also contained an 8-cm layer of wood shavings used as litter, and no fresh litter was added during the production period. Heating was provided by infra-red heaters, and economic white bulbs were used for lighting. A 24-h light regime was applied during the first 3 days. Light was incrementally decreased over days 3-14 to 20 hours and then remained constant until 6 weeks, after which natural lighting (app.14 h/day) was applied until slaughter. After 6 weeks of age, birds in the outdoor 'free-range' system were given 24-hour access to outdoor pens measuring 14x3.5 m through a single doorway measuring 50x90 cm.

All birds were fed *ad libidum* with the same commercial broiler starter diet based on corn and soybean meal (230 g CP and 12.8 MJ ME, 13.5 g lysine, 4.50 g methionine, 10.0 g Ca, 5.00 g P, 120 mg Mn, 15 mg Cu, 100 mg Zn, 12000 IU Vit. A, 4200 IU Vit. D per kg). Water was also provided *ad libidum*. All birds were individually weighed from hatch to 18 weeks of age with two weeks intervals. Feed consumption, feed conversion ratios and mortality and was evaluated by replication and given as mean value per guinea fowl.

Statistical analysis

Statistical analysis was performed using the software SPSS Version 16. Analysis of variance with a factorial arrangement (production system, age and sex) was used to test the effects of production system, age, and the interactions between production system, age and gender. A level of $P<0.05$ was considered statistically significant.

RESULTS AND DISCUSSIONS

All birds were kept indoors until 7 weeks. Live weight, feed consumption, feed conversion ratio and mortality at first 6 weeks were given in Table 1. Feed conversion ratio was increased with age and this was an excepted result. Total mortality was found as 2% at first 6 weeks.

Live weights of guinea fowls at different ages, after birds had access outdoor were given in Table 2. Production system had a significant effect on live weight until 14 weeks of age ($P<0.05$) and intensively reared birds had higher live weights.

Table 1. Live weight, feed conversion ratio (FCR) and mortality of guinea fowl in first 6 weeks

Age (weeks)	N	Live weight (g)	Feed consumption (g)	FCR	mortality
Hatch	200	26.73	-	-	-
2	197	74.57	124.53	1.65	1.5
4	197	161.97	289.53	1.79	1.5
6	196	382.37	711.35	1.86	2.0

But, after this age, the difference between the weights of free-range and intensive system was not significant. Mean body weight of guinea fowls in free-range system was 1196, 61 g, whereas 1203, 8 g in intensive system. These weights were lower than the findings of Tjetjoo et al., (2013) and Nahashon et al., (2006) who found the mean body weight around 1400 g at 9 weeks of age. Genotype is an important factor on body weight gain. The parent stock of the animal material of current study was not selected for meat production. This could be the reason of lower weights occurred in this experiment.

Production system x gender interaction was not found significant. Male guinea fowls had significantly higher weights than females at all ages in both production systems ($P<0.05$). Male guinea fowls reached 1241.67 g at 18 weeks and females had a mean body weight of 1158.74 g at the same period. This was also an expected result and similar to other poultry species.

Table 2. Effect of production system on live weight changes of guinea fowl

Production system	Gender	Live weight (g/weeks)					
		8	10	12	14	16	18
Free-range	Male	548.64	750.08	953.59	1077.15	1194.55	1236.62
	Female	453.44	641.58	849.59	990.79	1105.75	1156.60
Intensive	Male	575.61	780.48	979.23	1102.32	1197.40	1246.72
	Female	500.67	687.65	898.37	1009.17	1108.58	1160.88
SEM		5.42	6.31	5.31	4.66	5.47	5.63
Effects							
Production system		**	**	**	*	NS	NS
Gender		**	**	**	**	**	**
P.System x gender		NS	NS	NS	NS	NS	NS
Production system	FR	501.04	695.83	901.6	1033.97	1150.15	1196.61
	INT	538.14	734.07	938.8	1055.75	1152.98	1203.8
Gender	M	562.13	765.28	966.41	1089.73	1195.97	1241.67
	F	477.06	664.62	873.98	999.98	1107.16	1158.74

Cumulative feed consumption and feed conversion ratios of guinea fowls at different ages in two production systems were given in Table 3. Birds reared in free-range system had significantly higher feed consumption at all ages. Total feed consumptions in free-range and intensive system were found as 7693.g and 6983.5 g, respectively. More physical activity in outdoor could be resulted as higher feed consumption. Production system had significant effect on feed conversion ratio, and intensively reared guinea fowls had better FCR. Nahashon et al. (2009) found the FCR of guinea fowl broilers to be around 2.5 at 8 weeks. This result is in parallel with the findings of our study.

Table 3. Feed consumption and feed conversion ratio (FCR) of guinea fowl at different ages

Production system	Age (weeks)	Feed consumption (g)	FCR
Free-range	8	1280.9	2.56
	10	2231.6	3.21
	12	3496.1	3.88
	14	4702.7	4.54
	16	6655.1	5.79
	18	7693.6	6.43
Intensive	8	1274.2	2.37
	10	2179.3	2.97
	12	3293.9	3.51
	14	4430.4	4.19
	16	6043.0	5.24
	18	6983.5	5.80
		Effects	
	8	**	**
	10	**	**
	12	**	**
	14	**	**
	16	**	**
	18	**	**

CONCLUSIONS

The result of this study showed that, there was not significant difference between live weight of guinea fowls reared in free-range and intensive systems. This finding is also notable, given the importance that animal welfare has gained among consumers in recent years and the fact that back-yard production is the most common form of guinea fowl production in many countries and represents a significant economic activity in rural areas. Selection of guinea fowl in live weight and feed consumption will benefit to increase profit and decrease fattening period.

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APPLICATION OF PLANT EXTRACTS AS FEED ADDITIVES IN POULTRY NUTRITION

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Abstract

Various kinds of antibiotics have been used extensively as growth promoters in animal feeds for a large number of years, especially in the fields of poultry production. As a result of the decision to ban of the antibiotics in livestock production researches on plant extracts as alternatives to the use of growth promoters (antibiotics) has significantly increased. Many novel natural candidate replacements including probiotics, prebiotics, organic acids and plant extracts and essential oils have been applied to maintain good production. Recently, herb and plant extracts have been received a great attention to be fed to poultry as feed additives to improve and increase production. The most commonly studied plants to be used in animal nutrition are cinnamon, oregano, cumin, garlic, sumac, cloves, anise, mint, coriander and ginger. Researchers have shown that these extracts are the powerful stimulators of the immune and animal digestive systems as well as highly beneficial effects in poultry nutrition due to their antioxidant, antimicrobial, antiviral, anticoccidial and anthelmintic properties. The aim of this review is to provide an overview of the recent knowledge on the use of plant extracts in poultry feeds as feed additives and their effects on the poultry performance.

Key words*: plant extracts, nutrition, action mechanism, poultry.*

INTRODUCTION

Many kinds of antibiotics have been used as feed additives in animal nutrition to improve growth performance and health. Starting with a discovery in late of 1940's, the use of antibiotics as feed additives in pig and poultry industry has become a common application. However, the use of them as growth promoters in animal feed have been banned by the European Union and United States due to the possibility of developing resistant populations of bacteria (Hashemi and Davoodi, 2010). As a result of the decision to ban of the antibiotics in livestock production researches on plant extracts as alternatives to the use of growth promoters (antibiotics) has significantly increased. Many novel natural candidate replacements include probiotics, prebiotics, organic acids and plant extracts and essential oils have been applied to maintain good production (Fulton et al., 2002). Addition of plant extracts and essential oils obtained from plants into diets may play role in improving growth performance of animals and health status as well (Manzanilla et al., 2001; Denli et al., 2004). The aim of this review is to provide an overview of the recent knowledge on the use of plant extracts as feed additives in poultry feeds and their effects on the poultry performance.

PLANTS AND THEIR ACTIVE COMPOUNDS

The active compounds from the common plants used are presented in Table 1.

Table 1. Common plant extracts, their utilised parts, main active substances and reported properties (Richard, 1992; Charalambous, 1994)

Plant name	Utilized parts	Main Compounds	Reported properties
Aromatic spices			
Clove	Flower	Eugenol	Appetite Enhancer, Digestive Stimulant ,Antiseptic
Cinnamon	Leaf	Cinnamalde-hyde	Appetite Enhancer, Digestive Stimulant, Antiseptic
Coriander	Leaf-Seed	Linalol	Appetite Enhancer , Digestive Stimulant
Cumin	Seed	Cuminalde-hyde	Digestive, carminative, galactague
Anise	Fruit	Anethol	Digestion stimulant, galactagogue
Celery	Fruit, leaf	Phtalides	Appetite and digestion stimulant
Pungent spices			
Capsicum	Fruit	Capsaicin	Antidiarrhoeic, anti-inflammatory, stimulant, tonic
Pepper	Fruit	Piperine	Digestion stimulant
Ginger	Rhizome	Zingerone	Gastric stimulant

Aromatic herbs and spices			
Garlic	Bulb	Allicin	Digestion stimulant, antiseptic
Rosemary	Leaf	Cineol	Digestion stimulant, antiseptic, antioxidant
Thyme	Whole plant	Thymol	Digestion stimulant, antiseptic, antioxidant
Sage	Leaf	Cineol	Digestion stimulant, antiseptic, carminatif
Peppermint	Leaf	Menthol	Appetite and digestion stimulant, antiseptic

PLANT EXTRACT PROPERTIES

Plant extracts consists mainly of proteins, peptides, oligosaccharides, fatty acids, vitamins, micro minerals. Plant extracts have a wide range of activities and their active secondary plant metabolites typically belong to the classes of isoprene derivatives and flavonoids (Tajodini et al., 2015). They have a wide range of activities. A great number of plant extracts contain chemical compounds exhibiting antioxidant (Kähkönen et al., 1999; Hashemi et al., 2009), antimicrobial (Hammer et al., 1999; Hsieh et al., 2001), anti-inflammatory (Pradeep and Kuttan, 2004), anticoccidial (Arab et al., 2006) and anthelmintic (Hoste et al., 2006) properties. The cultivation area, climatic conditions, vegetation phase, genetic modifications and others are factors affecting their chemical and biological diversity (Miliauscas et al., 2004). These properties of plant extract are mainly due to the bioactive compounds such as flavonoids and glucosinolates isoprene derivatives found in nature (Kutlu and Erdogan, 2010). Additionally, the properties probably are the major mechanisms by which plant exert positive effects on the growth performance and health of animals (Hashemi, and Davoodi, 2011). They can exhibit their effects by stimulating feed intake and endogenous secretions or having antioxidant, antimicrobial activities.

PLANT EXTRACTS AS FEED ADDITIVES IN POULTRY NUTRITION

Various plant or herbal extracts are commonly included in poultry diets for promoting of growth performance and animal health especially when there are health challenge conditions. A lot of research have documented the benefit effects of plant extracts on the performance of poultry (Jamroz and Kamel, 2002; Tucker, 2002; Alçiçek et al., 2003; Denli et al., 2004). They reported that the supplementation of plant extracts or oils in to diets increased the body weight gain, feed intake and improved feed conversion rate in poultry (Table 2).

The main action of plant extracts or oils as feed additives is improving the ecosystem of gastrointestinal microbiota through controlling potential pathogens and digestive capacity in the small intestine (Hashemi and Davoodi, 2011). Wenk et al. (2000) observed that dietary plant extracts exhibit strong effects on stimulate endocrine system and intermediate nutrient metabolism. Tucker (2002) demonstrated a significant improvement of performance, survive rate in broilers fed diet with many kinds of plant extracts.

Beneficial actions of herbal extracts or their active compounds in animal nutrition may include the stimulation of appetite and feed intake, the improvement of endogenous digestive enzyme secretion (Rahimi et al., 2011). Tollba et al (2007) reported that at two, four and six week of age, the broiler receiving varying levels of black pepper showed better body weight gain.

Results from the most of studies exhibits beneficial actions of plant extracts on poultry productivity and health. However, some researchers reported that some plant extracts additives have no any effects on growth performance or health status of poultry. Al-Kassie et al. (2011) showed that no difference in broilers fed diet with black pepper. In addition, Aydin et al. (2008) reported that dietary black cumin seed at the level of 1, 2 or 3 % had no effects on body weight gain, feed conversion rate or feed intake in laying hens. Similarly, Hernandez et al. (2004) showed that supplementing broilers diets with essential oil extract from oregano, cinnamon and pepper affected the performance slightly, but this effect was not significantly.

Table 2. Effects of dietary plant extracts used as feed additives on poultry performance

Plant Extract	Dose	Performance effect	Literature
Chinese herbal medicine	0.5-1%	Better growth performance from 7 to 21 days	Guo et al. (2004)
Cinnamon	0.2%	Higher growth performance	Al-Kassie, (2009)
Red pepper extract	0.1%	No effect on live performance or in organ morphometrics.	Barreto et al. (2008)
Thyme extracts	3 and 6%	No improved the performance and carcass traits	Amouzmehr et al. (2002)
Thyme essential oil	0.2%	Improved growth performance	Denli et al. (2004)
Black cumin seeds	1%	Increased BW, improved feed efficiency	Khalaji et al. (2011)
Plant extract consisting ofcapsaicin, cinnamaldehyde and	0.1%	No affected the BW, feed efficiency was improved at 4.2 %	Jamroz et al. (2005)

CONCLUSIONS

The use of plant extracts as feed additives in poultry can be valuable because they allows maximize the overall performance and an improvement digestibility of poultry. The most of plant extracts tested in poultry experiments exhibited positive effects on the productivity and no any harmful effects on animal health and products obtained animal. Therefore they can use as an alternative feed additives in poultry production.

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PHYSICAL PERFORMANCE AND CARCASS CHARACTERISTICS OF HOLSTEIN AND BROWN SWISS CATTLE GROWN IN AN INTENSIVE BEEF SYSTEM

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Abstract

In this study, it was aimed to compare the physical performances and carcass characteristics of Holstein and Brown Swiss cattle grown in an intensive beef production system.
For this purpose, 20 Holstein and 20 Brown Swiss male cattle with an average age of 6 months old were assigned to two feedlot paddocks evenly and fed on the similar ration for 12 months in 2012. The average initial weights of Holstein and Brown Swiss breed animals were 158 and 132 kg respectively. General Linear Model (GLM) procedure was used for the statistical analysis of the data and initial weight and age were taken as covariates to eliminate the weight differences at the start of the experiment. It was found that at the end of the experiment, the differences in final weights were not statistically significant (P>0.05) and Holstein and Brown Swiss animals reached average final weights of 502 and 493 kg, respectively. Furthermore, there were no significant (P >0.05) differences in mean total gains (344 v. 361 kg) and average daily liveweight gains (0.985 v. 1.028 kg). The slaughter weight of the animals was 502 and 493 kg for Holstein and Brown Swiss cattle, respectively. Hot carcass weight was approximately similar for both breeds (264 kg) and Dressing percentages were 52.6 and 53.6% for Holstein and Brown Swiss cattle, respectively. Total fat values were 9.2 and 8.8 kg, Fat ratios were 3.52 and 3.45 % for Holstein and Brown Swiss cattle, respectively. Total bone values were 49.37 and 44.29 kg, Bone ratios were 19.8 and 18.3 % for Holstein and Brown Swiss cattle, respectively. There was only statistical differences (P<0.05) in total bone and bone ratio between breeds. The results indicated that under the Mediterranean climate conditions both breed animals performed similarly and there was no superiority of any breeds over each other although Brown Swiss cattle tended to show better performances. Therefore, both breeds can be recommended for an intensive beef system in the region.

Key words*: Holstein, Brown Swiss, beef cattle, Mediterranean, performance, carcass traits.*

INTRODUCTION

Traditional beef production is highly dependent on climate and soil, and very much linked to the availability of local resources either plant growth or the animal breeds reared. In addition, it is also related to the socio-economic conditions such as environment, the land tenure situation, quality of life and a reasonable degree of advanced technology. The animal production based on extensive systems have common features such as limited number of animals per unit area, relatively limited use of resources and advanced technology, low productivity per animal and hectare of land, feeding mainly based on natural grazing (Boyazoglu and Nardone, 2005).

Beef production constitutes an important part of the agricultural sector of many countries. The development of beef industry in many countries depends largely on climatic conditions and land types. It also depends on the size of agricultural holdings and the overall structure of the cattle industry especially the relationship between beef and dairy production (Allen and Kilkenny, 1984).

Meat is produced primarily as a by-product of milk production and the cattle are mainly dual purpose for milk and beef in Turkey, where there is a much smaller range of farming

environments divided mainly into smaller farms.

Feedlot beef production systems have gained a big interest due to its low investment and operational costs for the last decade in Turkey and beef producers have been facing a big challenge in meeting the great demand for red meat consumption of the population along with its rapid growth rate (Ecevit, 1999). The Average carcass weight has increased from 200 kg to 275 kg within a decade in the country.

While there is some information on the comparative feedlot performance of Holstein with Brown Swiss cattle breeds, there is not much information on comparative evaluation of carcass characteristics of both breed especially under the Mediterranean climatic conditions. Therefore, this study was aimed to provide some information about physical and carcass performance comparisons of breeds grown in intensive beef system in the Mediterranean part of the country.

MATERIALS AND METHODS

Animals

The experiment was conducted in Isparta province located in the west Mediterranean part of Turkey at the Süleyman Demirel University Research Farm. The data composed of a total of 40 beef animals with a mean initial weight of 145 kg; including 20 Holstein and 20 Brown Swiss breeds. The initial average weights of cattle were 132 and 158 kg for Brown Swiss and Holstein respectively.

Animal Management

Animals were obtained from local cattle markets with approximately six months and were initially weighed at the beginning of the experiment and were randomly allocated according to their weights into two groups, each group having the same type of breed and were kept in feedlots with two pens The free access of the experimental animals to water was available throughout the experimental period. Each group was weighed and monitored on a fortnightly basis, using electronic weighing scale (True-Test2000 SmartUnit). The experiment lasted for 12 months and at the end of the experiment, the animals were slaughtered at a commercial abattoir.

Diets

Dried alfalfa and hay as roughages and ground barley and cattle fattening feed as concentrates were provided to obtain a target of 1 kg daily live weight gain (DLWG) and the rations were rearranged according to live weight changes of the animals as required.

The rations were weighed out into bags and fed twice a day. The chemical compositions of concentrate diets used in this study are shown in Table 1.

Table 1. Chemical composition of concentrate diets

Dry matter %	88	Sodium %	0.3-0.6
Crude Protein %	14	Metabolic Energy, Kcal/kg	2600
Crude Fibre %	14	Vitamine A, I.U/kg	5000
Crude Ash %	9	Vitamine D3, I.U/kg	700
Calsium %	1.0-2.0	Vitamine E, Mg/Kg	30
Phosphate %	0.5		

Statistical Analysis

The data for breed types and seasons were analysed by GLM (General Linear Model) procedure (Minitab v.16), using the following model:

$$Y_{ijk} = \mu + \alpha_i + \beta_j + \varepsilon_{ijk}$$

where Y_{ijk} is the ijk th observation of animal weight,

μ is the overall mean,

α_i is the effect of breed type,

β_j is the effect of initial weight and,

ε_{ijk} is the residual effect or random error associated with the individual animal

Breed type factor was fitted as fixed effect, and initial weight was included in the model as a covariate (average 145 kg approximately).

The data related to carcass traits were statistically analysed by Two-sample Student's *t*-Test.

Table 2. Overall physical performance comparisons of breed types

Breed Type	N	IW (kg)	s.e.	FW (kg)	s.e.	TWG (kg)	s.e.	DLWG (kg)	s.e.
Holstein	19	158	4.92	502	6.16	344	5.12	0.985	0.023
Brown Swiss	20	132	4.52	493	6.67	361	8.05	1.028	0.028

IW= Initial weight, FW= Final weight, TWG= Total weight gain, DLWG= Daily Live weight gain

Table 3. Carcass traits comparisons of breed types*

Breed Type	N	SW (kg)	s.e.	HCW (kg)	s.e.	DP (%)	s.e.	TF (kg)	s.e.	FR (%)	s.e.	TB (kg)	s.e.	BR (%)	s.e.
Holstein	19	502	6.16	264.1	2.5	52.6	0.36	9.2	1.0	3.52	0.42	49.4^{a}	1.1	19.8^{a}	0.42
Brown Swiss	18	493	6.67	263.8	6.1	53.6	0.3	8.8	0.49	3.45	0.18	44.3^{b}	1.4	18.3^{b}	0.32

SW = Slaughter weight, HCW = Hot Carcass weight, DP = Dressing Percentage, TF = Total Fat, FR = Fat Ratio, TB = Total Bone, BR = Bone Ratio.
* The statistically significant means are shown with the different superscripts within the same columns ($P < 0.05$).

RESULTS AND DISCUSSIONS

There were some health problems encountered during the winter and one Holstein was died of pneumonia and dismissed from statistical analysis.

The least-square means and standard errors for liveweights for breed types are shown in Table 2. There were no significant ($P > 0.05$) differences between breed types in FW, TWG and DLWG. Holstein cattle performed better than Brown-Swiss cattle in all parameters observed. Mean daily liveweight gains for Holstein and Brown-Swiss cattle were 0.985 and 1.028 kg respectively.

Final weights and overall weight gains of Holsteins (502 kg and 344 kg respectively) were no statistically higher ($P >0.05$) than those of Brown Swiss cattle (493 kg and 361 kg respectively). The least-square means and standard errors for carcass parameters for breed types are shown in Table 3.

The slaughter weight of the animals was 502 and 493 kg for Holstein and Brown Swiss cattle, respectively. Hot carcass weight was approximately similar for both breeds (264 kg) and Dressing percentages were 52.6 and 53.6% for Holstein and Brown Swiss cattle, respectively. Total fat values were 9.2 and 8.8 kg, Fat ratios were 3.52 and 3.45 % for Holstein and Brown Swiss cattle, respectively. Total bone values were 49.37 and 44.29 kg, Bone ratios were 19.8 and 18.3 % for Holstein and Brown Swiss cattle, respectively.

As shown in Table 3, there were no significant ($P > 0.05$) differences between breed types in SW, HCW, DP, TF and FR. However, there were significant ($P < 0.05$) differences between breed types in TB and BR.

It was reported by Wilkinson (1985) that conformation and growth potential vary greatly between different breeds of cattle. While there are certainly differences between breeds in growth rate, the liveweight gain which can be achieved from a given area of grass or quantity of feed is similar for most breeds, provided that each breed is fed and managed according to its own particular requirements. This is supported by the results of this study that both breeds were fed on similar feeding conditions.

Bozkurt (2006, 2007 and 2011) reported about the superior weights of Holstein cattle, However, in this study the results were not in agreement with the results reported by Wilkinson (1985), Bozkurt and Ap Dewi (1996) and Bozkurt (2012). The results of this study showed that under the Mediterranean conditions Holstein and Brown Swiss cattle were performed similarly and both can be well-suited to the feedlot beef systems.

However, as Keane *et al.* (1989) and Keane and More O'Ferrall, (1992) reported that the results of these comparisons, including those reported in this study are not necessarily applicable outside the countries where comparison studies were carried out due to the differences in factors such as production systems, slaughter weights and climate, etc.

In relation to carcass traits, Onenc (2004) found that there were no statistically significant differences in hot carcass weights between Holstein and Brown Swiss cattle slaughtered in the Aegean region. These results were similar to those found in this study. However, there were differences in conformation and fatness between both breeds while no difference in fatness was found in this study.

Similar results to the findings of this study was reported by Diler et al. (2016) who worked on cold carcass traits of Holstein and Brown Swiss young bulls grown in the Eastern Anatolia Region. They reported that cold carcass weights as 237.4 and 248.6 kg for Holstein and Brown Swiss cattle respectively. Cold dressing percentages were found as 53.3 and 51.9 % for Holstein and Brown Swiss cattle respectively. Fatness scores were as follows; 2.2 and 2.1 for Holstein and Brown Swiss cattle respectively. Similar results were also reported by Aydin et al (2013) that there were no significant differences in the same carcass parameters observed in this study.

CONCLUSIONS

It can be concluded that under the Mediterranean climate conditions both breed animals performed similarly in terms of physical performance and carcass characteristics and there was no superiority of any breeds over each other although Brown Swiss cattle tended to show better performances. Therefore, both breeds can be recommended to be kept in an intensive beef system in the region.

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EFFECT OF USING A WINERY BY-PRODUCT WITH ANTIOXIDANT PROPERTIES IN LATER DIETS ENRICHED IN POLYUNSATURATED FATTY ACIDS, ON EGG QUALITY

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Abstract

Due to their multiple positive, acknowledged effects of the omega-3 unsaturated fatty acids, present day consumers are increasing interested in ways of incorporating these fatty acids in their daily diet. The production of eggs enriched in polyunsaturated fatty acids via the feeds given to layers is one way, but solutions have to found to preserve for a longer time the organoleptic properties of these enriched eggs. It appeared thus necessary to use antioxidants, natural mainly, in layer diets. Due to their high content of polyphenols, the natural antioxidants block oxidation by their reaction with the free radicals. The purpose of our study was to evaluate the effect of a winery by-product, grape seeds meal, displaying antioxidant properties, given to layers, on egg quality. The experiment was conducted at a commercial poultry farm owned by Avicola Lumina SA, for 4 weeks, on a total of 64.000 layers aged 27 weeks, Tetra SL LL hybrid. The layers were assigned to two groups, control (C) and experimental (E), of 32.000 layers each. The diets were based on corn, soybean meal and sunflower meal, and had the same protein and energy content. The diet of the experimental group differed by the inclusion of flax meal and camelina meal, as ingredients rich in unsaturated fatty acids, and of 1% grape seed meal, as natural antioxidant. Egg samples were collected in the end of the experiment and assayed for their quality. The higher concentration of omega-3 unsaturated fatty acids in the diet for group E (12.26 ± 0.15 g/100 g fat) compared to group C (7.28 ± 0.89 g/100 g fat) was also found in the yolk of the sampled eggs, which increased from 3.33 ± 0.20 g/100 g fat in group C, to 5.76 ± 0.30 g/100 g fat in group E. The higher antioxidant capacity, 7.157 ± 0.662 mM Trolox equivalent / g of the diet for group E, compared to 6.507 ± 0.401 mM Trolox equivalent / g of the diet for group C, also increased the antioxidant capacity of the eggs. Thus, the eggs harvested from group E had an antioxidant capacity of 126.353 ± 4.523 mM Trolox equivalent / g, compared to 115.300 ± 7.269 mM Trolox equivalent / g in the eggs collected from group C. These results that the winey by-product, grape seed meal, can be used as antioxidant feed additive in layer diets enriched in unsaturated fatty acids, thus improving the properties of the eggs.

Key words: *antioxidants, grape seeds meal, eggs, quality.*

INTRODUCTION

Recent researches in human diets show that a high level of omega-3 polyunsaturated fatty acids in the animal foods for human consumption have beneficial effects on human health (Riediger et al., 2009, Huang, 2010, Shapiro et al., 2010, Turner et al., 2011). Considerable research efforts are directed towards the nutritional manipulation of the fatty acids profile from the hen eggs (Criste et al., 2009). Fraeye et al. (2012) published a review of 26 studies conducted in 1991-2011, regarding the possibilities of enriching the hen eggs in omega-3 polyunsaturated fatty acids, which used flax seeds, fish oil and/or micro-algae as sources of omega-3 fatty acids. The changes in egg composition, following the supplementation, are quite varied.

The rather high concentration of polyunsaturated fatty acids in the yolk, make the egg susceptible to oxidative degradation during heat preparation and during storage. The Lipid oxidation produces free radicals, which cause several oxidative degradations and start the reactions of secondary oxidation (Ren et al., 2013). It appeared thus necessary to use antioxidants in layer diets enriched in polyunsaturated fatty acids, particularly natural antioxidants which, by their high concentration

of polyphenols, block oxidation by reacting with the free radicals. Many researches have been conducted during the past decade, which have shown that winery by-products can be used in poultry feeding, particularly the grape marc. The wine-making process produces large amounts of this waste, which can be used efficiently in corn-soybean diets for poultry (Su et al., 2008; Hu et al., 2013; Juśkiewicz et al., 2015).

The paper reports on the experiments aiming to evaluate the effect on egg quality of a winery by-product, grape seeds meal resulting from oil extraction, used as antioxidant in layer diets high in polyunsaturated fatty acids.

MATERIALS AND METHODS

The experiment was conducted in a commercial company, Avicola Lumina SA, for four weeks, on a stock of 64.000 TETRA SL LL layers aged 27 weeks. The layers were assigned to two groups, a control group (C) and an experimental (E) group, each with 32.000 layers. The two experimental halls (Photo 1) belong to module 5 of SC Avicola Lumina SA, where the layers are reared in enriched, EUROVENT 1500-type cages. These cages, manufactured by Big DUTCHMAN (Germany) are stacked on 2, 3 or 4 tiers. The area of each hall is of 2000 sq.m, with 6 rows × 33 cages each × 2 tiers each × 60 layers / cage. These cages for layers are fitted with nipple drinkers. Each hall is fitted with 8, 1.1 kW fans, with a capacity of 32.000 cubic meters per hour. Each hall is also fitted with a Big Dutchman computer which regulates the feeding, watering, air admission, ventilation and heating.

The diet formulations were based on corn, soybean meal and sunflower meal. The formulation for the experimental group also included flax meal and camelina meal as ingredients rich in polyunsaturated fatty acids, plus 1% grape seeds meal, as natural antioxidant. After 2 and 4 weeks of feeding (end of the experiment), egg samples (10 eggs per group) were collected and assayed for the fatty acids concentration in the yolk; for the concentration of total polyphenols; for the content of flavonoids and for the antioxidant capacity in the methanol extracts of yolk.

Standardized methods were used to assay the concentration of main nutrients in the feeds, as follows:

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

- The crude fibre (CF) was determined with the method with intermediary filtration, whose working principle involves weighing the sample boiled successively with solutions of sulphuric acid and sodium hydroxide. The resulting residue was filtered, dried, burnt and weighed. The method complies with *Regulation (CE) 152/2009 and standard SR EN ISO 6865:2002*. We used a FIBERTEC 2010 system – Tecator (Sweden);
- The ash (Ash) was determined by the gravimetric method, which involves sample decomposition by burning and weighing of the resulting ash. The method complies with *Regulation (CE) 152/2009 and standard SR EN ISO 2171:2010*. We used a Caloris CL 1206 furnace.
- The gross energy was determined by calculation using the gross chemical analysis (dry matter, protein, fibre, fat, nitrogen-free extractives and ash) using the equations of Burlacu et al. (2002).

To determine the concentration of polyphenols and the antioxidant capacity of the samples, we first extracted the phenol compounds in acidified methanol (methanol:HCl=80:20). To 1g sample we added 10 ml acidified methanol and stirred at room temperature for 48 h. The homogenate has been centrifuged twice at 10,000 RCF, for 15 min, at room temperature, and the final supernatant (methanol extract) has been preserved at 4^{o}C, until analysed. Instruments: orbital stirrer Heidolph Unimax 1010, Microstirrer Vepl Scientific, Centrifuge Eppendorf 5810R, RADWAG AS220/C/2 (10-220 mg) and PS600/C/2 (0.01-600 g) scales, pH metre WTW Senix-HW

- The polyphenol content of the methanol extracts has been determined according to the method described by Mihailovic et al. (2013), modified. The reaction mixture consisted of: the methanol extract in proper dilution according to the analysed sample, Folin-Ciocâlteu reagent and a solution of 7.5% de Na_2CO_3. The reaction mixture was maintained for 30 min. at room temperature, thereafter absorbance was read at 765 nm. Three replicates have been done for the same sample, and the average value of the readings, representing the total concentration of phenols, was expressed in equivalents gallic acid/g fresh matter (mg EAG/g sample). We used a UV-VIS Thermo Scientific spectrophotometer.
- The flavonoids content of the methanol extracts has been determined according to the method described by Zhishen et al. (1999).
- The determination of the antioxidant capacity of the methanol extracts has been done using the DPPH method proposed by Marxen et al. (2007). The antioxidant capacity has been estimated by calculating the difference between the control and the sample, compared to a standard curve which used Trolox (synthetic antioxidant analogue to α-tocopherol), as standard antioxidant. Three replicates have been done for the same sample, and the average value of the readings, representing the antioxidant capacity has been expressed in Trolox equivalents/g fresh matter (mM Trolox/g sample). We used a UV-VIS Analytik Jena Specord 250 Plus spectrophotometer with thermostatic carousel.

The results of the experiment as presented as mean values with standard deviations, the statistic processing being done with Origin 5 software, using the t-Test (2 populations). The differences were considered statistically significant for $P \leq 0.05$.

RESULTS AND DISCUSSIONS

The grape seeds meal, resulting from oil extraction by a Romanian company, Chimia Bistrita SCM, was characterized by 11.59 ± 0.534 % protein, 5.29 ± 0.303 % fat, 42.83 ± 1.635 % fibre and 2.87 ± 0.136 % ash, values comparable with literature data (Olteanu et al., 2014; 2015).

Table 1. Data on the antioxidant properties of the grape seeds meal

Item	Total polyphenols (mgEAG/g)	Flavonoids (mg ERU/ g)	Antioxidant capacity (mM ET/g)
Sample 1	12.022	16.367	157.603
Sample 2	11.093	15.988	160.120
Sample 3	10.913	14.912	145.755
Sample 4	10.926	16.715	145.163
Average	*11.239*	*15.996*	*152.160*
Standard deviation	±0.529	±0.781	±7.810
Coefficient of variation	0.047	0.049	0.051

EAG – gallic acid equivalents; ERU –Rutin equivalents; ET – Trolox equivalents.

Table 1 shows the concentration of polyphenols and flavonoids, as well as the antioxidant capacity of the grape seeds meal determined on 4 samples.

The concentration of total polyphenols in the grape seeds meal, 11.239 ± 0.529 mg gallic acid equivalents / g sample, resembles to other plants such as coriander - 8.80 mg gallic acid equivalents / g sample; dill – 9.80 mg gallic acid equivalents / g sample, chilli – 8.60 mg gallic acid equivalents / g sample (Bin Shan et al., 2005), birthwort – 11.04 mg gallic acid equivalents / g sample (Papuc et al., 2010). Poudel et al. (2008) cited by En-Qin Xian et al. (2010) has shown that the antioxidant capacity of the grape seeds ranges between 16.8-92 mM Trolox equivalents /g sample, while it ranges between 157-113.3 mM Trolox equivalents /g sample in the grape peels.

The nutrients from the feed samples, collected after manufacturing, have shown that nutritionally, they meet the quality parameters, being isoprotein (about 19.5 % protein) and isoenergy (about 16.5 MJ /kg gross energy).

The compound feed for group E has been enriched in omega 3 polyunsaturated fatty acids using two unconventional forages (flax and camelina), as meal. The analytical results from CF analyses show a significantly ($P \leq 0.05$) higher concentration of omega 3 fatty acids in group E (12.26 ± 0.15 g / 100 g fat), given mainly by the α-linolenic acid, compared to just 7.28 ± 0.89 g / 100 g fat in group C.

As expected, the use of 1% grape seeds meal as natural antioxidant, in the feeds for group E, increased by 2.36 % the polyphenols concentration, by 3.18% the flavonoids concentration, and by 9.99% of the antioxidant capacity, compared to the corresponding values for group C (Figures 1, 2 and 3).

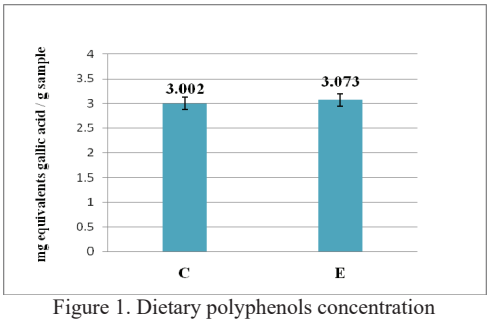

Figure 1. Dietary polyphenols concentration

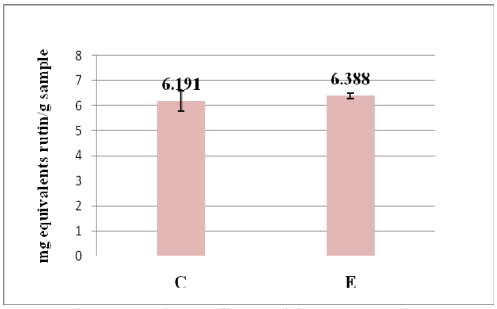

Figure 2. Dietary flavonoids concentration

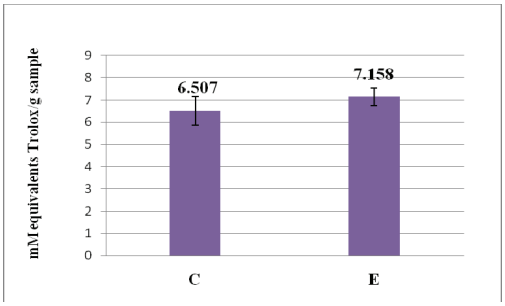

Figure 3. Dietary antioxidant capacity

At the same time, as shown in Figure 4, there was a good correlation between the antioxidant capacity and the polyphenols concentration in the feeds.

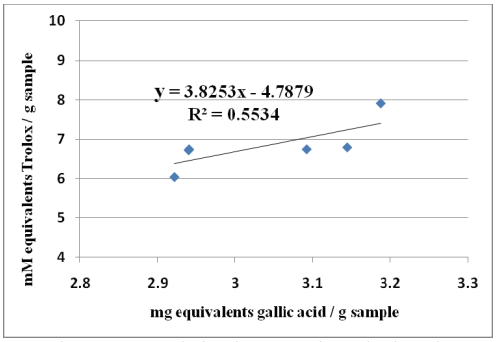

Figure 4. Correlation between the polyphenols concentration and the antioxidant capacity of the feeds

The quality evaluation of the eggs collected at the end of the trial has shown that the concentration of omega 3 fatty acids in the yolk from group E was of 5.76 ± 0.30 g / g fat, significantly ($P \leq 0.05$) higher compared to 3.33 ± 0.20 g / g in the yolk from group C. this is due to the higher concentration of omega 3 fatty acids in the feed for group E compared to group C.

Regarding the oxidative status of the eggs (Figures 4 and 5), one can notice that the polyphenols concentration in the yolk of eggs from group E was 0.466 ± 0.039 mg gallic acid equivalents / g sample, while in the yolk of the eggs from group C, it was 0.491 ± 0.044 mg gallic acid equivalents / g sample, with no significant differences.

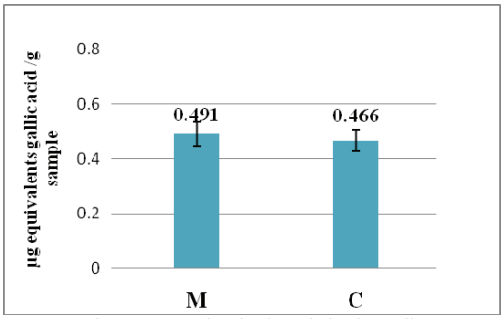

Figure 5. Total polyphenols in the yolk

On the other hand, the antioxidant capacity of the yolk from group E eggs was 126.353 ± 4.524 mM Trolox equivalents / g sample, significantly (P≤0.05) higher, by 9.59%, compared to group C, 115.300 ± 7.270 mM Trolox equivalents / g sample (Figure 6).

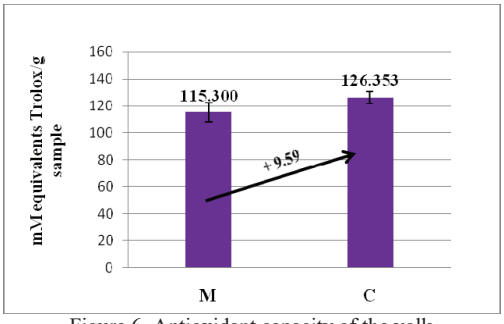

Figure 6. Antioxidant capacity of the yolk

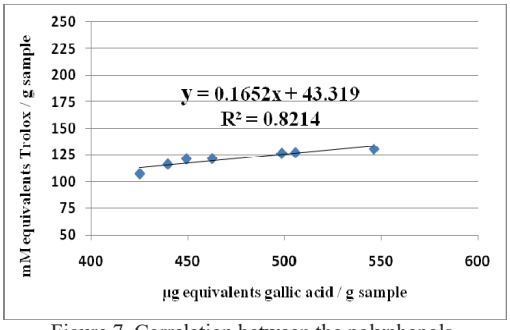

Figure 7. Correlation between the polyphenols concentration and the antioxidant capacity of the yolk

As shown, there has been a rather close correlation between the polyphenols concentration and the antioxidant capacity of the eggs, particularly of the yolk ($R^2 = 0.8214$), as shown in Figure 7.

CONCLUSIONS

Although the concentration of polyunsaturated fatty acids was higher in the feeds for the experimental group, the addition of 1% grape seeds meal, balanced the oxidative status of the compound feed, allowing a good correlation between the polyphenols concentration and the antioxidant capacity of the feeds.

The determinations of polyphenols concentration and antioxidant capacity performed on yolk samples, revealed a higher antioxidant capacity (by 9.59%) compared to the control group, confirming the existence of a close correlation between the antioxidant capacity and the polyphenols concentration ($R^2 = 0.8214$).

The experimental results show that this winery by-product, grape seeds meal, has antioxidant properties and can be recommended as feed additive for layer diet formulations enriched in polyunsaturated fatty acids, enhancing thus egg quality.

ACKNOWLEDGEMENTS

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TESTING OF THE NUTRIENT SUPPLEMENT ENRICHED WITH BIOMASS OF AQUATIC ALGAE IN THE BEE'S FEED

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Abstract

The purpose of this study was to test in the bee's feed the biomass of aquatic microalgae Scenedesmus quadricauda, hereinafter referred to as bioactive supplement "Scenecuadri" and elaboration on its basis of a process of feeding of bee families during the end of winter and start of spring (february-march), poor harvesting period in nature. The researchers was conducted on the Apis mellifera Carpatica bee families at the experimental apiary of the Institute of Zoology of the Academy of Sciences. For testing of biomass in bee's feed at the end of February, they were formed three batches of bees families, to which once for each frame with bees were administered 200 g of nutritional paste, prepared by mixing the powdered sugar with honey in proportion 7:3. The batch I - control, bees have received only nutritional paste, prepared by mixing the powdered sugar with honey. The batch II - the bees have received paste enriched with nutritional supplement "Apispir + Cr" in quantity of 200 mg of active substance per 1 kg of paste. The batch III - bees have received nutritional paste enriched with bioactive supplement "Scenecuadri" in a quantity equivalent to 200 mg of dry substance per 1 kg of paste. Research results have shown that feeding of bee families with nutritional supplement enriched with biomass of aquatic microalgae S. quadricauda help to increase, compared to the control batch, queen prolificacy up to 125 eggs or 7.8%, the amount of capped brood with 14.7 hundreds cell or 7.7%, family power by 0.30 kg or 9.3%, the amount of bee bread accumulated in nest with 14.0 hundreds cells or 15.5%, the amount of wax increased by 0.04 kg or 13.3%, resistance to disease by 1.6 or 1.8%, brood viability with 11 points or 1.2% and the amount of honey in the harvesting 3.01 kg, or 27.6%. The result is due to increasing nutrient assimilation and accessibility of biomass, given the fact that the microalgae Scenedsmus quadricauda is covered with a thin protective membrane and the biomass is rich in biologically active substances, in particular proteins, carbohydrates, lipids, essential amino acids, micro - and macro elements, antioxidants (beta carotene), which have a catalytic role in the metabolism of substances nitrogenous to worker bees, participates in the synthesis of enzymes, improves the qualitative composition of royal jelly and stimulates its secretion by wet nurses-bees, so indirectly influence (by feeding with royal jelly of the queen) on the reproductive system of the queen, intensifiing the ovogenesis and eggs laying. All of this largely determines the queen prolificacy, development of the larvae and brood from the nest, contributing to the increased family strength and their productive potential as a whole.

Key words: *bees, nutritional supplement, biomass, microalgae*, *Scenedesmus quadricauda*.

INTRODUCTION

At the end of winter (February) and early spring (March) reserves of natural food in the nest of bee family is exhausted and the deficiency of bioactive nutrients in the body of bees appears, especially of carbohydrates, protein, micronutrients, vitamins which have a decisive role in the physiological processes of vital activity of the bees organism, determining the reproduction and further development of the bee family on the whole (Cebotari et al., 2012; Cebotari et al. 2013a and 2013b; Toderaş et al., 2014).

In order to compensate the deficiency of nutritive substances in bees feeding during critical periods of harvesting in nature, most of the beekeepers fed the bees familie with sugar syrup, in the composition of which a number of important biologically active substances, excluding of carbohydrates, are absent. In these circumstances, identifying of available sources of biologically active substances for enriching the nutritional supplements from alimentar ration of bees in periods of poor harvest in nature, becomes an actual problem.

In recent decades researchers microbiologists have drawn attention to the biomass of mono- or multicellular microalgae, as important sources of biologically active substances. Among these the most studied became microalgae *Chlorella vulgaris* and multicellular microalgae (cyanobacteria) *Spirulina platensis* (Кожухарь et al., 1971; Baccep et al., 1989; Ionov et al., 2003; Mazo et al., 2004; Rudic et al., 2008a; Luca, 2012). Research has shown that biomass of microalgae *Ch. vulgaris* contains an important set of biologically active substances. According to certain authors (Luca, 2012), *Chlorella* is surnamed the "*supplement of energy and vitality*", having therapeutic properties, improving the health of the the organism in general and in particular fortifying the immune system, increases body resistance against infections. This microalgae is rich in β-carotene and is able to remove pesticide residues from the body, ingested through food, extract deposits of mercury, and is therefore a powerful detoxifier. Testing of suspension of biomass

of microalgae in in the feeding of the bees has helped to increase the rhythm of development of the colonies with 17.0-22.4% (Eremia et al., 2013).

Among the multitude of species of algae, the most studied is *S. platensis* (Rudic et al., 2006b; Rudic et al., 2006a; Bulimaga et al., 2006a and 2006b; Rudic et al., 2007; Rudic et al., 2008a and 2008b). For over 20 years this multicellular filamentous cyanophytes microalga has been explored as a food source. The World Health Organization and the 3rd International Congress of Food Science and Technology unconventional defined Spirulina as an essential source of up to 50 bioactive substances, which ensures the normal vital processes of the human and animal body.

To strengthen the vigor and disease resistance of bee families, some experts have proposed to enrich the nutritional supplements with biomass of strain *Bifidocbacterium globosum* + biomass of *Streptococcus faecium* + carbohydrate + oxide and aluminum hydroxide + ascorbic acid (Панин et al., 2001), suspension the *Karnitinhlorid* (Кузин et al., 2003).

In beekeeping are known also other proceeding to stimulate growth of bee families by feeding with sources of biologically active substances, in particular sugar syrup mixture of 50% enriched with biomass microalgae *S. platensis (Nordst.) Geitl CALU-835* (Toderaş et al., 2003). The disadvantage of this proceeding is the low efficiency, because the cells of that cyanophytes microalgae are covered with relatively thick a protective membrane, which stagnates the digestion process of nutrients from biomass by bees, in addition, the sugar syrup can not be used in winter when air temperatures are low.

Among the known proceedings (Toderaş et al., 2012, 2012b and 2012c), the nearest solution after technical essence and the obtained result is the proceeding of feeding bee families *Apis mellifera* MD 476Z 2012.09.30. (Toderaş et al., 2012b), which includes the feeding of bees in spring with a mixture of solution of 1% mas. biomass extract of microalgae *S. platensis* CNM-CB-02 and 50% sugar syrup in the ratio of 1: 500, respectively. Previously, to obtain biomass of *S. platensis,* microalgae has been cultivated in the presence of coordinative organic compound of chromium and potassium alum - $KCr(SO_4)_2 12H_2O$, which increases the permeability of living cells. The feeding of the bees with this mixture was carried out every 2 days, for two weeks in an amount of 100 ... 130 ml of a mixture on bee frame.

The disadvantage of this proceeding is the fact that the technology of obtaining of the extract biomass of microalgae *S. platensis* cultivated in the presence of coordinative compound is too complicated and expensive, and the mixture of sugar syrup enriched with supplement of bioactive substances can not be used during winter or early spring, because of high humidity created by a large amount of releasing vapor in the nest during the period when bees are in hibernation skein. For these reasons, some researchers have proposed as a source of biologically active substances biomass aquatic microalgae (Ungureanu et al., 2015), which are more accessible and less expensive.

In this context, the aim of this paper was to test in feeding of bees biomass of aquatic microalgae *Scenedesmus quadricauda* and elaboration on its basis of a proceeding for feeding of bee families during the end of winter and start of spring (february-march) poor harvesting period in nature.

MATERIALS AND METHODS

The researches were conducted on the *Apis mellifera Carpatica* bee families at the experimental apiary of the Institute of Zoology of the Academy of Sciences To achieve the purpose, experimental plan has been made that included the feeding of bee families at the end of winter during poor harvesting in nature, when atmospheric temperatures were low, with the nutrition paste wich was prepared by mixing powdered sugar with honey in proportion 7:3 and bioactive supplements. As a bioactive supplement the biomass of aquatic microalgae *S. quadricauda* was used, that was mixed with the pasta. The feeding of the bee families with enriched paste was performed by its distribution in the form of expellers in the nest, above the frame. Usually one expeller for every frame with bees was put.

Bioactive supplement, called by us "*Scenecuadri*", represents a suspension of biomass of 2%, greenish yellow coloured, dry matter contains 47-49% proteins, which includes the complete set of essential and non-essential amino acids, 40-46 % of carbohydrate, 11.9 to 12.2% of lipids, vitamins, micro- and macro elements and other important bioactive substances. In 100 mg of dry matter of the supplement there is 0.28 to 0.31 mg of beta-carotene, one of the main components with antioxidant properties and catalyzation of regeneration processes of cells and reproductive tissues of the queen and function lactogenic at the bee working. Considering that the monocellular microalgae Scenedesmus quadricauda is covered by a relatively thin protective membrane, bioactive substances from biomass are available for digestion in the digestive tract of honey bees.

To estimate the efficiency the proceeding of the bees feeding with the supplement, at the end of february were initiated experiments of comparative testing of the paste on bee colonies divided into three batches, 13 to 15 families each batch. The supplement was administered once, 200 g nutritional paste (a cake) for every frame with bees. The batch I - control, bees have received only nutritional paste, prepared by mixing the powdered sugar with honey in previously mentioned proportion. The batch II – the closest solution, the bees have received paste enriched with nutritional supplement “Apispir + Cr" ” (Toderaş et al., 2012b) in quantity of 2 ml solution with a concentration of 10% (200 mg of active substance) per 1 kg of paste. The batch III - the proposed invention, bees have received nutritional paste enriched with bioactive supplement "*Scenecuadri*" in a quantity 10 ml of suspension with a concentration of 2.0% (200 mg of dry substance) per 1 kg of paste.

In 100 days after feeding bees with the nutritional bioactive supplement (which coincided with the first

harvest) principal morph-productive characters of reproduction, development and productivity of bee families in the experimental batches were evaluated, according to Zootechnical norme regarding breeding of bee families, the growth and certification of genitor beekeeping material, approved by Government Decision no. 306 of 28.04.2011 (Zootechnical norme, 2011). The obtained in experience data were statistically processed using computer software "STATISTICA - 6" and evaluated their certainty, according to variation biometric statistics, by methods of Плохинский, 1989.

RESULTS AND DISCUSSIONS

The test results showed that the feeding of bees with the supplement "*Scenecuadri*" at the end of winter during poor harvest in nature, has contributed to a significant increase of value of principale morpho-productive characters. (Tab. 1).

It was found that the biologically active substances in the supplement "*Scenecuadri*" indirectly have caused stimulation of reproductive functions of the queen (oogenesis) contributing to the growth of egg-laying and to the increasing of the capped brood in the nest and as a result, the development of the bee family. Because, the queen does not consume nutritional supplement administered in the nest, but is constantly fed by worker bees with royal jelly, we can say that the biologically active substances in the supplement have a stimulating impact on lactogenic functions of the nurses bee and on the qualitative composition of royal jelly, stimulating, thus, reproductive functions of the queen.

As a result, the queen's prolificacy in hives from the batch III increased significantly compared with those in batch I (control) and batch II (the nearest solution), respectively, with 125 and 100 eggs/24 hours, or 7.8 and 6.2% (td = 3.5 and 2.8; P<0,001 and P<0.01).

Also, the quantity of capped brood at the hives from the batch III was significantly higher compared to batch I and batch II, 14.7 and 12.0 hundred cells, or 7.7 to 6.2% (td = 3.4 and 2.7, P<0.001 and P<0.01), respectively.

The increasing of the queens prolificacy and amount of capped brood, indirectly led to a significant increase of family strength, expressed by the total population of bees in the nest. Thus, the power of bee families from the batch III was significantly higher compared to group I and group II (the nearest solution), with 0.30 and 0.27 kg of bee, or 9.3 and 8.3% (td = 3.5 and 3.4; P < 0.001), respectively.

Due to higher family strength, the colonies from experimental batches II and III have obtained more pronounced feature of accumulation of the final bee products in the nest, for which, actually, are bred and exploited the bees.

The quantity of bee bread accumulated in the nest was also positively influenced by nutritional supplements enriched with algal biomass. Thus bee families from experimental batches II and III, who received nutritional supplements enriched both with extract of biomass of *Apispir*+Cr and biomass of microalgae *S. quadricauda* significantly exceeded the families from the control batch after the quantity of bee bread accumulated in the nest, respectively, 7.6 and 14.0 hundred cells, or 8.4 to 15.5% (t_d=2.6 and 3.4; P<0.05 şi P<0.01). Also compared with batch II, the amount of accumulated bee bread in the nest at the bee families in group I, who received in food nutritional supplement enriched with biomass of *Scenecuadri*, only had a tendency to increase by 6.6% (t_d=1.6; P<0.1).

The amount of wax accumulated during this period in the nest was also, positively influenced by the nutritional supplement enriched with biomass of microalgae *S. quadricauda*. Thus, bees families in the experimental batch III significantly exceeded the families from the batch after amount of wax accumulated at the first harvesting with 0.04 kg or 13.3% (t_d=2.9; P<0.01) and families from batch II with 0.03 kg or 9.7% (t_d=2.1; P<0.05).

Finally, the amount of honey accumulated in the nest, the morpho-productive character with important economic value, was also the most positively influenced by the biologically active substances contained in supplements enriched with both extract of biomass of *Apispir* + Cr and bioactiv suppliment *Scenecuadri*. Thus after the amount of honey collected at the first harvesting, the bees families from experimental batches II and III vastly exceeded families in the control batch I with 1.03 and 3.01 kg, or 9.6 and 27.6% (t_d=2.0 and 7.2; P<0.05 şi P<0.001). The data presented in table reveals that bees families from the batch III had greatest capacity of accumulation of products in the nest, and after honey production, significantly exceeded the families from the experimental group II, with 1.98 kg or 16.8 %. This difference is veridical with the highest threshold of certainty without error probability forecasts after Stiudent (t_d = 4.5; P<0.001).

More obvious influence of biologically active substances from the extract or biomass of microalgae on the morpho-productive characters of bee families is presented in histogram 1.

In the histogram it can be seen that the characters of accumulation in the nest of bee products such as bee bread, wax and honey had the greatest growth rates. In particular the quantity of honey had rose the most obvious at the bee families who received by food biologically active substances from supplements enriched with biomass of aquatic microalgae.

However, we have to mention that biologically active substances from biomass of microalgae have had a beneficial influence on disease resistance features and viability of the brood.

So the brood viability of bee families of batches II and III, who received supplements enriched, respectively, with extract of biomass Apispir + Cr and biomass Scenecuadri, was significantly higher compared with controls, with 1.3 to 1.2% (t_d=3.1 and 2.2; P<0.01 şi P<0.05). Given the fact that biological variability of this feature is very narrow, the

significance of this difference (small at first sight, as the absolute dimension) is quite high and corresponds

Table 1. The test results of feeding of bees families with nutritional supplements fortified with algal biomass

Batch and name of bioactive substances	Nr. of bee fam.	The value of the character at first harvesting, M ± m	The difference compared to batch I (control)			The difference compared to batch II (the nearest solution)		
			d	%	t_d	d	%	t_d
Prolificacy of queen, eggs/24 hours								
I control	14	1593 ± 25	-	-	-	-25	1.6	0.7
II Apispir+Cr	15	1618 ± 26	+ 25	1.6	0.7	-	-	-
III Scenecuadri	13	1718 ± 25	+ 125**	7.8	3.5	+100**	6.2	2.8
Quantity of capped brood, hundred cells								
I control	14	191.5 ± 3.1	-	-	-	-2.7	1.4	0.6
II Apispir+Cr	15	194.2 ± 3.2	+ 2.7	1.4	0.6	-	-	-
III Scenecuadri	13	206.2 ± 3.0	+ 14.7**	7.7	3.4	+12**	6.2	2.7
Family strength, kg								
I control	14	3.22 ± 0.05	-	-	-	-0.03	0.9	0.5
II Apispir+Cr	15	3.25 ± 0.04	+ 0.03	0.9	0.5	-	-	-
III Scenecuadri	13	3.52 ± 0.07	+ 0.30**	9.3	3.5	+0.27**	8.3	3.4
Quantity of bee bread, hundreds of cells								
I control	14	90.1 ± 2.1	-	-	-	-7.6*	8.4	2.6
II Apispir+Cr	15	97.7 ± 2.1	+ 7.6*	8.4	2.6	-	-	-
III Scenecuadri	13	104.1 ± 3.6	+ 14.0**	15.5	3.4	+6.4	6.6	1.5
Quantity of honey, kg								
I control	14	10.74 ± 0.35	-	-	-	-1.03*	9.6	2.0
II Apispir+Cr	15	11.77 ± 0.37	+ 1.03*	9.6	2.0	-	-	-
III Scenecuadri	13	13.75 ± 0.24	+ 3.01***	27.6	7.2	+1.98***	16.8	4.5
Quantity of wax, kg								
I control	14	0.30 ± 0.01	-	-	-	-0.01	3.3	0.7
II Apispir+Cr	15	0.31 ± 0.01	+ 0.01	3.3	0.7	-	-	-
III Scenecuadri	13	0.34 ± 0.01	+ 0.04**	13.3	2.9	+0.03*	9.7	2.1
Resistance to disease, %								
I control	14	88.9 ± 0.8	-	-	-	-1.5	1.7	1.6
II Apispir+Cr	15	90.4 ± 0.5	+ 1.5	1.7	1.6	-	-	-
III Scenecuadri	13	90.5 ± 0.6	+ 1.6	1.8	1.6	+0.01	0.1	0.01
Broods viability, %								
I control	14	90.0 ± 0.3	-	-	-	-1.1**	1.2	3.1
II Apispir+Cr	15	91.1 ± 0.2	1.1**	1.2	3.1	-	-	-
III Scenecuadri	13	91.1 ± 0.4	1.1*	1.2	2.2	0.0	0.0	0.0

Remark: * P<0.05; ** P<0.01; *** P<0.001;

to a high threshold of certainty, according to probability theory predicts without error Student [Плохинский, 1989].

Similarly, bees families of these experimental batches had a tendency to have increased resistance to the diseases. Despite the fact that the biologically features like brood viability and disease resistance are largely determined by heredity (h2 = 0.7-0.8), however, the test data demonstrates that feeding of bees with biologically active substances certainly have contributed to fortify immunity and strength of bee families, as the result - to the increase of their productivity.

Therefore, the technical result of the use of biomass of microalgae in the nutrition of bees consist in stimulate functions of ovogenesis and egg laying at queens, increasing the quantity of capped brood and of the number of hatched worker bees, which in its turn, led to the quantitative increasing of the power of bee families, the harbinger of higher productivity. The result is due to increasing nutrient assimilation and accessibility of biomass, given the fact that the micro S. quadricauda is covered with a thin protective membrane and the biomass is rich in biologically active substances, in particular proteins, carbohydrates, lipids, essential amino acids, micro and macro elements, antioxidants (beta carotene), which have a catalytic role in the metabolism of substances nitrogenous to worker bees, participates in the synthesis of enzymes, improves the qualitative composition of royal jelly and stimulates its secretion by nurses bees, so indirectly influence (by feeding with royal jelly of the queen) on the reproductive system of the queen, intensifiing the ovogenesis and laying eggs.

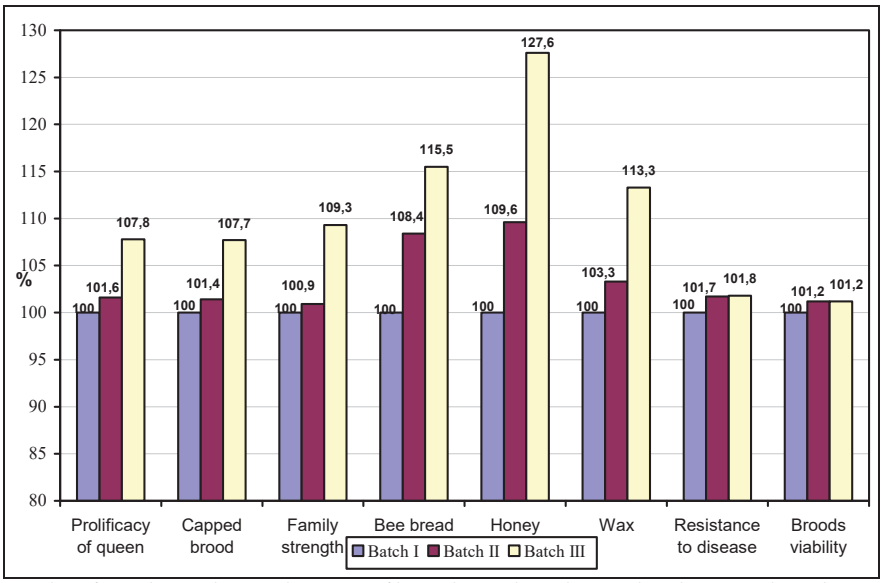

Fig. 1. Value of morpho-productive characters of bee colonies depending on the administered supplement

All of this determines largely prolificacy of the queen, development of larvae and brood in the nest, contributing to increased strength of bee families and their productive potential entirely.

On the base the carried investigations it was elaborated a new proceeding of feeding of bee families in poor harvesting period in nature, at the end of winter - early spring (February-March), when atmospheric temperatures are low. The proceeding provides the enrichment of the nutritional supplements with biologically active substances, which are a little cheaper and easier to obtain, more accessible and more easily digested by bees, ensuring at the same time, the nutritional needs of bees during this period of year. Biologically active substances, added to the food, stimulates prolificacy of bees queen, increase the power and productivity of bee families of *Apis mellifera.*

CONCLUSIONS

1. The feeding of bee families with nutritional supplement enriched with biomass of aquatic microalgae *S. quadricauda* help to increase, compared to the control batch, the queen prolificacy with 7.8% ($P<0.01$), the amount of capped brood with 7.7% ($P<0.01$), family power with 9.3% ($P<0.01$), the amount of bee bread accumulated in nest with 15.5% ($P<0.01$), the amount of wax increased with 13.3% ($P<0.01$), resistance to disease with 1.8% ($P<0.1$), brood viability with 1.2% ($P<0.05$) and the amount of honey in the harvestig with 27.6% ($P<0.001$).

2. On the base the carried investigations it was elaborated a new proceeding of feeding of bee families in poor harvesting period in nature, when atmospheric temperatures are low. This proceeding ensuring the increase of productivity of bee families.

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EFFECTS OF DIETARY ADDITION OF CHLOROGENIC SUBSTANCES ON GROWTH PERFORMANCE, INTESTINAL MICROFLORA POPULATION AND SERUM BIOCHEMISTRY IN BROILERS

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Abstract

The aim of this study was to evaluate dietary effects of chlorogenic substances (Igusafe) on growth performance, intestinal microflora population and serum biochemistry in broilers. A total of 144 Ross 308 one-day-old male broiler chicks were randomly allocated to 3 treatment groups consisting of 8 replications of 6 chicks per pen for 42 days. There treatments were used: chicks were fed by basal diet as control group, basal diet plus 80 mg Igusafe/kg diet, and basal diet and 160 mg Igusafe/kg diet. Results showed that the addition of 80 mg Igusafe to the diet resulted in significantly higher body weight gains and lower aspartate amino transferase (AST) and serum total protein (TP) concentrations compared with control group (P<0.05). Intestinal tract weight was also significantly higher in the group fed 160 mg Igusafe/kg diet (P<0.05). Supplementing both levels of Igusafe significantly reduced Escherichia coli count (P<0.05). There were no treatments effects on carcass yield, liver weight and Lactic acid bacteria population (P>0.05). In conclusion, our results indicated that supplementation of Igusafe may be useful as enhancer of growth performance by reducing Escherichia coli count with a positive trend to decrease the broilers mortality.

Key words*: chlorogenic substances, growth performance, serum biochemistry, microbial population, broiler.*

INTRODUCTION

Many kinds of antibiotics have been used extensively as growth promoters in animal feeds for a large number of years, especially in the fields of poultry production. Antibiotic feed additive bans have forced in investigators to research novel approach for alternative feed additives in poultry production.

Various herbs, plant extracts, spices, essential oils, prebiotics, and probiotics have received great attention being used as feed supplements to improve growth performance. These alternatives are of great interest to the poultry industry. In recent years, several studies have been done to identify the potential beneficial effects of plants or plant extracts and spices or their active compounds in animal nutrition. Various kinds of plant extracts were being used as feed additive to improve growth performance of animals (William and Losa, 2001).

Previous studies suggested that several herb and plants compounds indicated antimicrobial, anti-inflammatory and antioxidant activities (Hernandez et al., 2004; Lawrence, 2005; Khalaji et al., 2011). Beneficial actions of herbal extracts or their active compounds in animal nutrition may include the stimulation of appetite and feed intake, the improvement of endogenous digestive enzyme secretion (Rahimi et al., 2011). In addition Allen et al. (1998) reported beneficial effects in the control of coccidiosis in poultry. However, there is limited published data concerning with using plant extracts as liver protector in poultry. Igusafe has developed with choleretic, cholagogue and antioxidant properties which protect the liver cells reinforcing the liver functions and enhancing performance in the animal.

The aim of the present study was to investigate the effect of dietary supplementation with chlorogenic substances (Igusafe) on growth performance, intestinal

microflora population and serum biochemistry in broilers.

MATERIALS AND METHODS

This trial was performed at the Dicle University, Animal Research Center according to the guidelines for animal experimentation of Dicle University and approved by the Ethical Committee.

A total of 144 Ross 308 one-day-old male broiler chicks were randomly allocated to 3 treatment groups consisting of 8 replications of 6 chicks per pen for 42 days. There treatments were used: chicks were fed by basal diet as control group, basal diet plus 80 mg Igusafe/kg diet, and basal diet and 160 mg Igusafe/kg diet. Two diets were formulated according to the NRC (1994) recommendations to meet the nutrient requirements of broilers from d 1 to 21 (grower diet) and from d 22 to 42 (finishing diet). The composition of the basal diets is presented in Table 1. Feed and water were provided *ad libitum* throughout the experiment.

Table 1. Composition of experimental diets (%)

Ingredients	Starter (1-22 day)	Finisher (23-42 day)
Maize	57.4	59.5
Soybean meal (46 % CP)	28.3	28.9
Full fat soybean	6.8	4.0
Fish meal (60 % CP)	4.7	-
Sunflower oil	-	4.7
Dicalciumphosphate[a]	1.7	2.1
NaCl	0.35	0.40
Vitamin premix[b]	0.10	0.10
Mineral premix[c]	0.15	0.15
Lysine	0.26	0.18
Calculated composition		
Crude Protein	23.0	20.0
ME (kcal/kg)	3010	3240
Calcium	1.0	0.91
Available phosphorus	0.5	0.47
L-lysine	1.3	1.02
Methionine+cystine	1.1	0.62

[a] Contains 240 g Ca and 17.5 g P/kg;
[b]Provided (per kg of diet): vitamin A, 8,000 IU; vitamin D3, 1,200 IU; vitamin E, 10 IU; vitamin K3, 2 mg; thiamine, 2 mg; riboflavin, 5 mg; pyroxidine, 0.2 mg; vitamin B12, 0.03 mg; pantothenic acid, 10 mg; niacin, 50 mg; biotin, 0.1 mg; folic acid, 0.5 mg; iron, 80 mg; zinc, 40 mg; manganese, 60 mg; iodine, 0.8 mg; copper, 8 mg; selenium, 0.2 mg; cobalt, 0.4 mg
[c]Provided (per kg of diet): Iron, 80 mg; zinc 40 mg; manganese 60 mg; iodine 0.8 mg; copper, 8 mg; selenium, 0.2 mg; cobalt, 0.4 mg.

The experiment lasted 42 days, including 21 days on the grower diet and from day 22 to 42 on the finishing diet. Igusafeis is a commercial product which has developed by Igusol Advance SA, Spain. It is a combination of a standardized mixture of plant extracts with choleretic, cholagogue and antioxidant properties which protect the liver cells reinforcing the liver functions and enhancing performance in the animal.

The body weight gains of chickens were measured individually and feed intake, and feed conversion rate per cage were recorded weekly from day 7 to 42 (n = 6). Mortality was recorded daily throughout the experimental period.

At the end of the experiment, blood samples (2 mL per bird) were collected from 10 chickens per treatment for serum biochemical determination. Within 1 h, the serum was obtained by centrifugation (2.500 × *g* for 15 min) and stored at −80°C until further analysis. Serum biochemical parameters were measured by using Architech System Reagents and an automatic clinical chemistry analyzer. The concentration of total protein (TP) was measured by following the Biuret method; uric acid (UA) by following the uricase method; cholesterol by following the cholesterol esterase-peroxidase method; respectively; triglyceride by following the glycerol phosphate oxidase method; and the enzymatic activities of alkaline phosphatase (ALP), aspartate aminotransferase (AST) by using the recommended International Federation of Clinical Chemistry and Laboratory Medicine reference methods. After taking blood samples, chickens were euthanized with an intravenous injection of sodium pentobarbital and immediately intestinal tract, liver and spleen were removed and weighed (data expressed as relative organ weight; grams of organ per 100 g of BW). Small intestine was immediately removed and digesta contents (from final part of small intestine) from 40 chickens (10 chickens per treatment) were collected separately, cooled at once used for microbial assays *(Escherichia coli and Lactobacillus).* The data were analyzed by using the ANOVA procedure of SPSS 16.0 (2011). Tukey's multiplerange test was used to detect the differences ($P<0.05$)

RESULTS AND DISCUSSIONS

The body weight gain, feed intake, feed conversion rate and mortality are given in

Table 2 Data from this study showed that supplementation of broiler's diet with Igusafe at the level of 80 mg/kg feed significantly increased body weight compared to the control (P<0.05). Also, the mortality rate of chickens fed diet supplemented Igusafe was lower than control. However, there were no differences (P > 0.05) in feed intake and feed conversion rate between treatment groups. (P>0.05).

Table 2.Effects of dietary inclusion of Igusafe on growth performance of broilers

Treatments	Body Weight Gain (BWG, g)	Feed Intake (FI, g)	Feed conversion rate (FI/BWG)
Control	2395.6[b]	4072.4	1.69
Igusafe (80 mg/kg)	2526.2[a]	4089.4	1.66
Igusafe (160 mg/kg)	2452.0[ab]	3913.1	1.65
SEM	20.66	41.95	0.013
P-Value	0.030	0.170	0.486

Pooled SEM: pooled standard error of the mean
[a,b,] Means within column with different superscripts differ significantly P < 0.05

Mortalities were 3 and 1 for control, and Igusafe (80 mg/kg) respectively. No mortality was seen in the Igusafe 160 mg/kg feed group. Many researchers obtained that various plant extracts as mixed preparations may play role in improving growth performance and health status of broilers (Manzanilla et al., 2001; Alcicek et al., 2004; Rahimi et al., 2011). Our BWG findings are in agreement with those of Brzoska et al. (2010) who investigated the effects of herbs and herbal products in broiler diets and those of Shafey et al. (2013) who used olive leaves extract in broiler diets.Table 3 shows the effects of treatments on serum concentration of cholesterol, creatinine, total proteins, triglyceride and uric acid and activities of ALP and AST. No significant differences were observed among treatments in serum concentration of cholesterol, triglyceride, creatinine uric acid (P<0.05). These results are in agreement with the results of Amouzmehr et al. (2012) that showed plant extracts had no significant effects on these serum parameters in broilers. Liver enzymes activities have been reported to be sensitive indicators of hepatocellular damage (Shi et al., 2006). Our results showed that chickens fed diet with Igusafe had significantly lower AST and higher total protein compared with the control group (P<0.05). It is possible that the antioxidant properties of Igusafe protected hepatocellular.

Table 3. Effects of dietary inclusion of Igusafe on serum biochemistry of broilers

Treatments	ALP (U/L)	AST (U/L)	CHOL (mg/dL)	CRE (mg/dL)	TP (g/dL)	TRG (mg/dL)	UA (mg/dL)
Control	1649.6	320.6[a]	109.3	0.297	3.43[ab]	55.0	6.70
Igusafe (80 mg/kg)	1401.2	275.6[b]	112.2	0.314	3.33[b]	46.1	7.12
Igusafe (160 mg/kg)	1443.6	258.9[b]	119.2	0.306	3.78[a]	49.7	7.05
SEM	57.41	9.22	2.44	0.004	0.07	2.20	0.24
P-Value	0.170	0.013	0.242	0.380	0.038	0.260	0.760

Pooled SEM: pooled standard error of the mean
[a,b,] Means within column with different superscripts differ significantly P < 0.05
ALP = alkaline phosphatase; AST = aspartate amino transferase; TP = total protein; UA = uric acid; CRE =creatinine
CHOL = cholesterol; TRG = triglyceride.

The measures for carcass yield, liver weight and intestinal tract weight are presented in Table 4.

No significant differences were detected for carcass yield and liver weight, whereas, intestinal tract weight was significantly higher in chickens fed with Igusafe at the level of 160 mg/kg feed (P < 0.05).

Table 4. Effects of dietary inclusion of Igusafe on internal organs weight and carcass yield in broilers

Treatments	Carcass Yield, (%)	Liver weight, (g/BW, %)	Intestinal tract weight (g/BW, %)
Control	75.9	2.40	4.81[b]
Igusafe (80 mg/kg)	76.2	2.35	4.79[b]
Igusafe (160 mg/kg)	75.6	2.39	5.55[a]
SEM	0.44	0.04	0.12
P-Value	0.877	0.871	0.010

Pooled SEM: pooled standard error of the mean
[a,b,] Means within column with different superscripts differ significantly P < 0.05

Plant extracts are feed additives which beneficially affect the host by improving its intestinal microbial microflora to animal health and nutrition. Beneficially affect can be more evident when animals are challenged by pathogens or chemicals (Yirga, 2015). In earlier studies, significant reductions of *E. Coli* number have been obtained after application of various plant extract (Jamroz et al. 2005; Tiihonen et al. 2010). In the present study, reduction of *E. coli* and increase of *Lactobacillus* spp. were observed in the end of the 42 days old chickens from the Igusafe supplemented groups (Table 5).

Table 5. Effects of dietary inclusion of Igusafe on *E.coli* and *Lactobacillus* numbers in ileal contents of broilers.

Treatments	***E. coli* (log CFU g-1)**	***Lactic acid bacteria* (log CFU g-1)**
Control	5.55[a]	7.78
Igusafe (80 mg/kg)	4.06[b]	7.11
Igusafe(160 mg/kg)	4.11[b]	7.98
SEM	0.152	0.162
P-Value	0.0001	0.064

Pooled SEM: pooled standard error of the mean

[a,b] Means within column with different superscripts differ significantly $P < 0.05$

CONCLUSIONS

Results of this study showed that the addition of Igusafe to broilers diets may be useful as enhancer of growth performance by reducing *Escherichia coli* count with a positive trend to decrease the broilers mortality.

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RESEARCHES REGARDING THE CHEMICAL COMPOSITION AND GROSS ENERGY OF SORGHUM IN COMPARISON TO OTHER FORAGES FOR FEEDING CATTLE AND PIGS

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Abstract

Sorghum is recommended to be grown in drier areas, being able to exploit the salty soils where the cereal growing is more difficult. For an efficient use in the animal organism, the grains of sorghum should be prepared by grinding, being also possible to be used in the compound feed recipes or in the mixtures of concentrates, and the sorghum plants through ensilaging. Some varieties of sorghum contain higher amounts of tannin, which negatively affects the animal performances. The purpose of the current study was to compare the chemical composition of two sorghum hybrids with other feeds for cattle or swine feeding. The recorded data showed that the chemical composition of the two analyzed sorghum hybrids was close, as there were no differences between the samples of grain or of pickled forage. The chemical composition of sorghum grains was close to that of maize, except for the crude protein, which was higher and that of crude fat, which was lower. Sorghum silage showed a higher content in ash and crude fiber, while crude protein, crude fat and N-free extractives were lower compared to maize silage. The calculated values for gross energy of analyzed forages are within the recommended values from the speciality literature.

Key words*: sorghum, grains, ensilaged forage, chemical composition, gross energy.*

INTRODUCTION

Sorghum (*Sorghum bicolor* (L.) *Moench*) is one of the cereal species that achieves a much greater importance, because it may be considered an alternative to maize crops in dry areas as it ensures high productions even in the conditions of high temperatures during summer.

Sorghum has a high genetic variability, existing a multitude of hybrids that can be used in obtaining the necessary forage for the livestock sector and food industry, but also obtaining biofuel (Wrigley et al., 2016).

At an early phase the sorghum plants contain cyanogen glycoside called "durrhina", which through hydrolysis and in contact with the emulsin enzyme from the stomach of animals decompose and forms the hydrogen cyanide (Joshi, 2015). This toxic substance is favored by plant age, drought, low temperature, weeding, excessive fertilizing with nitrogen, irrigation. In order to avoid poisoning of animals, sorghum should not be grazed, and at the stall it is administered after the plants wilt, when the hydrogen cyanide breaks down into non-toxic compounds. Also, the sorghum grain does not contain this substance.

The purpose of the paper is to make a comparison regarding the chemical composition of sorghum and of other feed for cattle or swine feeding in the subsequent purposes of compound feed rations or recipes.

MATERIALS AND METHODS

During the undertaken research samples of sorghum were analyzed, belonging to Euralis ES Alize and Arkanciel hybrids, which were grown in the south of Romania and harvested for both grain and pickled forage. Both hybrids are mid early, very resistant to drought and shaking.

In parallel were analyzed samples of cereal grains (maize, barley, oats, triticale), as well as silage (maize), all the cultures were obtained in the same area.

To determine the raw chemical composition 90 laboratory samples have been analyzed (10 samples for each type of forage).

The preparation of samples for analysis and determination of the chemical composition (Table 1) was conducted according to the standard methods and legislation in force, namely: dry matter (DM) by drying in an oven at 105°C; crude ash by calcination at 550°C; crude protein (CP) through the Kjeldahl method; crude fat (Ether Extract EE) through the Soxhlet method; crude fiber (CF) through the method with intermediate filtration.

Table 1. Experimental scheme

Type of analyzed forage	Number of probes	Followed objectives
ES Alize sorghum hybrid	10	- Chemical composition (Dry Matter, Ash, Crude Protein, Crude Fat, Crude Fiber, Neutral Detergent Fiber, Acid Detergent Fiber, Nitrogen-Free Extract)
Arkanciel sorghum hybrid	10	
Maize	10	
Barley	10	
Oat	10	
Triticale	10	
ES Alize hybrid sorghum silage	10	
Arkanciel hybrid sorghum silage	10	
Maize silage	10	

Because the structure of the components forming the crude fiber varies greatly from one forage to another, having different nutritive effects, nutritionists take into account other categories of cellulose, respectively NDF and ADF.

NDF is the short form for Neutral Detergent Fiber, which determines the total insoluble fibers in feed after treating them with a "neutral detergent." NDF is composed of cellulose, hemicellulose and lignin in forage.

ADF is the abbreviated form for Acid Detergent Fiber, which sets the total insoluble fibers in feed and food, after treating them with an "acid detergent". ADF is composed of cellulose and lignin in forage.

It is believed that ADF refers more to ration digestibility and NDF to the intake of dry matter, respectively the workload of rumen.

NDF and ADF are determined by applying the Van Soest method, using the FOSS Fibertec systems.

Nitrogen-Free Extract was determined by calculation: NFE = DM - (ash + ether extract + crude protein + crude fiber).

Based on the chemical composition of forages, it was computed the amount of gross energy expressed in kcal or kj gross energy, in terms of per kg of forage or kg of dry matter.

Gross energy (GE) refers to the total energy in feed, which is determined by complete oxidation (burning) of the feedstuff and measurement of the heat produced in bomb calorimeter. Common feedstuffs are similar in gross energy content, but differ in feeding value because of the differences in digestibility. Thereby the amount of gross energy is exclusively dependent on the chemical composition of the feed, but it cannot help to predict the energetic transformation efficiency, gross energy as such is meaningless in animal production, because it does not take into account any losses of energy during ingestion, digestion and metabolism of feed.

Gross energy of various organic substances is different, the values were: 4.2 kcal/g for carbohydrates; 5.7 kcal/g for protein; 9.5 kcal/g for lipids.

Taking as standard these values, there have been proposed several ways of calculating GE of feed based on their chemical composition. The researchers from the Institute O. Kellner of Rostock formulated the following relationship calculation, which was adopted by INRA in France (Stoica, 2001).

GE (kcal/kg) = 5.72 x CP + 9.5 x EE + 4.79 x CF + 4.17 x NFE,

where CP, EE, CF, NFE (g/kg) represents protein, ether extract (fat), fiber and nitrogen free extract resulted from the chemical analyzes.

RESULTS AND DISCUSSIONS

The chemical composition of sorghum grains, compared to the cereal grains, is presented in Table 2, and in Table 3 the obtained values are expressed as percentage of dry matter.

From the presented data it is seen that the two sorghum hybrids showed similar values in terms of content in the main raw nutrients. The crude protein content ranged between 10.25 and 10.75%, which is superior to the maize grains (8.75).

Crude fat of sorghum hybrids fat was located at an average value between the analyzed cereals, being of 2.52-2.88%.

Brute cellulose registered values close to those of maize grains (2.49-2.74%). Also, the content

of ADF and NDF was relatively similar to that of maize (3.33% ADF and 9.05% NDF for the ES Alize sorghum hybrid, 3.20% ADF and 8.88% NDF for the Arkanciel sorghum hybrid compared to 3.11% ADF and 8.75 % NDF for maize).

Considering the obtained values it can be appreciated that the sorghum grain can substitute maize in compound feed recipes, being mostly used as a cereal grain energy source and is a good feedstuff for poultry, pigs and ruminants.

Similar values were obtained by Heuze et al. (2015), who estimates that crude protein content in grain sorghum ranges from 9 to 13% DM and is slightly higher than that of maize, though much more variable depending on growing conditions. Also, similar results were obtained by other researchers (Stoica, 2001; Pop et al., 2006; Dragotoiu et al., 2014).

To assess the possible use of sorghum at ruminants harvesting of sorghum was done to achieve the ensilaged forage in the wax phase of grains.

The chemical composition of sorghum silage compared with that of corn silage is presented in Table 4.

Table 2. The chemical composition of sorghum grains and cereal grains (%)

Forage type	Dry matter	Ash	CP	EE	CF	ADF	NDF	NFE
Sorghum								
Hybrid ES Alize	85.44 ±10.21	2.18 ±0.09	10.75±1.27	2.88 ±0.08	2.74 ±0.04	3.33 ±0.09	9.05 ±2.01	66.89 ±7.11
Hybrid Arkanciel	84.35 ±9.11	2.05 ±0.11	10.25±2.64	2.52 ±0.06	2.49 ±0.07	3.20 ±0.12	8.88 ±2.54	67.04 ±5.89
Maize	86.58 ±13.09	1.42 ±0.12	8.75 ±1.76	4.36 ±0.09	2.51 ±0.05	3.11 ±0.07	8.75 ±1.88	69.54 ±6.58
Barley	85.76 ±11.75	2.36 ±0.08	9.15 ±1.85	1.87+0.03	5.02 ±0.10	5.84 ±0.21	14.75 ±2.78	67.36 ±8.11
Triticale	86.36 ±10.54	1.95 ±0.10	11.72±1.64	1.55 ±0.05	3.95 ±0.08	4.22 ±0.18	12.35 ±3.12	67.19 ±7.43
Oat	87.25 ±15.03	2.83 ±0.14	10.19±2.38	3.86 ±0.08	10.42 ±0.12	12.55 ±1.12	24.98 ±2.97	59.95 ±6.94

Table 3. Chemical composition of cereal grains (% of dry matter)

Forage type	Ash	CP	EE	CF	NFE
Sorghum					
Hybrid ES Alize	2.55	12.58	3.37	3.21	78.29
Hybrid Arkanciel	2.43	12.15	2.99	2.95	79.48
Corn	1.64	10.10	5.03	2.90	80.33
Barley	2.75	10.67	2.18	5.85	78.55
Triticale	2.26	13.57	1.79	4.57	77.81
Oat	3.24	11.68	4.42	11.94	68.72

Table 4. Chemical composition of sorghum silage and maize silage (%)

Forage type	Dry matter	Ash	CP	EE	CF	NES
Sorghum silage						
Hybrid ES Alize	28.11 ±3.45	2.07 ±0.06	2.55 ±0.05	0.75 ±0.002	8.57 ±0.65	14.17 ±0.09
Hybrid Arkanciel	29.56 ±3.85	2.22 ±0.05	2.46 ±0.04	0.85 ±0.001	8.83 ±0.54	15.20 ±0.11
Maize silage	35.35 ±2.28	1.98 ±0.06	3.62 ±0.06	1.52 ±0.002	7.15 ±0.62	21.08 ±0.08
% of dry matter						
Sorghum silage						
Hybrid ES Alize	100	7.36	9.07	2.67	30.49	50.41
Hybrid Arkanciel	100	7.51	8.32	2.87	29.87	51.43
Maize silage	100	5.60	10.24	4.30	20.23	59.63

It is observed that sorghum silage is characterized by a higher content in ash and crude fiber compared to maize silage (7.36% vs. 5.60% ash of the dry matter, respectively 30.49% compared to 20.23% crude fiber of dry matter), while the content in crude protein was higher (9.07% vs. 10.24% crude protein of dry matter).

Similar results were obtained by Podkowka (2011) who investigated the sweet sorghum (*Sorghum saccharatum*) silage, corn (Zea mays) silage, and sorghum and corn (1: 1) silage and observed that in sorghum silage, the concentration of crude ash and crude fiber was higher, and that of crude protein, crude fat and N-free extractives were lower compared to the maize silage.

Oliveira et al. (2013) analyzed the varieties of sorghum (*Sorghum bicolor* L. *Moench*) with low and high tannin content, that were ensilaged, having a moisture content of 29.32 and 30.73%.

The values for gross energy of analyzed forages are presented in Table 5. The calculated values are within the recommended values from the speciality literature (Jarrige et al., 1988; Stoica, 2001).

Table 5. Gross energy of analyzed forages

Forage type	GE (kcal/kg forage)	GE (kcal/kg DM)	GE (MJ/kg forage)	GE (MJ/kg DM)
Grains				
Sorghum:				
Hybrid ES Alize	3790	4435	15.85	18.55
Hybrid Arkanciel	3740	4434	15.65	18.54
Corn	3930	4539	16.44	18.99
Barley	3740	4361	15.64	18.24
Triticale	3810	4412	15.94	18.45
Oat	3950	4527	16.52	18.94
Silage forages				
Sorghum silage:				
Hybrid ES Alize	1210	4303	5.06	18.00
Hybrid Arkanciel	1270	4296	5.31	17.97
Maize silage	1560	4413	6.52	18.46

CONCLUSIONS

The chemical composition of the two analyzed sorghum hybrids was relatively similar, as there are no significant differences, both in the case of grains and that of ensilaged forage.

The chemical composition of sorghum grains is roughly similar to that of maize, but it is particularly rich in crude protein.

Fat content of sorghum grains is slightly lower than in maize, so its utilization can request a addition of vegetal oils or animal fat in the compound feed recipes.

Sorghum silage presented a higher content in ash and crude fibre, while crude protein, crude fat and N-free extractives were lower compared to maize silage.

The calculated values for gross energy of analyzed forages are within the recommended values from the speciality literature.

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THE UTILIZATION OF ENZYMES IN NON RUMINANT'S ANIMAL NUTRITION AS A WAY FOR REDUCTION OF SOIL AND WATER POLLUTION BY PHOSPHORUS

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Abstract
Albania continues the reforms and developed good institutional and regulatory capacities for managing environmental issues. The roles of the public and private sectors need to be considered according the pollution intensity (solid pollution, the potential collapse of water, water contamination from agricultural or industrial pollutants, energy inefficiency, and threats to natural resources. Groundwater contamination by nitrate-nitrogen and eutrophication of surface waters by phosphorus originating from land application of fertilizers and animal manure are well documented in some most populated areas like, Durres, suburb of Tirana, Lushnja, Fier etc. One of the important reasons of soil and water pollution in Albania is the agricultural techniques and animal manures. Soil and water are compounding parts of it are before the risks of pollution presenting problems for the change of environmental equilibrium. Nevertheless the agricultural is always in front of difficulties from the influence of agronomic techniques and the animal rests. Excessive animal manure and fertilizer inputs do cause various environmental problems, related to the accumulation and elevated leaching, runoff of nutrients (N and P) and heavy metal to ground water and surface water. This is particularly true in areas where animal production has been geographically concentrated. Interest in phytase for non ruminant animals takes place in regions, where soil and groundwater pollution due to animal wastes is a serious problem and phosphorus is a major concern.

Key words*: livestock development, environment, water and soil pollution, animal manure, enzymes.*

INTRODUCTION

It's important to evidence that Albania is a mountainous country and only 16% of its territory is located at elevations of less than 100 m a.s.l. The agricultural land is distributed as follows: 43.3% in the plan or flat areas, 34% in the hilly zones and the remaining 22.7% in the mountainous region.

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Nonpoint source nutrient pollution of ground water and surface water by agriculture is a major, longstanding environmental issue in the United States (Sims, 2005). As comprehensive nutrient management planning has become more widespread in the U.S., it has become increasingly apparent that the primary causative factor for nonpoint nutrient pollution in many regions is nutrient imbalance. Nutrient surpluses, usually due to imports of feed and fertilizer far in excess of exports in crops and animal products, lead to the buildup on nutrients in soils and increase the likelihood of poorly timed applications of manures.

It is now widely accepted that a fundamental tenet of agro-environmental policy must be restoring nutrient balance on farms, especially those referred to as ''Concentrated Animal Feeding Operations" (CAFO-s). To achieve nutrient balance on farms or in watersheds

requires a number of political, social, economic and logistic challenges (Sims, 2005).

Case Study-Durres

Durrës is one of the biggest cities in Albania. Also, there are the most concentrated poultry, pig farms and industries in this area. There are nearly: 1 million broiler chickens, 16.000 cattle and swine industry. Population in this area is nearly 200.000 habitants and arable surface: 16.000 ha.

Table 1. The average quantity of organic fertilizer belongs to the species in Albania (Piu and Locher 2001)

Species	Live weight (kg)	The quantity (ton)/year
Cow	300 400 500	7 8 10
Sow with piglets in maternity	200	2
Pigs	100	1.2
Piglets	30	0.9
Chicken	2	0.07
Sheep	40	0.6
Goat	30	0.4
Horse	500	6

Table 2. N and P amended in the cultivated soil with manure (Sulce and Veizaj, 2006)

	Number of livestock	Annual manure production (in 000/tons)	N (tons)	P (tons)
Cattle	32 000	170 (35% moisture)	1300	380
Chicken	977 000	70 (60% moisture)	1200	300
Pigs	4 000	4 (45% moisture)	150	25
Sheeps/goats	40 000	20	600	100
Total			3250	805

Table 3. The composition of organic fertilizer in Kg/ton, (Piu and Locher 2001)

	N	P_2O_5	K_2O	Mg
Organic manure				
Cow manure	2	3		
Horse manure	2.4	3	6.3	1
Sheep manure	3.2	3.3	8	0.8
Poultry manure	10	28	16	4
Pig manure	3.3	3.2	2.3	0.6
Liquid manure	6	5	5	3

Animal nutrition and environmental problems

As a result of growing concern about the environment, intensification of animal production in many European countries is considered as potential source of air pollution and threat to soil and drinking water quality, (Eeckhout and De. Paepe, 1994).

Cereals composition

Nutritive ration of non ruminant animals (pigs and poultry) contains 90% cereals seed (corn, wheat, barley, rye, and oat). The majority of "P" in cereal grains is organically bound as phytic acid or phytate. This form of P is nutritionally unavailable to non-ruminant animals due to the lack of phytase in their digestive tract. Indeed, plant phytate is the major form of plant phosphorus and phytate phosphorus itself has low availability (Kirby and Nelson, 1988).

The P-excretion on the pigs and poultry faeces is potential source for soil and water pollution, due to his high level on the subsoil water and destruction of the ecosystem. In such situation the solution is utilization of phytase on pigs and poultry nutritive rations.

MATERIALS AND METHODS

Thirty two piglets (Large White x Landrace) of four litters were transferred after weaning to flat-decks and allocated to 2 groups (A and B) with 16 animals, respectively. Two piglets from different litters (1 male and 1 female), with the same body weight were housed in every box (experimental unit). The control group (A) was feed with a balanced diet, containing mono calcium phosphate. The experimental group (B) was feed with low level of P, without inorganic phosphorus. All the phosphorus in this group originates from soybean meal. This group was supplemented with NATUPHOS phytase 750 FTU/kg feed. The diets were offered *ad libitum* and animals had free access to water.

RESULTS AND DISCUSSIONS

The supplementation of microbial phytase preparation (*Aspergillus niger*, NATUPHOS) 750 FTU/kg feed was reduced the P-excretion. The P-excretion was reduced by 20-25%, provided that pig's diets can be supplemented

with an economical and efficacious level of phytase that will allow all of the supplemental inorganic P to be removed from the diet (Cromwell and Coffey, 1991).

Table 4. Efficacy of supplemented phytase in low phosphorus diet for piglets.

Parameters		Control group	Experimental group
Production	n[1]	X±SE	X±SE
Initial BW,kg	16	12.2 ± 0.90	12.6 ± 0.48
Final BW,kg	16	23.2 ± 1.06	24.5 ± 0.96
DWG,g	16	369 ± 10.0	396 ± 7.33
FCR	16	2.44 ± 0.11	2.43 ± 0.10

With the industrial production of phytase, application of this enzyme to pig's diet to increase P availability and improve animal performance, as well as reducing environmental pollution has gained widespread attention. The beneficial effects of supplementary phytases on P digestibility and animal performance have been well documented (Rao et al., 1999, Ravindran et al., 1999).

The efficacy of any enzyme preparation depends not only on the type, inclusion rate and level of activity present, but also on the ability of the enzyme to maintain its activity in the different conditions encountered through the gastrointestinal tract and the conditions used for the pre-treatment of a feedstuff or diet.

CONCLUSIONS

Agricultural techniques and animal rest is a serious problem for soil and water pollution, especially in the areas with a big concentration of livestock farms. An original solution is the utilization of microbial phytase on the nutritive rations of non ruminant animals. Nowadays, phytase supplementation is considered as a good way to reduce phosphorus excretion in non ruminant animals.

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FATTENING PERFORMANCE AND CARCASS CHARACTERISTICS OF LAMBS FED DIETS WITH DIFFERENT SHARES OF NON-DEGRADABLE PROTEIN

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Abstract

The effects of the level of non-degradable protein in diet on fattening performance and carcass characteristics of intensively fattened lambs are presented in the paper.The aim of this study was to determine the optimal level of non-degradable protein in the diet of weaned lambs fattened intensively.The experiment was conducted on 60 lambs of MIS population weaned at 60 days of age, divided into three groups.The effect of the use of three concentrated mixtures which differed in terms of the share of non-degradable protein at the rumen level was studied: 43% (I), 51% (II) and 58% (III).The average daily gain of lambs included in the treatments I : II : III was 0.169 : 0.205 : 0.227 kg, respectively. Conversion of dry matter (kg/kggain) on analogue treatments was 4.54 : 3.71 : 3.30; energy (MJ NEM/kg): 33.77 : 29.37 : 26.25; total protein (g/kg): 732 : 596 : 549. Values of yield of warm carcass with offal, observed per treatments I : II : III were as follows: 58.70 : 58.02 : 57.42%. Meat yield of category I (thigh, loin) on analogue treatments amounted to 37.27 : 37.35 : 37.51%, category II (back, shoulder, neck) 33.9 : 32.67 : 32.83% and category III (chest with a forearm and shanks): 27.78 : 29.59 : 29.10%. The ratio of muscle and bone tissue in the treatments with 48 : 51 : 58% non-degradable protein was 2.5:1; 2.9:1; and 2.8:1.This research has shown that the best fattening performance was achieved by lambs on treatment with 58% RUP while meat yield and morphological composition of the carcass side were not under significant influence of the studied treatment.

Key words*: lambs, non-degradable protein, gain, carcass characteristics.*

INTRODUCTION

Protein in foods that reach the small intestine of ruminants represent sum of two protein fractions: microbial protein and protein non-degradableat the rumen level (Ma et al., 2015;Rezaei et al., 2014; Ma et al., 2013; Zuo et al., 2011;Ramos et al., 2009; Webster et al., 2003). Microorganisms in the reticulo-rumen break down dietary proteins to peptides, amino acids and ammonia, and subsequently use these substances for the synthesis of own proteins. With each of these processes of decomposition and synthesis certain losses occur which ultimately means that reduced amount of amino reaches the place of digestion and adoption of proteins (Grubić et al.,1992). If the extent of degradation is larger proportionally lower is the amount of amino acids that directly remains for the animal (Grubić et al.,1992). The specific role of amino acids absorbed from the small intestine of lambs is protein synthesis, which contributes to increase of physical tissues. In order to ensure the optimal amount of amino acids for a particular production, it is necessary to provide in the diet for lambs protein fraction, which avoids degradation in reticulo-rumen (non-degradable protein). This is especially important in intensive fattening of lambs, since with the increasing genetic capacity of lambs also their requirements increase, especially in this part of the protein that passes through the rumen undegraded and together with the microbial protein reaches the duodenum (Ružić-Muslić, 2006).

MATERIALS AND METHODS

The trial included 60 lambs of MIS population weanedat the age of 60 days, divided in 3 homogeneous groups. The average body mass of lambs at the beginning was about 18.0 kg, and at the end of trial around 35.0 kg. Lambs were fed fodder mixtures,in a group and ad libitum, while the amount of hay was limited and equally distributed to animals. Three isoprotein feed mixtures were studied which differed in terms of the share of non-degradable protein in the reticulo-rumen: 43: 51: 58%. Nutrient calculation was performed based on

the French system recommended by INRA (1988) and Obračević (1990). The structure of the feed mixtures used is shown in Table 1, and their nutritional value in Table 2.

Changes in body weight, the total gain, the average daily gain, feed conversion and nutrients, are controlled at 15 day intervals. At the end of the trial, to determine the yield and quality of the meat, 18 animals were randomly selected (6 per each treatment).

Table 1. Structure of concentrate mixtures for fattening of weaned lambs (%)

Feeds	Levels of non-degradable protein(%)		
	43	**51**	**58**
Corn	73	79	82
Sunflower meal	23	5	7
Soybean meal	0	12	0
Fish meal	0	0	7
Livestock lime	2	2	2
Salt	1	1	1
Premix	1	1	1

Table 2. Nutritional value of mixtures

Nutritional indices	Levels of non-degradable protein (%)		
	43	**51**	**58**
Dry matter, g kg-1(*)	870	860.5	860.8
OFU(*)	1.2	1.2	1.2
NEM,MJ(*)	7.51	7.98	7.91
UFV(**)	0.99	1.05	1.04
Total protein,g/kg(*)	142	137	141
RUP	**43**	**51**	**58**
PDIN/g/animal/day(**)	102	103	107
PDIE/g/animal/day(**)	102	112	118
Ashes(*)	25	23	27
Ca,g(*)	8.4	8.2	10.6
P,g(*)	4.6	3.7	5.0

RUP- rumen non-degradable protein; PDIN - protein digested in small intestine depending on the fermenting nitrogen; PDIE - protein digested in small intestine depending on the fermenting organic matter.
**INRA (1988)*Obračevic (1990)

The following relevant parameters were recorded at slaughtering and usual primary processing, carried out in in the experimental slaughterhouse of the Institute for Animal Husbandry: pre-slaughter mass of the animals, the mass of warm carcass with head and edible by-products.

The carcasses were identified/marked using numbers that were placed on the carcasses during slaughtering procedure. After cooling for 24 hours at a temperature of 0 to + 4^0C, the weight of cold carcass with head and offal was determined as well as net mass of cooled carcass without offal and subsequently carcasses were cut along the spinal line into two symmetrical carcass sides.

The meat yield was calculated based on the ratio of pre-slaughter body weight of lambs and mass of warm carcasses with head and offal and net mass of cooled carcasses without offal. Then, the left carcass sides were cut into the major carcass parts and their weights recorded by the method stipulated in the Rulebook on cutting and categorization of mutton ("Official Gazette of SFRY" no. 34 of 1974).

The meat of category I includes the thigh and sirloin. Back, shoulder and neck are category II meat, and chest with a forearm and shanks are categorized as category III meat. The tissue ratio of the carcass side (morphological composition) was studied using the three rib cut (9th, 10th and 11th rib), by way of dissection and measurement of specific muscle, fat, bone and connective/binding tissue.

Statistical analysis of the data was performed using the computer program Stat.Soft, Inc. (2003). STATISTICA (data analysis software system), version 6, using standard mathematical - statistical methods.

RESULTS AND DISCUSSIONS

Data on average fattening parameters by dietary treatments are shown in Table 3.The highest daily gain (0.227kg) and the lowest conversion of dry matter (3.30 kg) were realized by lambs on treatment with 58% RUP in the mixture. Results similar to ours, in regard to the effects of non-degradable protein source are reported by (Orskov et al., 1971; Miller, 1978; Grubić et al.,1991; Walz et al., 1998; Peter et al., 2000; Memiši et al., 2002; Grubić et al., 1991).

In the analysis of the relationship between average daily gains and protein values of the diet expressed by the total digestible and degradable proteinsin the diet, we have found the highest correlation coefficient (r = 0.76) between the daily gain and the share of non-degradable protein in the diet, slightly lower between gain and crude proteins (r = 0.72) and the lowest between gain and digestible proteins (r = 0.68).

Table 3.Production performance of fattening lambs

Traits	Levels of non-degradable protein (%)		
	43	51	58
Initial body mass (beginning of the trial), kg	18.12	18.08	18.17
Age of lambs at the beginning of the trial, days	60	60	60
Final body mass (end of the trial), kg	30.78a	33.52b	35.17b
Total gain, kg	12.70a	15.40b	17.00b
Average daily gain, kg	0.169a	0.205b	0.227b
Utilization of dry matter r kg/kg of gain	4.54	3.71	3.30
Utilization of total proteins g/ kg of gain	732	596	549
Utilization of NEM, MJ/kg of gain	33.77	29.37	26.25

Difference between a and b is statistically significant at the level (P<0.01)

With the increase of the share of non-degradable in total proteins, there was a reduction in energy consumption by 4.4-7.5 MJ NEM for each kilogram of gain. Data on feed conversion and conversion of nutrients which are obtained in these studies, are consistent with the results of other authors (Kozarovski, 1988; Grubić et al., 1992; Mekić, 1994; Grubić et al., 1991) in their examination of the effect of concentrate mixture with a share of NP in total proteins of 38: 55: 62%, have recorded conversion of DM on analogue treatments of 3.75: 3.44: 3.30 kg. In fattening of Ile de France genotype lambs reared to 90 days of age with concentrate mixtures containing different shares of NP in total proteins (40: 50: 60%), Mekić (1994) has found that with increasing levels of RUP the consumption of concentrates decreased: 2512: 2493: 2357 g per kilogram of gain of lambs.

Table 4. Average values of carcass mass and yield

Traits	Levels of non-degradable protein (%)		
	43	51	58
Lamb pre-slaughter mass, kg	30.78±4.73	33.52±4.99	35.17±5.34
Warm carcass mass with offal, kg	18.07±2.86	19.45±3.02	20.19±3.31
Warm carcass yield with offal,%	58.70±1.14	58.02±1.65	57.42±1.47
Cold carcass yield with offal, kg	56.49±1.37	55.97±1.78	55.38±1.22
Cold carcass mass without offal, kg	15.44±2.22	17.21±2.40	17.36±2.57
Cold carcass yield without offal, kg	46.45±1.25	46.14±1.72	45.42±1.27

The established differences in meat yield values ranged within the limits of random deviation (P>0.05), which means that different levels of RUP in diets for lambs had no significant effect on the observed trait (Table 4).

The obtained results are consistent with results of Mekić et al.(1999)who examined the effects of different shares of non-degradable protein (41: 50: 60%) in the total mass of diet on fattening and slaughter performance of lambs of genotype Ile de France until 88 days of age, and found that different levels of RUP protein have no significant impact on the meat yield value of warm carcass with offal, since the values obtained were: 54.16: 56.54: 57.36%. Also, Shahrbabak et al. (2009) suggest that different levels of non-degradable protein: 19.86: 26.47: 33.08 g/kg DM in diets for fattening Kermani lambs, did not affect the yield, weight of edible parts and the surface of the MLD. Al Jassim et al. (1991) point out that the effect of non-degradable protein in the rumen was greater in lambs compared to goats,especially in regard to the efficiency of feed utilization but not on the carcass quality.

Table 5. Yield of meat of different categories

Indicators	Levels of non-degradable protein (%)		
	43	51	58
Left carcass side, kg	7.69±1.12	8.63±1.27	8.65±1.28
I category meat, %	37.27±2.68	37.35±1.35	37.51±2.11
II category meat,%	33.19±2.21	32.67±1.68	32.83±1.36
III category meat,%	27.78±2.71	29.59±2.53	29.10±2.35

According to the data presented in Table 5, the share of I category meat (leg and loin) in the mass of the left carcass side in lambs on treatments 43: 51: 58% was as follows: 37.27: 37.35: 37.51%.

Meat of category II (back, shoulder, neck) were represented as follows: 33.19: 32.67: 32.83%. The relative share of meat of category III (breast with the forearm, shank) was: 27.78: 29.59: 29.10%. Thus, the studied nutritive treatment did not affect the mass of carcass sides and shares of different categories of meat, as realized differences were not statistically significant (P>0.05).This is consistent with the statements of a number of authors (Butterfield, 1988 quoted by Petrović, 2000; Shahrbabak et al., 2009), who point out that the variability in the quality of the meat mostly depends on the genotype and age of animals at slaughter, and less on the diet.

Table 6. Tissue ratio in three rib cut

Properties	Levels of non-degradable proteins,%		
	43	51	58
Mass of three rib cut, g	245.67±4.39	265.50±5.01	275.53±5.28
Ratio of certain tissues,%			
Muscle	43.52±4.61	42.27±1.80	41.92±3.12
Fat	26.68±6.68	31.76±3.07	30.68±4.42
Bone	28.23±6.33	25.06±3.14	25.93±5.60
Connective	1.19±0.64	0.93±0.52	1.01±0.53
Meat bone ratio (muscle and fat tissue together)	2.5 : 1	2.9 : 1	2.8 : 1

Morphological composition of the carcass sidewas determined by establishing the ratio of tissues in three rib cut, the values are presented in Table 6. The results of the relative share of certain tissues in three rib cut show that the levels of non-degradable protein in feed mixtures for lambs did not have a statistically significant impact on the morphological composition of carcass sides (P>0.05). However, the most favorable meat to bone ratio was recorded in the treatments with 51% and 58% RUP. Lambs on treatment with 43% RUP achieved 0.4 kg less meat per kg of bones than lambs on treatment with 51% NP and 0.3 kg less than lambs on treatment with 58%.

The confirmation of these results is found in studies ofŠokarovski et al. (1988), Tahirović and Mašnić (1979). The obtained results relating to the characteristics of the carcass and the share of certain tissues (muscle, fat, bone and connective tissue) showed that they were not influenced by dietary treatment, which is consistent with the results of Atti et al. (2007). The explanation lies in the fact that the lambs had similar weights of the empty carcasses and composition of carcasses, as they were slaughtered with similar finalbody weights. these parameters mainly depend on pre-slaughter body weight (Colomer-Rocher and Espejo, 1972; Atti et al., 2003).

CONCLUSIONS

Based on results obtained in the present study of the effect of different levels of non-degradable protein in the diet on fattening parameters and characteristics of the carcass of intensively fattened lambs, the following can be concluded:

The highest average daily gain (0.227 kg) was realized by lambs fed diet containing 58% RUP.The best DM conversion (3.30 kg/kg gain) was realized by lambs on treatment with 58% RUP.

The values for yield of warm carcass with offal, in the treatments 43: 51: 58% were as follows: 58.70: 58.02: 57.42%.

The most favorable meat to bone tissue ratio was recorded in the treatments with 51 and 58% of non-degradable protein, without statistical significance. The level of non-degradable protein in diets for weaned lambs had no significant effect on theyield of meat categories.

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THE EFFECT OF CHITOSAN IN THE RATION ON TEGAL DUCK PERFORMANCE

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Abstract

The aim of the study is to examine the effects of chitosan in the ration on Tegal duck performance. The dose of chitosan used ranging from 0.0%, 0.5%, 2% and 2.5%, mixed into the basal ration. The basal diet used iso-protein and iso-energy, with protein content of 15.34% and Metabolic Energy 2809 kcal / kg (NRC, 1994). Parameters measured were feed intake, duck day production (DDP), total egg weight and feed conversion. This study uses a completely randomized design (CRD) consisting of 4 treatments and 5 replicates and each replicate consisted of two ducks. The basal diet (R0) = without chitosan as a control, R1 = 0.5% chitosan, R2 = 2% chitosan, and R3 = 2.5% chitosan. Data were analyzed using SPSS 16 statistical program (Statistical Package for Social Science). Results indicated that chitosan showed no significant effect (P> 0.05) on feed consumption, duck day production, total egg weight and feed conversion. In this study, treatment of chitosan 2.5% (R2) gives the best results on day duck production, total egg weight and feed conversion. From the daily egg production (DDP), treatment R2 has a result of 59%, larger than R0 (57.76%), R1 (44.1%) and R3 (46.9%). Total egg weight for R2 = 3597.7 (73.42 g / grain) also show a greater number than the treatment R0 (2769.72), R1 (2662.46), and R3 (3403.14). On feed conversion, R2 showed the smallest (1.93) compared to R0 (3.06), R1 (7.4) and R3 (2.31). This means duck in treatment R2 more efficient of feed consumption, 1.93 kilograms to produce one kilogram of eggs.

Key words*: chitosan, rations, performance, Tegal ducks.*

INTRODUCTION

Performance of duck production is highly dependent on the farm management such as seed, feed and disease prevention. Some of the advantages of duck eggs by reference are as follows:

1. Duck egg was spot used as an option to meet community nutrition. This is due to the nutrient content of duck eggs are very complete and easy to digest. Total protein content of duck eggs is 13.10% (Winarno and Koswara, 2002);
2. Economically, the selling price of duck eggs is more expensive than chicken eggs, so it is an alternative for farmers' additional income;
3. The advantages of duck are more resistant to disease and more tolerant of crude fiber, making it easier to choose the raw material feed.

Productivity of duck is determined by the farm management, especially feeding factors. Feed should contain nutrition according to the needs of duck, especially for basic living needs and production. Feed can also be added with a feed supplement or feed additives to improve livestock productivity.

Chitosan is poly-glicosamin, an animal fiber origin of crustaceans which are very abundant in nature. Chitosan has the characteristics of an anti-germ, antioxidants, enzymes mobilization and fat binder. If fed to livestock as feed additive, it is predicted to be able to launch the body's metabolism.

Anti-bacterial characteristic of chitosan when mixed in the ration will protect feed from pathogenic bacteria contamination; inhibit the growth of pathogenic bacteria in the gastrointestinal tract of duck. Therefore, it would increase a large number of good bacteria to optimize digestive metabolism. Optimizing metabolism of digestive enzymes would optimize the absorption of nutrient in the small intestine.

The result of this study is expected to improve the performance of the duck, so increase the productivity.

MATERIALS AND METHODS

The material used in this study was 40 Tegal ducks of production period (aged 10 months) were kept in a cage colony (2 ducks per unit). Size per unit is 1 x 1 meter^2, with a total of 20 units equipped with feed and drink. Cages are also equipped with a lamp as lighting at night. Rations are prepared with 15.34% protein and 2809 kcal/ kg metabolizable energy (NRP, 2004). Feed materials are yellow corn, rice bran, soybean fish meal, coconut meal, flour shells and premix. Chitosan is given as treatment with doses of 0%, 0.5%, 2% and 2.5%. Rations are given in the form of pasta, two times daily (morning and afternoon), while drinking water is given *ad libitum.*

The observation was carried out for 7 weeks. Ducks lay eggs per unit enclosure is recorded and weighed each day. The leftovers of the ration to the ducks also collected and weighed once a week. The parameters measured in this study were: feed intake, daily egg production, egg weight and total feed conversion.

Feed consumption during the study (7 weeks) is calculated based on the amount of rations given minus the leftovers ration for a week.

Daily egg production (Duck Day Productions) is calculated based on the number that indicates the average number of eggs entirely on ducks produce at a certain time and stated in percentage (Scott, 1992, cited by Manin, 2003). Total weight of the eggs is obtained from multiplying the number of eggs with egg weights.

Feed conversion is the number which indicates the duck ability to change the sum of rations into the production of one kilogram (kg) of eggs within a certain time unit.

The weight of the egg is the number which indicates the average weight of the eggs produced in a given period expressed in grams per egg. Data were statistically analyzed using SPSS 16, following the pattern completely randomized design (4 treatments, 5 replicates), if the treatment showed significant differences then it is continued with Duncan's multiple range test.

RESULTS AND DISCUSSIONS

Effect of Treatment of Consumption Rations

The average feed consumption of ducks trial during the study (7 weeks) in all treatments ranging from 6.759-7.222 g/duck. If converted, the amount of feed consumed by ducks every week was 965.6 to 1,031.7 grams (Figure 1).

This means that the experiment ducks spend ration amount from 137.9 to 147.39 grams/head/day.

This figure is much lower than Alabio ducks feed consumption which is 215-248 g/head/day. The local ducks feed intake over 20 weeks of age, in one week between 900-1.100 grams (Hardjosworo, 2001).

Chitosan which acts as a feed additive in duck ration is thought to contribute to protect the quality of ration nutrition from extreme environmental influences, so the predicted balance of protein and energy available in the feed is sufficient for life and production of duck.

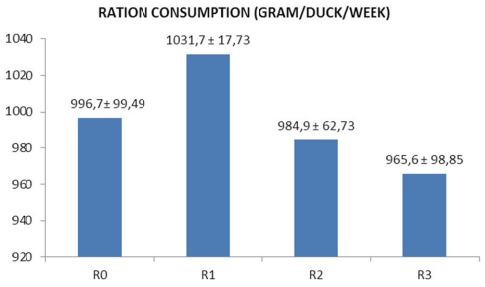

Figure 1. Diagram of Tegal Ducks Feed Consumption

Effect of Treatment of Egg Production (Duck Day Production)

The average of egg production (duck day production) for 7 weeks between 44.1 to 59%.

This figure is slightly lower than the egg production of Alabio duck, which is 58.92 to 64.63% (Setioko, 2001). This is thought because of the difference of age and the ducks species used in the research.

Beside genetic factors and the duck age, the amount and quality of feed will affect the number of eggs produced. If seen from the production percentage of trial duck eggs produced per treatment (Figure 2), the treatment with 2.5% chitosan in the ration, provided a better yield (59%) and a small ration consumption figure (984.9 g/head/week), although statistically did not show significant differences with the other treatments (P> 0.05).

Giving of 2.5% chitosan is thought to provide a good influence in protecting the nutritional quality of feed because of its characteristics as

anti-oxidant, in addition to chitosan role as dietary fiber and prebiotic (gives a positive effect on microflora proliferation which is beneficial in the duck gastrointestinal tract, so that it will facilitate the digestive system.

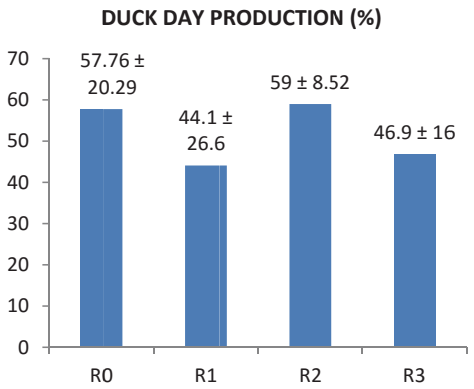

Figure 2. 7 Weeks Tegal Duck Day Production

Effect of Treatment on Mean of Eggs Total Weight

Total weight of the eggs is representing the egg production (egg weights) and obtained by multiplying the number of eggs with egg weights (Figure 3). The average total weight of egg during 7-weeks is 2662.46 to 3597.7 grams. This means that the weight of the egg treatment R0 = 56.52, R1 = 54.34, R2 = 73.42 and R3 = 69.45 grams/egg.

Based on the observation, total weight of the eggs in treatment R2 with 2.5% chitosan shows heavier than other treatments (R0, R1 and R3).The total egg weight of 3597.7 (R2 = 2.5% chitosan) is higher, presumably because this dose chitosan is able to activated the digestive enzymes in the duck gastrointestinal tract; so the nutrient absorption is better, especially the protein and fat; as the egg primary material The egg composition consisted of the albumin with 11% protein content and 0% fat, while the yolk contains 17% protein and 35%.

Effect of Treatment on Feed Conversion

The average of feed conversion was between 1.93 to 7.4. (R0 = 3.06 ± 1.98, R1 = 7.4 ± 11.23, R2 = 1.93 ± 0.16, and R3 = 1.02 ± 2.31). The lowest average of the feed conversion (1.93) is achieved from treatment R2 (2.5% chitosan in the ration). These results indicated that the treatment R2 has the highest efficiency with a reduced ration feed conversion by 6.93% (from 3.06 to 1.93) compared with the control diet (R0 = ration without chitosan).

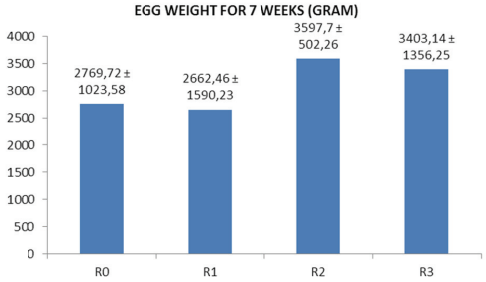

Figure 3.Total Egg Weight of Tegal Duck

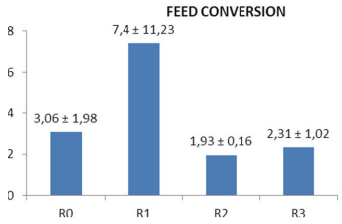

Figure 4.Tegal Ducks Feed Conversion

This result is more efficient than the feed conversion for the Indonesian ducks is 3.2 to 5.0 (Ketaren, 2007). It is further explained that the poor feed conversion in laying ducks in Indonesia is allegedly caused by three factors, namely genetic quality, the number of scattered pellet, and nutritional value of the ration supplied which does not match the needs of the ducks.

In this study it is predicted that, with a dose of 2.5% chitosan, it is able to protect the quality of the nutrient rations from extreme contamination of germs or other environmental influences, so the health of duck is well maintained and able to efficiently utilize the ration.

CONCLUSIONS

The 2.5% chitosan in the ration (R2) is able to provide better production performance (feed consumption 984.9 grams/week, duck day production 59%, egg total weight of 3597.7 grams or 73.42 grams per egg, and feed conversion of 1.93), when compared to other treatments (R0, R1 and R3).

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THE EFFECT OF SOME MICROORGANISMS IN GASTRO-INTESTINAL TRACTS ON THE NUTRITIVE VALUE OF BROILER DIETS

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Abstract

A 2x2 factorial experiment was carried out to determine the effects of two levels of diet supplemented with and without microorganisms in combination with and without sterilized feed on the nutritive value of broiler diets with four replicates in each treatment. Some microorganisms from the gastrointestinal tract of chicken were supplemented in commercial broiler diets. They were bacterial (BC-NA-01), actinomycetes(BI-NA-03, BC-NA-02 and BL-NA-02), Aspergillus niger sp.(BD-PDA-01), Mucor sp.(BL-PDA-02), Rhizopus stolonifer sp.(BI-PDA-02) and Trichoderma sp.(BL-PDA-02). The results of proximate analysis revealed that a diet supplemented with microorganisms had a lower percentage of dry matter and crude fiber in the starter diet(0-3 wks), grower diet(4-5wks) and finisher diet(last period) than the diet without microorganisms ($p<0.05$). They were higher in the percentage of phosphorus in the starter diet and calcium in both the grower diet and finisher diet than the diet without microorganisms ($p<0.05$). The sterilized diet had a higher percentage of moisture than the non-sterilized diet ($p<0.01$). Also, they were higher in the percentage of crude protein and ether extract in the starter diet and crude fiber, calcium in both the grower diet and finisher diet than the non-sterilized diet ($p<0.05$). The diet supplemented with microorganisms and sterilized was higher in crude protein, ether extract, crude fiber, ash and metabolizable energy than the others in the starter diet ($P<0.01$). However, crude protein, and metabolizable energy were not significantly different with the control. Also, the crude protein, ash and metabolizable energy were higher than the others except the control in grower and finisher diet ($p<0.01$).

Key words: *microorganisms, gastro-intestinal tracts, nutritive value, broiler diets.*

INTRODUCTION

The chicken's gastro-intestinal tract contains approximately 40 species of microorganisms with more than three different types. They plays an important role to enhance nutrient absorption and improve growth performance, feed efficiency, and in reducing mortality from enteric pathogens (Larbier and Leclercq, 1994 cited by Wood, 2016). The uses of direct-fed useful microbial to chickens by administering with feed or drinking water is commonly practiced in commercial broiler production. The multispecies of microorganisms are more effective than monospecies (Timmerman et al., 2004). This is due to the interactive effects of anaerobes and facultative anaerobes. There are bacteria, photosynthetic bacteria, actinomycetes, and other types of organism. The microorganisms isolated from the digestive tract of chickens could increase the broiler productivity by 1.84-3.72% based on daily weight gain, feed efficiency, and mortality. The differences in the administration of microorganisms and timing of administration might affect the efficacy of microorganisms. The uses of microorganisms in the drinking water resulted in a lower average daily gain than using via the feed (Timmerman et al., 2005). The colonization patterns of chicks are instable and susceptible to pathogens. Initial colonization is important to the host because of a pioneer bacteria, which are the first to arrive in the gut, are capable of effectively blocking growth of other bacteria introduced later in the ecosystem. These pioneer bacteria also inhibit production of toxins by pathogenic bacteria (Ducluzeau, 1993). The microbial community of the gastrointestinal tract reflects the co evolution of microorganisms with their host and the diet adopted. Changes in the composition of the animal's microflora can have beneficial or detrimental effects on health, growth, and maturation of the animal host. Each region of the gastrointestinal tract developed its own unique bacterial community as the chicken matured (Lu et al.,2003). The objectives of this experiment were to determine the

effect of some microorganisms from the gastro-intestinal tracts of chicken and feed sterilization on the nutritive value of broiler diets.

MATERIALS AND METHODS

Isolation and Identification

The microorganisms were isolated from the broiler chickens' intestinal tracts content which was modified from Gonzalez-Pastor et al. (1994). The contents of duodenum, jejunum, ileum, caeca and large intestine were separated and removed under sterile conditions. Samples from each site were serially diluted in normal saline plated onto a NA (Nutrient Agar) medium for bacteria and actinomycetes and PDA (Potato Dextrose Agar) medium for fungi. They were incubated anaerobically and observed under compound microscope for morphological characteristics.

Enzyme production analysis

The isolated microorganisms were selected for study. They were screened for their ability to produce extracellular degradative enzymes such as amylase, protease, lipase, cellulase, hemicellulase and ligninase. Four replicates of each treatment were assayed and non-inoculated plates with substrates served as negative controls. The chemical indicators were added to assay enzyme activity and activity zones were measured.

Number of microbe determination

The isolated microorganisms which were capable to produces enzymes were selected for study as indicated in Table 1. The pour plate technique was used to determine the number of microbial in a plate. The range for total CFU/plate was between 135 to 160 colonies/plate for bacteria and 160 to 190 colonies/plate for actenomycetes, respectively. The fungal spore/plate was counted by Haemacytometer as follows; $3.1x10^8$ for *Aspergillus niger*, $1.7x10^8$ for *Rhizopus stolonifer*, $2.9x10^8$ for *Trichoderma* sp., and $2.1x10^8$ for *Muco*r sp.

Table 1. The efficiency of microorganism from Gi-tracts of broiler chickens on enzyme production.

Microorganism	Enzyme Production					
	Amylase	Protease	Lipase	Cellulase	Hemicellulase	Ligninase
Bacteria						
BC-NA-01	+		+	+	+	+
Actinomycetes						
BI-NA-03					+	+
BC-NA-02	+			+	+	+
BL-NA-01			+	+	+	+
Fungi						
Aspergillus niger (BD-PDA-01)	+	+				
Mucor sp. (BL-PDA-02)	+			+	+	+
Rhizopus stolonifer (BI-PDA-02)	+				+	
Trichoderma sp. (BL-PDA-02)	+		+	+	+	+

Diet sample preparation

Three rations of commercial broiler diet were used in this experiment. There were 22%, 20%, and 18% of crude protein for starter, grower, and finisher diet, respectively. The selected microorganisms were used at 5-7 days of age. Each plate of all selected fungi, bacteria and actenomycetes were mixed or non-mixed with 500 grams of broiler diets. They were sterilized or non-sterilized at 121°C with 15 psi. for 30 minutes. All diet samples were kept at room temperature for 30 days and their nutrients composition was determined by Proximate analysis.

Statistical analysis

The nutrient composition means served as the experimental unit with four replication, each for statistical analysis. Data were initially analyzed using the GLM procedure of SAS (SAS Institute, 1999) as a Completely Randomized Design (CRD) with factorial arrangements of microorganism supplementation level, sterilization level, and the 2-way interactions between microorganism supplementation and sterilization levels. Significant differences among treatments were determined at $P<0.05$ using Duncan's New Multiple Range Test (DMRT).

RESULTS AND DISCUSSIONS

Effects of microorganism supplementation on nutrients composition

The nutrients composition was varied among treatment in three rations of broiler diets as indicated in Table 2. The microorganism supplementation was decrease crude fiber percentage in all three rations ($p<0.01$). The microorganisms that were used in this experiment were capable of producing enzyme cellulase, hemicellulase, and lignin which they can digest fiber in feedstuff. Furthermore, the microorganism supplementation in the diet was to improve the phosphorus percentage in the starter diet ($p<0.01$). Also it was to improve the calcium percentage both in the grower and finisher diet ($p<0.01$). However, the dry matter percentage was decreased in the starter, grower and finisher diets ($p<0.01$). The crude protein percentage was decreased only in the starter diet ($p<0.01$). Both the percentage of ash and metabolizable energy were not significantly different among treatment in the starter, grower, and finisher diets. The microorganism supplementation could not improve the importance nutrient composition as crude protein, ether extract, nitrogen free extract, and metabolizable energy because these selected microorganism in the experiment have less capability to produces enzymes which can digest protein, fat, carbohydrate, and energy in the diet.

Table 2. Effects of microorganism supplementation on nutrients composition of broiler diets.

Chemical Composition (%)	Starter		Grower		Finisher	
	With microorganism	Without microorganism	With microorganism	Without microorganism	With microorganism	Without microorganism
Moisture	10.75±0.07 [a]	9.42±0.08 [b]	11.27±0.10 [a]	9.66±0.11 [b]	11.50±0.0 [a]	9.91±0.07 [b]
Crude protein	19.42±0.16 [b]	20.25±0.1 [a]	17.51±0.39	18.54±0.46	16.69±0.63	16.98±0.25
Ether extract	5.60±0.22 [b]	6.33±0.16 [a]	7.19±0.18 [a]	7.80±0.20 [b]	6.43±0.26	6.94±0.19
Nitrogen free extract	55.00±0.47	54.33±0.29	56.03±0.42 [a]	55.49±0.58 [b]	57.34±0.75	57.18±0.32
Crude fiber	3.43±0.06 [b]	3.49±0.10 [a]	2.84±0.04 [b]	3.18±0.09 [ab]	2.89±0.07 [b]	3.51±0.04 [a]
Ash	5.75±0.16	6.14±0.10	5.14±0.10	5.32±0.11	5.15±0.06	5.47±0.11
Calcium	0.80±0.02 [b]	0.83±0.02 [a]	0.96±0.02 [a]	0.78±0.01 [b]	1.11±0.03 [a]	1.00±0.02 [b]
Phosphorus(%	0.46±0.02 [a]	0.31±0.02 [b]	0.19±0.02	0.42±0.02	0.30±0.03 [b]	0.32±0.02 [a]
Metabolizable energy (k.cal./kg.)	3,789.12 ±33.50	4,044.18 ±28.88	3,850.19 ±56.97	4,100.69 ±19.10	3,773.39 ±31.78	4,035.37 ±10.14

[a–b]Means within a row with no common superscripts differ highly significance ($P\leq0.01$).

Effects of sterilization on nutrients composition

The sterilized diet was varied in nutrient composition of broiler diets as indicated in Table 3. The crude protein and ether extract percentage was only increased in the starter diet ($p<0.01$). Also, the nitrogen free extract percentage was only increased in the finisher diet ($p<0.01$). The crude fiber and calcium were increased in both the grower and finisher diets ($p<0.01$). However, the dry matter percentage was lower in the starter, grower, and finisher diets ($p<0.01$) due to the moisture increased from the sterilization process. Metabolizable energy, ash, and phosphorus percentage were not significantly different among treatment in the starter, grower, and finisher diets. The crude protein percentage was also not significantly different among treatment in the grower and finisher diets.

Table 3. Effects of sterilization on nutrients composition of broiler diets.

Chemical Composition (%)	Starter		Grower		Finisher	
	Sterilize	Non-sterilize	Sterilize	Non-sterilize	Sterilize	Non-sterilize
Moisture	10.44±0.06 [a]	9.72±0.09 [b]	10.79±0.08 [a]	10.14±0.09 [b]	10.98±0.06 [a]	10.43±0.07 [b]
Crude protein	19.96±0.17 [a]	19.72±0.14 [b]	17.90±0.63	18.14±0.21	16.87±0.42	16.80±0.46
Ether extract	6.14±0.19 [a]	5.79±0.20 [b]	7.34±0.26 [b]	7.65±0.12 [a]	6.71±0.21	6.66±0.24
Nitrogen free extract	54.04±0.48 [b]	55.29±0.28 [a]	55.57±0.71	55.95±0.20	56.87±0.53 [a]	57.65±0.54 [b]
Crude fiber	3.45±0.08	3.47±0.08	3.14±0.06 [a]	2.88±0.08 [b]	3.24±0.03 [a]	3.16±0.07 [b]
Ash	5.92±0.16	5.97±0.09	5.23±0.07	5.23±0.12	5.31±0.09	5.30±0.07
Calcium	0.77±0.02 [b]	0.86±0.02 [a]	0.93±0.01 [a]	0.81±0.02 [b]	1.10±0.03 [a]	1.01±0.02 [b]
Phosphorus	0.39±0.02	0.38±0.02	0.30±0.02	0.30±0.02	0.32±0.03	0.30±0.01
Metabolizable energy(k.cal./kg.)	3,915.84 ±44.93	3,917.46 ±17.45	3,963.04 ±46.17	3,987.84 ±29.90	3,917.28 ±16.13	3,891.48 ±25.80

[a–b]Means within a row with no common superscripts differ highly significance ($P\leq0.01$).

Effects of microorganism supplementation and sterilize on nutrients composition

The interaction between of microorganism supplementation and sterilization on the nutrients composition was varied among treatments in the starter, grower, and finisher diets as indicated in Table 4-6. The microorganism supplementation with sterilization was higher in crude protein, ether extract, crude fiber, ash, and metabolizable energy than other treatments in the starter diet ($p<0.01$). However, crude protein, and metabolizable energy were not significantly different with the control treatment. Although, crude protein, ash and metabolizable energy were higher than other treatment but they were not significantly different with the control treatment in grower and finisher diets.

Table 4. Effects of microorganism supplementation and sterilize on nutrients composition of starter diets.

Chemical Composition (%)	With microorganism		Without microorganism	
	Sterilize	Non-sterilize	Sterilize	Non-sterilize
Moisture	9.63 [c]	10.24 [b]	11.26 [a]	9.21 [d]
Crude protein	20.26 [a]	19.19 [c]	19.66 [b]	20.25 [a]
Ether extract	6.78 [a]	5.71 [bc]	5.50 [c]	5.88 [b]
Nitrogen free extract	53.56 [c]	55.47 [a]	54.53 [b]	55.11 [ab]
Crude fiber	3.58 [a]	3.54 [a]	3.32 [b]	3.40 [b]
Ash	6.16 [a]	5.82 [b]	5.69 [b]	6.12 [a]
Calcium	0.66 [d]	0.71 [c]	0.89 [b]	1.01 [a]
Phosphorus	0.27 [d]	0.41 [b]	0.52 [a]	0.35 [c]
Metabolizable energy (k.cal./kg.)	4,053.49 [a]	3,800.04 [b]	3,778.20 [b]	2,034.88 [a]

[a–d]Means within a row with no common superscripts differ highly significance ($P\leq0.01$).

Table 5. Effects of microorganism supplementation and sterilize on nutrients composition of grower diets.

Chemical Composition (%)	With microorganism		Without microorganism	
	Sterilize	Non-sterilize	Sterilize	Non-sterilize
Moisture	9.76 [c]	10.73 [b]	11.81 [a]	9.55 [d]
Crude protein	18.33 [a]	17.54 [b]	17.48 [b]	18.74 [a]
Ether extract	7.77 [ab]	7.47 [b]	6.91 [c]	7.82 [a]
Nitrogen free extract	55.59 [b]	56.51 [a]	55.54 [b]	55.38 [b]
Crude fiber	3.21 [a]	2.60 [c]	3.08 [b]	3.15 [ab]
Ash	5.31 [a]	5.12 [b]	5.15 [b]	5.33 [a]
Calcium	0.94 [b]	1.00 [a]	0.92 [b]	0.62 [c]
Phosphorus	0.42 [a]	0.19 [b]	0.18 [b]	0.41 [a]
Metabolizable energy (k.cal./kg.)	4,819.75 [a]	3,864.05 [b]	3,836.32 [b]	4,111.63 [a]

[a–d]Means within a row with no common superscripts differ highly *significance* ($P\leq0.01$).

CONCLUSIONS

The supplementation of microorganism was decreased fiber and increased calcium, and phosphorus in broiler diets. The sterilization except the control treatment. method produced decreased dry matter in the diet. The supplementation of microorganism and sterilize method was higher in crude protein, ash, and metabolizable energy than the others

Table 6. Effects of microorganism supplementation and sterilize on nutrients composition of finisher diets.

Chemical Composition	With microorganism		Without microorganism	
	Sterilize	Non-sterilize	Sterilize	Non-sterilize
Moisture	10.02 [c]	11.06 [b]	11.94 [a]	9.8 [d]
Crude protein	17.05	16.69	16.68	16.90
Ether extract	6.95 [a]	6.36 [b]	6.49 [b]	6.93 [a]
Nitrogen free extract	57.10 [ab]	58.04 [a]	56.64 [b]	57.27 [ab]
Crude fiber	3.38 [b]	2.67 [d]	3.10 [c]	3.64 [a]
Ash	5.49 [a]	5.16 [b]	5.13 [b]	5.44 [a]
Calcium	1.09 [a]	1.11 [a]	1.10 [a]	0.91 [b]
Phosphorus	0.17 [b]	0.13 [b]	0.46 [a]	0.46 [a]
Metabolizable energy (k.cal./kg.)	4,318.05 [a]	3,750.26 [c]	3,796.51 [b]	4,312.69 [a]

[a–d]Means within a row with no common superscripts differ highly significance ($P\leq0.01$).

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USE OF ADDITIVES IN DURIAN PEEL SILAGES MAKING

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Abstract

The uses of three additives as sodium chloride, sodium nitrite, and yeast on durian peel silage making were determined. The Completely Randomized Design (CRD) with four replications in each treatment were used in the trial. The silage samples were kept tightly sealed in plastic containers and stored at room temperature for 21 days. The results of physical characteristics, chemical composition and fiber analysis of the durian peel silage were indicated that the color appearance of the durian peel silage was yellowish green for sodium chloride, a green brown color for sodium nitrite, and a red green color for yeast. The aroma of the durian peel silage was aromatic and acidic like pickled fruit. The aroma of the durian peel silage mixed with sodium chloride was sweeter than the durian peel silage from sodium nitrite and yeast. The chemical composition analysis of the non-fermented fresh durian peel for dry matter, protein, fat, fiber, ash, NDF, ADF, ADL, calcium, phosphorus and energy were 92.39%, 6.83% ,0.54% ,33.83% ,4.77% ,42.08% ,51.06% ,7.04% ,0.15%, 0.19%,and 3,843.85 kcal/kg, respectively. The durian peel silage made with sodium chloride, sodium nitrite, and yeast was highly significantly different in dry matter, fiber, ash, NDF, ADF, ADL and energy ($P<0.01$). However, protein, fat, calcium and phosphorus were not significant differences among treatments. Durian peel silage treated with $NaCL_2$ was the highest potential to degrade NDF, ADF, and ADL, respectively.

Key words: *durian peel, silage, additive.*

INTRODUCTION

Durian is one of the economic fruits of Thailand. This product is mostly used for fresh fruit consumption and processing products within the country and exported in terms of fresh fruits and frozen fruits. There are large amounts of durian peels which are left out from fresh fruit consumption and processing products such as durian chips. Thus the manufacturer and municipality must dispose of this large amount of durian peel' waste to alleviate this problem for a green environment. Now they try to make a value added aspect of durians and their by-products. Also, the use of durian peels contribute to useful material for the industrial sector such as packaging, paper pulp, insulator, combustible material, etc.

The chemical composition of durian peel is high in fiber which makes it is a good source of fiber for ruminant feed (Sorada et al., 2010).

Durian peel could be used as ruminant feed in silage forms to preserves the quality of its nutrient. Furthermore, it can be kept for a long time.

Durian peel silage can alleviate a mal-nutrition in ruminants during dry season or flooding time. It is a high quality silage because it is good in digestibility and palatability. It is easy for animal raisers to make durian peel silage for their animals by themselves.

This can help reduces animal feed cost and increase the quality of feed which is reflected in the high production performance of their animal. However, the quality of silage is depends on feed additive uses during making processes.

This research is aimed at selecting the suitable additives to apply to durian peel silage making for ruminant feed.

MATERIALS AND METHODS

Silage preparation

Durian peel was randomly taken from the durian products industry and chopped into 2-3 cm./pieces. The pre-silage material samples were mixed with three difference additives as; 1% $NaCl_2$, Sodium nitrate, and yeast. All samples were put in polyethylene bags and stored at ambient temperature for 21 days.

After 21 days of fermentation, a total of 25 g of each sample was dissolved in 100 ml of sterile water and stirred for 10 min. The pH values were measured for acidity changes by the pH meter (Polan et al., 1998). The silages color and aroma were described according to the indices score of Muhammad et al. (2008). For the color description, the silages were scored as 1 = dark or deep brown, 2 = light brown, 3 = pale yellow, and 4 = yellowish green. For the aroma description, the silages were scored as 1= putrid or rancid, 2 = pleasant, 3 = sweet, and 4 = very sweet.

Proximate analysis of silage

The 1,000 g of durian peel sample was randomly collected to determine the nutrient composition. The samples were oven dried at 60°C for 48 h. prior to proximate analysis. Dry matter (DM), ash, crude protein (CP), crude fiber (CF), ether extract (EE), nitrogen free extract (NFE) and organic matter (OM) were determined according to the methods of AOAC (1995). Neutral detergent fiber (NDF) and Acid detergent fiber (ADF) were determined according to the method of Van Soest and Robertson (1979). Proximate analysis was done two times as before and after fermentation. The experiments were repeated in three times.

RESULTS AND DISCUSSIONS

Physical characteristic of durian peel silage

After 21 days of fermentation, the polyethylene bag was opened and evaluated for gross characteristic as follows;

Color of durian peel silage

Durian peel silage mixed with 1% $NaCL_2$ had an olive yellow color which this shows a good characteristic of silage (Muhammad et al. 2008). For the durian peel mixed with $NaNO_3$ there was a dark brown color, and green red color for the durian peel that was mixed with yeast. Generally, the silage should have a darker color than fresh forage because the color of chlorophyll reacted with acid from fermentation. These changed them to become a magnesium free pigment phaeophytin. However, the carotene was a provitamin A which was suffered from oxidation at high temperature (Azim et al., 2000).

Smell of durian peel silage

Durian peel silage mixed with $NaCl_2$, $NaNO_3$, and yeast had a good smell like the pickled fruit. This smell was very aromatic and acidic for good silage (Merry et al., 2000). The sweet smell was caused by lactic acid bacteria which utilized sugar in the forage to produce lactic acid and volatile acid (McDonald et al., 1991).

General characteristic of durian peel silage

After 21 days of fermentation, the polyethylene bag was opened. The durian peel silage was a little subsided and fungi was found dispersed in the silage that was mixed with $NaNO_3$, and yeast. It occurred when the durian peel was compacted. Then, oxygen still remained in the polyethylene bag and it caused fungi to grow (Merry et al., 2000). However, the durian peel silage that was mixed with $NaCl_2$ was clear with non fungi occurring in the polyethylene bag.

Fig. 1. Characteristic of durian peel before and after fermentation
A = Before fermentation, B = Mixed with $NaCL_2$, C = Mixed with $NaNO_3$, and D = Mixed with yeast

Chemical composition of durian peel

The chemical composition of fresh durian peel revealed that DM, CP, EE, CF, Ash, Ca, P Energy, NDF, ADF and ADL were 93.39%, 7.39%, 0.59%, 36.62%, 5.17%, 0.21%, 4,160 kcal/kg, 55.27%, 45.54%, and 7.62%, respecttively (Table 1). These data was similar to Sorada et al. (2010) reported that the chemical composition of durian peel Montong variety were CP = 5.48%, EE = 0.82% and Ash = 3.58%.

Table 1. Nutrition value of fresh durian peel (%)

DM	CP	EE	CF	Ash	Ca	P	Energy (kcal/kg)	NDF	ADF	ADL
92.39	7.39	0.59	36.62	5.17	0.17	0.21	4,160.63	55.27	45.54	7.62

Changes in chemical composition of durian peel silage

The nutritive value of durian peel silage that mixed with $NaCl_2$, $NaNO_3$, and yeast was indicated in Table 2. The dry matter was changed before and after fermentation from 92.39% to 87.7%, 88.65%, and 90.47% for $NaCL_2$, $NaNO_3$, and yeast, respectively.

Durian peel treated with yeast was highly significant in dry matter than $NaCL_2$, but it was not significantly different with $NaNO_3$. The dry matter of durian peel silage that was treated with three kinds of additives was decreased when compared with fresh durian peel. This may have happened because the microorganism utilized carbohydrate in durian peel for their energy source to grow up and increased the number of bacteria (Suradej, 2005).

Durian peel silage treated with $NaNO_3$ was higher in protein (8.28%) than yeast (7.53%) and $NaCl_2$ (7.28%) but there were not significantly differences ($P>0.05$).

McDonald et al. (1991) reported that usually decreases in protein was due to the initially digestion by microorganism, while the increased of protein may occurs by the influence of salt, which it prevents *Clostridium* sp. to not destroy protein. There were not significantly differences in protein, ether extract, calcium, and phosphorus.

Durian peel silage were increased in fat before and after fermentation from 0.59% to 0.79%, 0.96%, and 0.77% for $NaCL_2$, $NaNO_3$, and yeast, respectively. However, there were not significantly differences among treatment ($P>0.05$). Fat had a little relation with fermentation processed, the increased of fat came from a cell wall released by anaerobic bacteria digestion (Suradej, 2005).

Durian peel treated with $NaNO_3$ was significantly lower in fiber (29.08%) than $Nacl_2$ (31.42%), and yeast (35.57%). However, there were not significantly differences between $NaNO_3$ and yeast. During fermentation, a decrease of fiber may have occurred by the digestion of *Lactobacillus* sp. to the cell wall which was the part of the fiber (Suradej, 2005; Sranya and Cnantakan, 1997; McDonald et al., 1991).

Durian peel silage treated with $NaCL_2$ was highly significant in ash (10.49%) than $NaNO_3$ (6.22%) and yeast (7.33%). There were not significantly differences between $NaNO_3$ and yeast. The increased of ash occurred by the utilization of plant organic substance and change to inorganic substance by microorganism during fermentation (Frame, 1994).

The energy of durian peel silage treated with $NaCL_2$, $NaNO_3$, and yeast were 3,979.21, 4,206.78, and 4,125.50 kcal/kg, respectively.

The fiber analysis revealed that $NaCL_2$ was highly significant degraded of NDF, ADF, and ADL (Table 3). Durian peel silage treated with $NaCL_2$ was significantly lower in NDF (50.19%) than yeast (61.96%). There were not significant differences in NDF percentages between $NaCL_2$ (50.19%) and $NaNO_3$ (51.06%). NDF was a part of the cell wall and carbohydrate structure of plants. It was utilized by microorganisms for their energy sources during fermentation, especially anaerobic microorganism. Furthermore, hemicellulose was hydrolysis by plant enzyme as a source of nutrient such as pentose (O'Kiely and Muck, 1998). Bustos et al. (2005) reported that when glucose was deficient, the *Lactobacillus pentosus* could produces acetic acid and lactic acid by using pentose from hemicellulose. The increase of NDF may occur by microorganisms utilizing sugar in plant cell for their growth and activity (Campbell and Smith, 1991).

The durian peel silage treated with $NaCL_2$ was significances lower in ADF (39.60%) than $NaNO_3$ (41.24%) and yeast (48.34%). There were not significantly differences between $NaCL_2$ and $NaNO_3$. Generally, a good range of ADF in dairy cattle' feed should be around 40 – 60 % to produces butterfat in milk (Somjit, 2006).

Durian peel silage treated with $NaCL_2$ was significantly lower in ADL (6.58%) than $NaNO_3$ (8.61%) and yeast (8.67%). There were not significantly differences between $NaNO_3$ and yeast.

The quantity of lignin, cellulose, and hemicellulose in feed are important for the forage crop of ruminants. A good quality of forage crop should be low in lignin (Flores, 1991).

Table 2.The Nutritive value of durian peel silage.[1/]

Treatment	DM	CP	EE	CF	Ash	Ca	P	Energy
1. $NaCL_2$ 1%	87.74^{b}	7.28	0.79	31.42^{ab}	10.49^{a}	0.22	0.15	3979.21^{b}
2. $NaNO_3$	88.65^{ab}	8.28	0.96	29.80^{b}	6.22^{b}	0.45	0.16	4206.78^{a}
3. Yest	90.47^{a}	7.53	0.77	35.57^{a}	7.33^{b}	0.22	0.12	4125.50^{ab}
CV. (%)	1.06	8.99	27.36	7.42	16.41	71.69	39.09	1.71

[1/]Mean followed by differences letter in each column are highly significantly differences (P<0.01)

Table 3. Fiber composition of durian peel.[1/]

Treatment	NDF	ADF	ADL
.1NaCl 1 %	50.19^{b}	39.60^{b}	6.58^{b}
2.$NaNO_3$	51.06^{b}	41.24^{b}	8.61^{a}
3.Yest	61.96^{a}	48.34^{a}	8.67^{a}
CV. (%)	4.36	5.16	7.84

[1/]Mean followed by differences letter in each column are highly significant differences (P<0.01)

CONCLUSIONS

Durian peel silage treated with NaCL2 may develops a biological feed for ruminant in Thailand. It was rapidly degraded of fiber within 21 days. It was the highest potential to degrade NDF, ADF, and ADL, respectively.

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THE UTILIZING OF PRODUCT BIOPROCESS SHRIMP WASTE AS NUTRIENT CONCENTRATE IN THE RATION ON PERFORMANCE NATIVE CHICKEN STARTER PERIOD

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Abstract

Bioprocess product of Shrimp waste can used as a source of nutrient concentrate in dietary of native chicken. Products of steps Bioprocess through by Bacillus licheniformis continued by Lactobacillus sp., and then by Saccharomyces cereviseae have a better protein digestibility value. The aim of the research was to evaluate the performance of native chickens to using Bioprocess product of Shrimp waste (Nutrient –concentrate) in the ration. One hundred and fifty day old native chickens were raised in cages until six weeks old. This experiment was conducted completely randomized design (CRD), six nutrient concentrate levels in the ration, namely R0 = basal ration without Bioprocess product of Shrimp waste (nutrient concentrate) with (crude protein 15%, ME 2750 Kcal/kg), R1= ration contained 5% nutrient concentrate (crude protein 15%, ME 2750 Kcal/kg), R2= ration contained 10% nutrient concentrate (crude protein 15%, ME 2750 Kcal/kg), R3= ration contained 15% nutrient concentrate (crude protein 15%, ME 2750 Kcal/kg), R4= ration contained 20% nutrient concentrate (crude protein 15%, 2750 Kcal/kg ME), and R5= standard ration high protein without content Bioprocess product of Shrimp waste (crude protein 18%, ME 2750 kcal/kg) and repeated five times. Data were analyzed using analysis of variance and Duncan's multiple range test. Feed consumption, body weight gain and feed efficiency were parameters observed. The results showed that treatment using bioprocess product of shrimp waste (nutrient concentrate) in the ration was significant effect on feed consumption, body weight and feed efficiency. The best performance was achieved by ration containing 10% nutrient concentrates (ration of 15% protein content) and equal in value to the standard ration (ration of 18% protein content).

Key words: *Bioprocess product, shrimp waste, nutrient concentrate, native chicken.*

INTRODUCTION

Native chicken as commodity is very popular among the Indonesian society in urban as well as in the rural areas, because it is being used as suitable alternative to increase the society income and important role as a nutritious food supply in the form of eggs and meat. To increase in population and production and also the business efficiency of native chicken, needs to improved from traditional system into agribusiness (Zakaria, 2004). Ration is the environmental factor that can affect poultry business success, and cause to production cost more or less 60-70 percent. The increasing of production cost can be overcome by finding other alternative feed ingredients which have good quality (Dutta and Mrigen, 2009). One of the alternative feed ingredient that have potential opportunity as a complement material for fish meal is waste-product frozen shrimp processing industry (cold storage) form of the skin and the head. Waste-product frozen shrimp processing industry (cold storage) is contains 43.41% crude protein, 18.25% crude fibre, 7.27% crude fat, 5.54% calcium, 1.31% phosphorus, 3.11% lysine, 1.26% methionine and 0.51% cystine, and the gross energy 3892 kcal/kg (Abun, 2008). Factors limiting the use of waste-products as ingredients of poultry feed is the presence of chitin in the amount of about 15-20%. Chitin bind strongly with proteins, fats and minerals covalent bond ß (1-4) making it difficult to digest by enzyme digestion of poultry (Leeson and Summers, 2001). Chitin is a chemical compound that cannot be digested

by the digestive enzymes of poultry (Leeson and Summers, 2001). Poultry have limitations in digesting food substances, especially those containing chitin and high crude fibre. This is because birds cannot produce the enzyme cellulase and chitinase, so that chitin and crude fibre can bind nutrients that can be digested out with feces (Tulung, 1987). In line with the facts found from the research the compound chitin shrimp waste without treatment is quite high, namely 20.11% (Abun, 2008). One effort to convert organic material into useful new products and has better nutritional value is to use microbes through bioprocess. Bioprocess waste-product can be done through the stages deproteinated using *Bacillus licheniformis*, and demineralizated with *Lactobacillus* sp. (Bisping et al, 2005). Bioprocess terminated by *Saccharomyces cereviseae* (Abun, 2008). Bioprocess product of shrimp waste used as ingredient of dietary of native chicken are expected to be better digestibility values because the nutrients have been relegated from the bonds of chitin. Bioprocess products by *Bacillus licheniformis*, followed demineralization by *Lactobacillus* sp. were the highest crude protein content in bioprocess treatment for 2 days in the amount of 47.60%. Finally fermentation by *Saccharomyces cerevisiae* release nutrient product with the lowest crude protein content of 43.5% and the highest 48.5%. The more nutrients are absorbed by the body, then the value of the higher digestibility. It is one indicator of the high quality of the feed.

The aim of this experiment is to get the optimal percentage of Bioprocess product of Shrimp waste as nutrient concentrate in the ration that produce the best to feed consumption, body weight gain and feed efficiency.

MATERIALS AND METHODS

One hundred and fifty native chickens were raised in cages until 6 weeks old, with the average of body weight was 27.83 grams (coefficient variance 7.57%). The birds kept in cage system, as much as 30 cages, and each cage consisted of 5 chickens. The ration consisted of yellow corn meal, fish meal, rice bran meal, soy-bean meal, nutrient concentrate meal, $CaCo_3$ and bone meal, in 15% protein, 2750 Kcal/kg of metabolizable energy and 18% protein, 2750 Kcal/kg metabolizable energy. The nutrient concentrate was made in Faculty of Animal Husbandry, Universitas Padjadjaran. The formula rations were :

R_0 Basal Ration without contained nutrient concentrate (15% crude protein, 2750 Kcal/kg ME)

R_1 Ration contained 5% nutrient concentrate (15% crude protein, 2750 Kcal/kg ME)

R_2 Ration contained 10% nutrient concentrate (15% crude protein, 2750 Kcal/kg ME)

R_3 Ration contained 15% nutrient concentrate (15% crude protein, 2750 Kcal/kg ME)

R_4 Ration contained 20% nutrient concentrate (15% crude protein, 2750 Kcal/kg ME)

R_5 Standard Ration without contained nutrient concentrate (18% crude protein, 2750 Kcal/kg ME)

Table 1. Composition of the formula rations (%)

Ingredients	Ration					
	R0	R1	R1	R3	R4	R5
Fish Meal	8.00	6.50	3.75	1.25	0.00	9.25
Nutrient concentrate	0.00	5.00	10.00	15.00	20.00	0.00
Yellow corn meal	58.00	58.00	58.00	58.00	60.00	56.00
Soy-bean meal	4.75	2.50	2.25	1.50	0.00	12.00
Rice bran meal	28.00	26.75	24.75	23.00	18.00	21.50
$CaCo_3$	0.50	0.50	0.50	0.50	0.50	0.50
Bone meal.	0.75	0.75	0.75	0.75	0.75	0.75

Completely Randomized Design (CRD) was used with 6 treatments, and each treatment was replicated 5 times. The data were analyzed by using Analysis of variance and if there are any significant effect then followed by *Duncan's multiple range* test (Steel and Torrie, 1989). The analyzed variables were feed consumption, body weight gain and feed efficiency.

Table 2. The nutrient and metabolizable energy content of ration

Ingredients	Rations					
	R0	R1	R2	R3	R4	R5
Crude Protein (%)	15.08	15.03	15.05	15.03	15.18	18.04
Crude Fat (%)	6.66	6.70	6.54	6.43	6.09	5.92
Crude Fiber (%)	4.89	4.97	5.08	5.69	5.92	4.51
Calcium (%)	1.05	1.27	1.39	1.54	2.03	1.16
Phosphorus (%)	0.58	0.65	0.68	0.72	0.84	0.63
Lysine (%)	0.97	0.95	0.90	0.86	0.86	1.21
Methionine (%)	0.35	0.38	0.40	0.42	0.45	0.40
Met + cystine (%)	0.67	0.69	0.70	0.71	0.73	0.75
ME (Kcal/kg)	2755	2770	2781	2792	2838	2781

Table 3. The average of feed consumption, body weight gain and feed efficiency native chicken

Variable	R0	R1	R2	R3	R4	R5
Feed Consumption(g)	591.92 **a**	557.62 **a**	642.22 **a**	558.52 **a**	560.68 **a**	618.18 **a**
Body weight gain (g)	261.60 **bc**	293.92 **a**	281.40 **a**	257.92 **bc**	240.76 **c**	305.00 **a**
Feed Efficiency (%)	39.13 **bc**	43.97 **a**	42.09 **a**	38.58 **bc**	36.01 **c**	45.62 **a**

Note: The Similar superscript in the same row no significant difference (P>0.05)

RESULTS AND DISCUSSIONS

The effect of dietary treatment on feed consumption, body weight and feed efficiency of native chicken starter period, is shown in Table 3

Feed Consumption

Feed consumption per chicks in each treatment during starter period shown in Table 3. From the Table 3, it can see an average feed consumption varying from 557.62 to 642.22 gram during starter period. The results of analysis of variance showed that using product bioprocess shrimp waste (nutrient concentrate) in the ration non significance (P>0.05) on feed consumption.

This means the use of nutrient concentrate products does not decrease feed consumption compared to a standard or control diet (R0 and R5). Its mean that the nutrient concentrate product from 5 – 20% in the ration still have palatable, but have limitedness on body weight gain and feed efficiency achievement.

In fermentation, microorganisms synthesize enzymes that can degrade the polymer into the substrate into simpler molecules, making them easier to digest.

Fermented products will have high quality such as high levels of digestibility and flavour and better texture.

This indicates that the product up to 20% in the diet does not cause smell, flavour, colour and texture that is not favoured by the chickens, so causing a decrease in feed palatability.

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 the poultry.

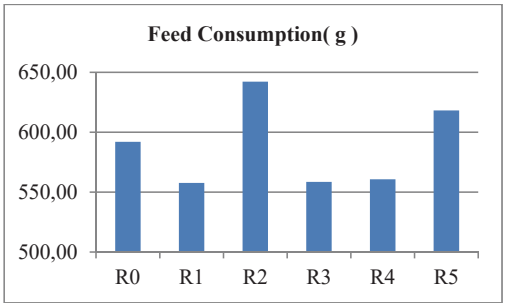

Figure 1. The average of feed consumption

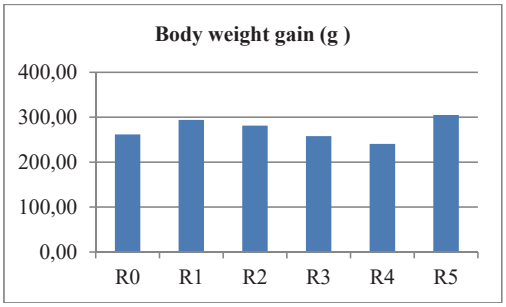

Figure 2. The average of body weight gain (g)

Daily Weight Gain

With Duncan's multiple range test on daily weight gain between the treatment use of nutrient concentrate products in the ration of 10% (15% crude protein) resulted in body weight gain similar to the standard ration which has a high protein content (18% crude protein). Adding the nutrient concentrate product meal until 10 percent in the ration native chicken still gave a good result.

This is because the bacterial species *Bacillus licheniformis* capable of producing *protease* and *chitinase* in relatively high amounts and acidic conditions created by *Lactobacillus* sp. mineral shed attached to the protein that has been unravelled. Further fermentation with *Saccharomyces cerevisiae* helps improve digestion with *carbohydrase* and *protease* enzymes it produces (Alam et al; 1996; Rahayu et al., 2004).

Bioprocess with microbes other than to break the bonds of polysaccharides also able to convert inorganic minerals into organic minerals that can improve metabolic processes and increased growth. By adding from 15 – 20% nutrient concentrate product in the ration, there was a tendency that body weight gain going to decreased ($P<0.05$). In treatment R3 and R4, the fibre content higher 5.69 % (R3) and 5.92% much more of basal and standard ration.

The higher fibre in ration will reduce feed consumption and intake energy is used in addition to make body balance. So the addition of 15 – 20% of nutrient concentrate product in the ration gave significant effect on body weight gain.

Lesson and Summer (2001) has told that if one of the ingredients in ration has high fibre, it will suppress performance of the chicken.

Feed Efficiency

In Table 3 can be seen that lowest of feed efficiency on native chicken which receiving 20 percent nutrient concentrate product in the ration R4 (36.01%), and the highest was R5, ration without nutrient concentrate product (45.62%).

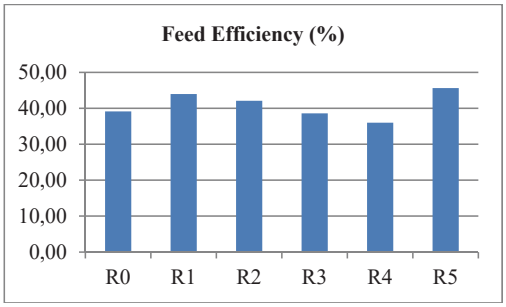

Figure 3. The average of feed efficiency

The results of variance analysis showed that the treatment by using of nutrient concentrate product gave significantly affected on feed efficiency. Leeson and Summers (2001) stated that the efficiency of the ration shows the efficiency of the use of feed to produce weight gain. Duncan results showed that the feed efficiency treatment of R1, R2 and R5 had similar and significantly higher than treatment R3, R4 and R0. Using the nutrient concentrate product 5 – 10% in ration can still support the good results in the feed efficiency. Bioprocess product of shrimp waste (nutrient concentrate) used as ingredient of dietary of native chicken are expected to be better digestibility values because the nutrients have been relegated from the bonds of chitin. By giving 15 – 20% nutrient concentrate product in the ration (R3 and R4) value feed efficiency become decreased, because ration R3 and R 4 received

much more chitin with used more product of shrimp waste in ration, so the crop and small intestine had extra work and more energy is used in addition to the balance of the body. According Leeson and Summers reported that a high efficiency value ration describe the less rations necessary to increase the weight per unit of weight, so the higher feed efficiency rate means better quality rations. Cheeke (2005) states that the value of feed efficiency can be used to measure the productivity of livestock.

CONCLUSIONS

The research showed that product bioprocess shrimp waste can be used as nutrient concentrates in the preparation of native chicken rations and by adding nutrient concentrate until 10% in the ration (ration with 15% protein) can still support the good results on the feed consumption, daily body gain and feed efficiency and equal in value to the standard ration (ration of 18% protein content) of native chicken starter period.

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THE CRYOGENIC RELATIONSHIP OF MORPHO-FUNCTIONAL PARAMETERS AND CHARACTERISTICS OF CARP GAMETES

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Abstract

The method of long-term preservation of mammalian semen in deep-frozen condition provides great opportunities for development and improvement of the reproduction system of farm animals. Using this method it is possible to check the breeders on the quality of offspring so as to maximum use the improvers. This allows performing the large-scale genetic selection in animal husbandry, which significantly increases the rate of mass improvement of breeding and productive qualities of animals. However, the existing cryotechnology not provide maximum preservation of the biological integrity of the reproductive cells. Comprehensive research has shown the possibility of increasing the efficiency of cryopreservation by improving of synthetic mediums and the development of optimum cryopreservation process parameters.

Key words*: synthetic mediums, cryopreservation, spermatozoa, efficiency of reproduction, farm animals.*

INTRODUCTION

Cryopreservation of reproductive cells of different species of fish, inclusive cyprinids achieved considerable success, allowing use cryoconservation method for scientific purposes to solve fish problem. Therefore it is advisable to carrying out research in different directions, such as: basic research to elucidate mechanisms of cryodamage and cryoprotection of reproductive cells; development of new methods for get 100% of surviving cells with intact genome; development of new methods for long-term storage of cells which allow to minimize costs for the maintenance of cryobanks (Alavai et. al., 2008; Cabrita et. al., 2010). The conservation of biological diversity of aquatic ecosystems and organisms acquires a special urgency nowadays, when sharply increased the impact of anthropogenic factors on the aquatic environment. From rivers and reservoirs used for industrial, agricultural and domestic uses large volumes of water, which leads to a significant deterioration of the conditions of reproduction, reduction of biodiversity and the extinction of many species and fish populations (Andreev et. al., 1996; Ananiev et. al., 1996).

The solution of nature protection problems in the fish farming is possible through the creation of cryobanks of valuable breeder semen (Ananiev et. al., 1996). However, advances of cryotechnology not widely used, due to the difficulty of reproducibility of the results (Andreev et. al., 1996; Cabrita et. al., 2010). According to research by E. Kopeika (Kopeika et. al., 2007), the number of surviving cells as a result of cryopreservation depends of spawning conditions, initial quality of sperm, its receipt conditions and other factors. It is shown that changes of structural and functional characteristics affect the qualitative variability and cryoresistance of fish sperm.

In the researches of fish reproduction the special importance attaches to the problem of preservation of reproductive products. The small time period of gametes preservation in native condition is one of the reasons of low efficiency of fish hybridization from different geographic area whose spawning occurs at different time of the year. Asynchronous maturation brings new difficulties to the working rhythm of fish farms. Therefore, the

practical importance of long-term preservation of carp gametes in cryopreserved condition attracts a lot of attention of many researchers (Boronciuc et. al., 2008; Cabrita et. al., 2010; Kutluyer et. al., 2015).

The most promising method of preservation known today, despite its complexity, is a method of low-temperature preservation which makes possible the creation of cryobanks of fish genomes and their uses for the conservation of biological diversity of the animal world (Ogretmen, İnanan, 2014; Asmad et. al., 2011).

Based on the foregoing, the main goal of the performed researches whose results are presented in this paper is the study of individual peculiarities of maintaining the functional activity of depreserved carp gametes.

MATERIALS AND METHODS

The experimental studies were carried out in the cryobiological laboratory of the Institute of Physiology and Sanocreatology of Academy of Sciences of Moldova and in conditions of experimental production. Sperm of common Carp was collected, evaluated and manipulated by using the general accepted methods. Through the methods of assessment of physiological indices we aimed to determine in sperm the concentration, mobility and survival of spermatozoa. Sperm cryoconservation was performed according to the classical scheme of cryopreservation in the form of pills at the liquid nitrogen temperature. The sperm of *Cyprinus carpio* were obtained after injection of pituitary extracts from male with weight 7-7.5 kg. In research for this paper was used the sperm of the carp 6-7 years. In the experiment was used variant in which roes were fertilized with cryopreserved sperm. Defrosted sperm was mixed with native caviar by means of the dry method (1/100), and then were sown in water on a labeled Petri dishes, placed on the bottom of a 0.6 l glass. In the control variant native material was used for the same purpose. The material was preserved in the form of granules of 0.1-0.2 ml volume which was subjected to cryopreservation on the surface of a fluoroplastic plate in liquid nitrogen vapour. The incubation of roe was carried out under production conditions using the Weiss apparatus: the water temperature is 22-23°C, the oxygen content of 7.0-8.5 mg/l flowage 3.0 L/min. The parameters of embryonic development of fish eggs were studied by method described by Jucinskii and Nedialcov (Jucinskii et. al., 1980). During the incubation of roe conducted observations of the quality of fertilization of eggs and visually calculated the percentage of fertilized roe at the ovum stage crushing. In addition was determined the percentage mortality of the ovules at the gastrulation stage and at the stage of separation of the caudal section of the yolk sac (corresponding stage of organogenesis). With the beginning of motor activity of the embryo were monitored the intensity of the movement of embryos in the experimental and control variants.

RESULTS AND DISCUSSIONS

One of the promising ways of preserve the genetic diversity of fish, not only rare and endangered species, but also the objects of aquaculture, is recognized the cryopreservation of sperm.

The main function is the preservation and transmission of genetic information by the reproductive cells from one generation to another. This can be realized in the normal course of the process of fertilization and embryonic development of organisms. This process is determined by the quality of gametes, the methods of fusion, the skill of the experimenter at artificial reproduction and environmental conditions.

In the solution of problems of carp sperm cryopreservation the important role is played by the creation of protective mediums and the establishment of optimal parameters which providing the maximal effect. The prior researches showed that the quality of carp sperm during cryopreservation undergoes considerable changes. Therefore was developed a new cryoprotective medium for carp sperm. It takes into consideration the necessity of stabilization of intermolecular interactions and binding of free water in cryobiological systems. For this purpose were used tris-(oxymethyl) aminomethane and 1.3-butylene glycol. The results of these experiments have provided a

new medium whose composition is shown in Table 1.

Table 1. Composition of medium for carp sperm cryopreservation

Names of components	Quantity
Tris-(oxymethyl)aminomethane, g	3.0
1,3-butylene glycol, ml	15.0
The yolk of hen eggs, ml	12.0
Water, ml	100.0

The highest effect the medium gives under separate cooling of the medium and sperm down to 4°C, the exposure of sperm at this temperature during at least 5 min, the dilution of sperm 1:1 takes place directly before freezing at 100-120°C during not less than 2 min.

However, the final results both of theoretical and applied researches can be evaluated from the data of production experiments (Table 2).

Table 2. The results of the production experiments on insemination of roe with cryopreserved carp sperm

Embryonic parameters	Female							
	Male 1		Male 2		Male 3		Male 4	
	Experiment	Control	Experiment	Control	Experiment	Control	Experiment	Control
1	2	3	4	5	6	7	8	9
The number of inseminated fish eggs (at the stage of ovicell segmentation)	50.0	90.0	60.0	90.0	40.0	90.0	45.0	90.0
Mortality of ovicells during transition from gastrula stage to the beginning of organogenesis	5.0	4.0	5.0	4.0	5.0	5.0	5.0	4.0
Mortality of embryos at the stage of separation of the tail part from the vitelline sac	10.0	4.0	10.0	5.0	10.0	8.0	10.0	6.0
Intensity of embryo movement in roe	N	N	N	N	N	N	N	N
Mortality of embryos at the stage of emergence out of roe	6.0	3.0	6.0	2.0	15.0	10.0	8.0	6.0
Embryos with anomalous development	2.5	3.5	2.5	3.0	4.0	3.0	3.0	3.5
Output of matured larvae (from native or cryopreserved sperm)	40.0	65.0	45.0	75.0	24.0	60.0	24.0	70.0

Table 2 shows important parameters such as the percentage of fish eggs fertilization and the output of matured larva using the sperm of different males in the experimental version varies from 60 to 40 and from 45 to 24%, respectively. It should be noted that these variations are observed on the background of similar fertilizing capacity of native sperm of both experimental males. However, the output of practical larva in this case is subjected to considerable variations even in the control version. One of the reasons of this phenomenon may be different cryo-resistivity of gametes of different sires found by A. Andreiev (Andreiev et al., 1996).

The established individual peculiarities of the male carp sperm quality provided the basis for similar researches under practical industrial conditions aimed at detection and analysis of such peculiarities in sex products of females irrespective of their native or cryopreserved condition (Table 3).

Table 3. The results of embryonic observations over fish eggs and prelarvae in experiments on carp sperm cryopreservation

Embryonic parameters, %	Male 1							
	Female 1		Female 2		Female 3		Female 4	
	Experiment	Control	Experiment	Control	Experiment	Control	Experiment	Control
1	2	3	4	5	6	7	8	9
Insemination of fish eggs (at the stage of ovicell segmentation)	35.5	73.5	60.0	96.0	20.5	80.0	77.0	97.0
Mortality of ovicells during transformation from gastrula stage onto the beginning of organogenesis	7.0	4.0	10.0	5.0	10.0	5.0	8.0	4.0
Mortality of embryos at the stage of tail portion separation from the vitelline sac	8.0	4.0	8.0	5.0	10.0	5.0	8.0	6.0
Intensity of embryo movement in roe	N	N	N	N	N	N	N	N
Mortality of embryos at the stage of emergence from roe	6.0	4.0	8.0	3.0	10.0	3.0	4.0	4.0
Embryos with abnormal development	3.0	2.5	3.5	2.6	3.5	2.0	2.5	2.0
Output of matured larvae (from native or cryopreserved sperm)	35.0	60.0	40.0	80.0	12.4	65.0	60.0	82.0

The data listed in table 3 show that the insemination of various female fish eggs in control version of the experiment varies from 80 to 97%. The output of viable larvae is also subjected to considerable variations from 65 up to 82%. In the experimental version the similar variations are even more pronounced. The insemination of fish eggs varies from 20.5 up to 77.0% (the maximum index is more than three times higher than the minimum one). The output of viable larvae varies within the range of 12.4-60.0% (almost five-fold difference in the studied material).

It should be noted that individual differences in quality of the sex products and the aggravation of these differences after sperm cryopreservation do not influence the quality of the obtained progeny.

Various indicators of the functional activity of the reproductive cells and the embryonal development of carp is explained by the initial quality of sperm and oocytes (Katkov, 2002), but to this must be added and the individual characteristics of both sexes reproducers, which have been previously detected by us in the experiments with the reproductive cells of other animal species (Boronciuc, Balan, 2008).

CONCLUSIONS

The researches allow making the following conclusions:

1. The individual peculiarities of functional activity of carp's genome are observed in male as well as in female experimental specimen.
2. The quality both of native and decreased carp reproductive material has a strictly peculiar individual characteristic for each male.
3. The greatest level of functional activity of decreased carp spermatozoa is achieved by using of 1.3-butylene glycol medium and optimal cryopreservation regimes.
4. During realization of programmes of sperm *Cyprinus carpios* cryopreservation it is necessary to take into account individual peculiarities of its quality and select the specimen with a high-quality and high-resistant material.

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IDENTIFICATION OF THE OPPORTUNITIES TO MAINTAIN THE REPRODUCTIVE HEALTH AS A RESULT OF THE SPERM LIPID SPECTRUM STUDY

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Abstract

Phospholipids as a component of the lipid spectrum represent the main part of structural integrity of sperm cell membranes. It is considered as natural fecundity factor which contribute to improving of spermatozoa cell membrane function by regenerative processes. Therefore, the purpose of the study was to examine the content of phospholipids, at various stages of a sperm cryopreservation. In results of the conducted researches is established that the content of phosphatidylcholine prevail above all fractions of phospholipids, which consistently decrease during cryopreservation. These changes are inherent to bull and boar spermatozoa. However, due to the fact that the content of phosphatidylcholine prevails over the other phospholipids, it is necessary to consider that this indicator can be the criterion of a fertility rate of spermatozoa which defines reproductive health of organisms relating to studied species.

Key words*: phospholipids, reproductive health, bull and boar spermatozoa, sanogenic status, cryopreservation.*

INTRODUCTION

By now has been accumulated a significant amount of information demonstrating that phosphatidylcholine and other phospholipids possess high biological activity relating to the maintenance of the integrity of the membrane systems of cells, the processes of differentiation, proliferation and regeneration of biological membranes. It is proved the participation of phospholipids in the regulation of metabolic processes through control of the membrane receptors and enzymes activities, both inside and outside the cell. Being a structural element of lipoproteins and also participating in the processes of blood coagulation, phospholipids determine hemodynamic and the cholesterol level in the blood (Quin P.J. et al., 1980).

A large variety of biological activities of phospholipids can be explained by feature of their chemical structure, which is characterized by: the presence of charged groups in the hydrophilic part of the molecule, the presence of biologically available phosphorus, polyunsaturated fatty acids and choline − the active component of phosphatidylcholine (Мэдди Э., 1979).

Thanks to its indispensability as a building material of biological membranes, cellular and subcellular structures, phosphatidylcholine takes an active part in a huge number of physiological and biochemical processes at all levels of biological objects organization. It has the most direct influence on the normal functioning of both somatic and reproductive cells. Formation of a sufficient number of reproductive cells contributes a significant, but not the major role to the maintenance of reproductive health. The significant influence has their qualitative indicators, such as: morphological features, mobility, life expectancy and the ability to fertilize. All of these properties are formed in a long process of the reproductive cells maturation. During this period occur certain sequences of changes of qualitative and quantitative composition of external and internal layers of the lipidic membrane of cells.

In many experimental researches is shown the positive impact of essential phospholipids on the toxic, allergic, metabolic and other damage of cell membranes. In this case takes place

inclusion of exogenous phospholipids in the damaged membrane, which is decisive in restoring of its morphology and functions (Quin P.J. et al., 1980). Regenerative effect of essential phospholipids refers to any types of cells. Phosphatidylcholine eliminates damage of cell membranes with the restoration of their functions and in the spermatozoa too. So, phosphatidylcholine showed the considerable activity in the protection and restoration of reproductive cells after their destruction under cold shock. In connection with this, phosphatidylcholine can be considered promising cryoprotector in the composition of cryopreservation medium (Шишкина Л.Н. et al., 2010).

Also, phosphatidylcholine is involved in the fertilization process. So, during movement to the ovule, the structure of the cellular membranes lipids of spermatozoa undergoes a series of successive changes−capacitation, and then the activation or acrosomal reaction.

In the inactive state, for spermatozoa it is characteristic the stable state of membrane supported by the asymmetry of the phospholipid layers composition. Asymmetry of the phospholipid composition is a typical phenomenon for all the cells. It is due to the activity of ATP and the sulfhydryl-dependent lipid pump. During the activation process the symmetry of local area of the spermatozoon membrane is restored (we are talking about acrosome), which contributes to the merger with the ovule. At this time in particular phosphatidylcholine has the triggering effect on the acrosomal reaction manifestation (Мэдди Э., 1979).

For the cells of the reproductive system phosphatidylcholine is a source of building blocks for the biological membranes, biological active substances as phosphorus, choline and essential fatty acids.

In connection with the above mentioned, the purpose of the conducted research was to study the cryogenic changes of phospholipids as an indicator that characterizing the sanogenic status of farm animals reproductive cells.

MATERIALS AND METHODS

In the experiments was used the sperm of the bulls of Holstein and boars of Large White breeds.

Extraction of lipids was carried out by the method which is based on a destruction of the protein-lipid connection by the polar solvents with the subsequent extraction of lipids by non-polar solvents which are united in one mixture, containing water in the ratio 5:10:4.

Another applied method was the colorimetric determination of phosphorus without burning. The colorimetry was performed at 820 nm using a spectrophotometer type SF-26. A calibration curve was built according to the standard solution of monopotassium phosphate. Translation of phosphorus in the phospholipids was made on the grounds that a molecule of phosphorus is 1/25 part of the phospholipid mass.

Research of spermatozoa phospholipid fractions were made using the method described by the M. Kates (Геннис Р., 1997) with the use of plates 9x12 cm, which was coated with silica gel «L» and «LSL», of the firm «Hematol» in the ratio of 5:3. Extract of lipids was received according to the above described method, then extract was dried in rotary evaporator and the pellet was dissolved in 0.5 ml of mixture of chloroform:methanol (1:1). The received mixture of lipids was used for chromatography, and their separation was carried out in the system of chloroform:methanol:water (65:25:4).

The phospholipids identification was performed by comparing them with the standard solution of lecithin, Rf and chromatograms of the chicken eggs (Кейтс М., 1975).

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

RESULTS AND DISCUSSIONS

Phosphatidylcholine is the main and the most versatile phospholipid, as presented in the various cell membranes. Therefore, in the studies were examined the changes of phospholipids under the influence of cryopreservation factors.

As a result of conducted experiments is established, that in the gametes of all types of studied animals in the greatest numbers are represented by a fraction of phospholipids, such as phosphatidylcholine (PC), phosphatidylethanolamine (PEA) and cholesterol (C) (Table 1).

Table 1. Content of phospholipids in the technological processing of the animal sperm, mg/100g of spermatozoa

Lipids	Elements of technological processing		
	dilution	refrigeration	defrosting
	M ± м	M ± м	M ± м
Boar, n = 8			
Phosphatidylserine	94.7 ± 13.6	66.3 ± 9.5	52.1 ± 6.9*
Sphingomyelin	356.1 ± 16.3	307.8 ± 11.2	274.6 ± 9.5*
Phosphatidylcholine	1468.8 ± 39.1	1344.8 ± 24.4*	1226.3 ± 24.1*
Phosphatidylethanolamine	639.2 ± 14.5	562.5 ± 11.9*	518.0 ± 12.2*
Cardiolipin	217.8 ± 13.9	151.5 ± 10.1	113.6 ± 10.1*
Cholesterol	482.0 ± 4.0	456.4 ± 10.4	424.2 ± 11.4*
The ratio of PC/SM	4.13	4.37	4.46
Bull, n = 7			
Phosphatidylserine	221.9 ± 9.9	189.4 ± 8.3	156.9 ± 5.4*
Sphingomyelin	238.1 ± 24.4	189.4 ± 20.2	140.7 ± 17.9*
Phosphatidylcholine	1649.4 ± 40.6	1253.3 ± 64.0*	979.4 ± 78.5*
Phosphatidylethanolamine	607.1 ± 11.3	541.1 ± 16.7*	389.6 ± 39.0*
Cardiolipin	216.5 ± 17.9	173.2 ± 16.2	140.7 ± 18.0*
Cholesterol	415.6 ± 10.9	379.0 ± 10.6	342.0 ± 10.6*
The ratio of PC/SM	6.93	6.63	6.94

Note: * P<0.05 the difference is statistically authentic in compared with diluted sperm.

In the quantitative content of the individual phospholipids and cholesterol are observed species peculiarities. So, in gametes of the boar less contained phosphatidylserine (PS) and phosphatidylcholine while the sphingomyelin (SM), phosphatidylethanolamine and cholesterol are in large quantities. Minor components of the boar gametes are presented by the phosphatidylserine and of the bull gametes – by the phosphatidylserine and cardiolipin (CL).

In addition, as a result of the conducted research it was shown that in the total content of phospholipids of the fresh dilute gametes of bulls and boars were not revealed significant specific differences.

Cooling and retention of the diluted bull seed at the temperature of 4˚C lead to a significant decrease of the total quantity of sperm phospholipid fractions.

These changes take place due to the fractions such as phosphatidylcholine and phosphatidylethanolamine. These, in our opinion, testify to the high vulnerability of these fractions, in the process of preservation-depreservation, compared with other fractions of phospholipids.

The results presented in the table 1 also show that the ratio of PC/SM is more than 67% in the bull sperm comparing with boar sperm. This follows that the phospholipids of their membranes have higher bilayer mobility.

In further technological processed can be traced decrease of the rest phospholipids factions.

Therefore, at freezing-defrosting sperm of bull and of boar the changes of content of phospholipids fractions, unlike protein fractions, have unidirectional character – their content is decreased. In technological processing of the semen of agricultural animals the content of different phospholipids fractions in gametes, regardless of the species of animal, go down which proves the non-specific nature of changes in their content at cryopreservation.

The output of membrane-bound phospholipids as a result of the influence of low temperatures is due to a breach of hydrophobic, ionic and lipid-protein interactions. A much larger decline was observed in the content of total lipids in bull spermatozoa after the freezing of sperm in comparison with boar spermatozoa, apparently fits mainly on the above type of loss, because observes more uniform change in phospholipid composition on all fractions.

Low-temperature processing of sperm stimulates the development of lipid peroxidation (LPO) (Абрамченко В.В., 2001) and apparently activates phospholipases. LPO, in turn, at biological freezing is an important element of pathology, contributing to the reduction of mobility and respiratory activity of spermatozoa. At the present it is known that the easily oxidable phospholipids are the CL and PEA, but PC on the contrary, inhibits oxidation processes. The last is present in large quantities in bull sperm, and sensitive to the LPO "PEA and CL" – in boar sperm, which indicates

higher stability of bull spermatozoa to redox influence.

The obtained data in the light of modern understanding of the role and functions of lipids in maintaining and strengthening reproductive health, with adequate certainty shows that the lipid composition of the sperm of different types of farm animals, possessing specific characteristics, determines their cryoresistance. Phospholipids are interrelated in membrane structure and correlate with sanogenic condition of cells. Protective effect of exogenous lipids, as well as specific changes in their composition in the area of low temperatures, indicates a significant role of these compounds in maintaining of the homeostasis in extreme environmental conditions.

The fact that, when cooled significantly reduced amount of PC and PEA suggests that this factor affects biological membranes on both sides at the same time as the first phospholipid is located mainly on the outer, and the second – on the inner side (Борончук Г.В. et al., 2008).

Significant role in preserving bilayer structure of biological membranes belongs to the PC and SM. Phosphatidylcholine is the main structural component of the membranes. It has a high interchange activity, represents specific activator of a many membrane-bound enzymes, serve as a metabolic precursor to SM as well as a source of lipid messengers and bioactive compounds: lysophosphatidylcholine, diglycerides and arachidonic acid; it has a great importance for the membrane permeability.

Unlike the PC, SM performs regulatory role, his presence in the membrane betrays special rigidity. Also, SM promotes the increase of microviscosity of the lipid phase of the membrane (Скатков С.А., 2003). On this basis it should be assumed that the PC may serve as a marker for characterizing of the functional spermatozoa state.

CONCLUSIONS

Generalization of the results of the conducted researches allows making the following conclusions:

1. In the process of sperm cryopreservation of farm animals, there is a reduction of the content of phospholipids at all technological stages.
2. From all spermatozoa phospholipids, quantitatively most representative is phosphatidylcholine.
3. The predominance of PC in bull spermatozoa and SM in boar spermatozoa indicates more rigid membranes structure of the latter species of animals.
4. The ratio PC/SM can be used as a criterion for the functional state of thawed spermatozoa of farm animals that predetermine their reproductive health.

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PHOSPHOLIPIDS, PHOSPHOLIPID FRACTIONS AND POSSIBILITY OF THEIR STABILIZATION DURING HYPOTHERMIC STORAGE OF BOAR SEMEN

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Abstract

Lipids are integral and essential components of the membranes that perform multiple functions in the life of germ cells. They provide the energy reserve, the solubility of various vitamins, the physical thermoregulation of activity of membrane-bound enzymes and other. In accordance with modern data about the molecular organization of biological membranes, the lipids are represented mainly by amphiphile connections: phospholipids, glycolipids and cholesterol. At the same time phospholipids make up more than 60% of the total number of membrane lipids. In combination with cholesterol they provide fluidity and plastic properties of biological membranes. Based on the above, the purpose of the conducted research was to study the content of phospholipids, their fractions and to determine the possibility of their regulation during hypothermic storage of boar semen at 16-18˚C. Using the method of thin-layer chromatography allowed allocating and identifying 6 phospholipid fractions in the reproductive cells of the boar. Among them prevail phosphatidylcholine and phosphatidylethanolamine, whereas phosphatidylserine and cardiolipin presents minor factions. In the process of dilution of sperm is observed multidirectional change in their content. It is established that targeted stabilization of the content of phospholipid fractions at the level of native spermatozoa is possible by improving of synthetic mediums. The obtained experimental data can be used for solving the problems of reproduction of pigs.

Key words*: lipids, biological membranes, phospholipid fractions, spermatozoa, boar semen.*

INTRODUCTION

Reproduction is one of the significant problems in the complex of measures aimed at further increasing the livestock of pigs which with the development of the industry is becoming increasingly topical.

Currently, the main method allowing in the shortest terms to implement a massive improvement of the breed qualities and increase their productivity, is artificial insemination, enabling intensive use of high-value boars-improvers. The efficiency of breeders is determined not only by their breeding advantages, but also by the quality of the semen, which varies among different breeders and varies depending on feeding, housing, use of animals, from ambient temperature and other factors (Яковлев, 2007). Therefore there is a need for a careful checking of the material used. Among the qualitative indicators should include the content of phospholipids, which have high biological activity relative to maintaining the integrity of membrane systems of cells, the processes of differentiation, proliferation and regeneration of biological membranes. It is proven the involvement of phospholipids in the regulation of the membrane receptors, enzymes and metabolic processes both inside and outside the cell (Скатков, 2002). Being structural elements of lipoproteins, as well as participating in the process of reproduction of animals, phospholipids determine the resistance of germ cells to adverse environmental conditions (Наук, 1991). A great variety of biological activity of phospholipids can be explained by the peculiarity of their chemical structure, which is characterized by amphipathicity (the ability of the phospholipids to hydrophobic and hydrophilic interactions), the presence of charged groups in the hydrophilic part of the molecule, the presence of biologically available phosphorus, of polyunsaturated fatty acids and choline - the active component of phosphatidylcholine (Борончук et al., 2008).

The main role in preserving of the fertilizing capacity of ejaculate is assigned to the homeostasis of the components of seminal plasma, to the maintenance of the viability of spermatozoa, the stability of their membranes, protection against the acidic environment of the genital tract of females (Oresti et al., 2011). The lack of phospholipids leads to a reduction of reproductive function of spermatozoa (Антонов et al., 2012).

In connection with the stated above, the purpose of the conducted research was to study the phospholipid spectrum of boar spermatozoa, as carriers of genetic information and to explore the possibility of its stabilization during the hypothermal phenomenon.

MATERIALS AND METHODS

In our experiments we used the sperm of boars breeders of Landrace breed which contained in the conditions of relevant veterinary requirements of the breeding enterprise "Moldsuin hibrid". The sperm was received by fractional method. After receiving it was divided into three parts. The first variant served as a control (native sperm). In the second variant, the sperm was diluted 1:1 with medium containing optimal amounts of L-carnitine, in the third variant, the semen was diluted with medium containing L-carnitine 1:1 and one hour after reaching the temperature of 16-18 ºC - with medium 2:1 containing ferric sulfate. As a basis it was used the medium consisting of glucose, EDTA and sodium citrate. Spermatozoa were separated from plasma by centrifugation with subsequent laundering of their with isotonic solution of sodium chloride. Thin-layer chromatography of lipids was performed on glass plates coated with a mixture of silica gels types "L" and "LSL" of company "CHEMAPOL" (Кейтс, 1975). Fractionation of phospholipids was carried out in the system chloroform-methanol-distilled water in the ratio 28:12:2. Staining of phospholipid fractions was carried out in the iodine vapor. The densitometry of chromatographic bands was performed using a computational technique using a special program "Sorbfil TLC Videodensitometer". The identification of chromatographic bands performed against standard solutions of lecithin and cholesterol, by special staining reactions, on Rf value and the comparison results of original photos and lipids in our studies (Наук, 1991).

Digital material was processed using the Student's t-test.

RESULTS AND DISCUSSIONS

One of the main technological parameters at the preservation of semen used for artificial insemination of sows is its dilution. Improving of the mediums for dilution of sperm, still, remains an actual problem in the field of reproduction of farm animals. The boar sperm in native form to a large extent diluted by the secrets of the accessory glands and exposed to the action of internal and external environmental factors. Among them we should mention, lipid peroxidation and accumulation of toxic products of this process (Alvarez et al., 1995; Goni et al., 2008). However, redox reactions play an essential role in maintaining the functional activity of the reproductive cells. In this connection great interest represents not only inhibition but also the activation of these reactions. The results of conducted researches are presented in figure 1.

Analysis of the chromatograms shows that as a result of the fractionation of lipids were found 7 fractions: 1. sphingomyelin (SPH); 2. phosphatidylcholine (PC); 3. phosphatidylethanolamine (PE); 4. phosphatidylserine (PS); 5. cardiolipin (CL); 6. cholesterol (Chol) and 7. phosphatidic acid (PA). The most expressed content of such phospholipid fractions are SPH, PC which represent saturated phospholipids and PE. In addition, it is assumed that PC includes plasmenylcholines, and the fraction of PE – plasmenylethalomines (Наук, 1991).

It turns out that the predominant fractions perform an essential role in the activity of biological membranes. So, the phosphatidylcholine is the basic, most versatile phospholipid, which is great represented in membranes and lipoproteins of different cells and tissues. This is the phospholipid, which is paramount structure-forming function. But along with this it also performs several other functions in particular – serves as a metabolic precursor for the other two major kinds of phospholipids – PE and SPH.

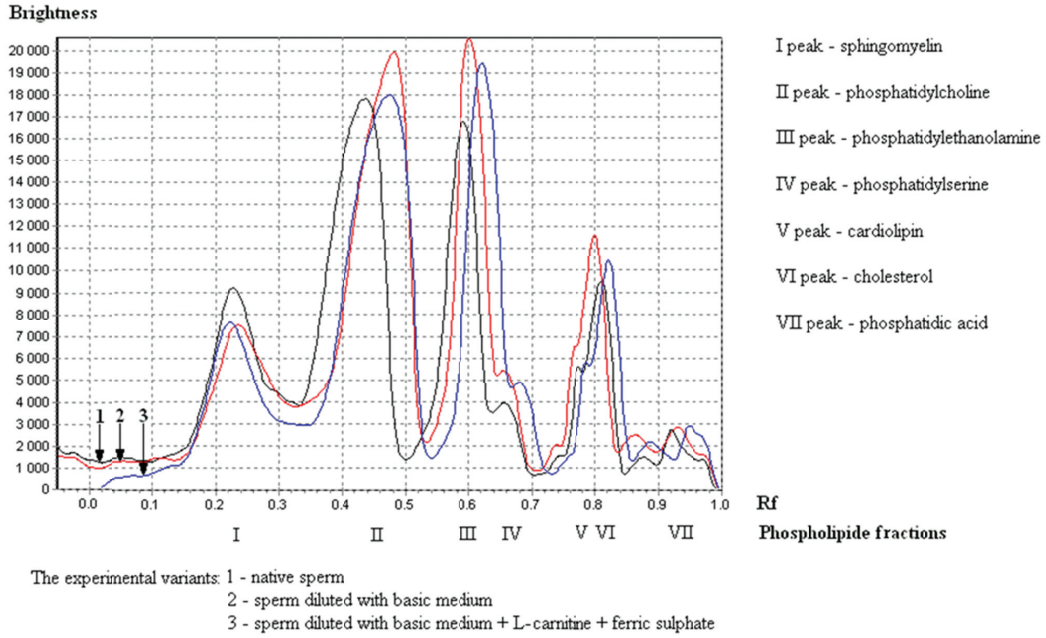

Figure 1. Phospholipid spectrum of boar spermatozoa during the hypothermal preservation and the influence upon it of the synthetic mediums of different composition

Important and indispensable role of PC, as a primary membrane phospholipid in the functioning, growth and development of cells is manifested in all tissues of animal origin, being significant the role of biological membranes in the activities of the cell and organism as a whole (Pomorschi et al., 2001). This is especially important for the sexual cells which are renewing relatively quickly – in a consequence of their high metabolic activity.

PE which represents an amine of phospholipids is involved in series of processes such as cell adhesion and endocytosis (Bazzi et al., 1992).

The SPH as a structural element of cell membranes is studied for many years, but only recently obtained proof of its regulatory role in important cellular processes. It is shown that, the presence in the membranes of SPH with its long-chain base and saturated fatty acid confers the stiffness to membrane.

It is established that the superficial changes of cell surface prevent the addition of SPH, on the basis of which it is assumed that the determining factor of morphological changes in the apoptotic cell is not the formation of ceramide, but the elimination from the surface of the SPH and its hydrolysis (Paris et al., 2001).

When the temperature is lowering to 16-18 ºC the content of phospholipid fractions varies depending on the composition of mediums used for dilution and storage of semen of the boar. Thus, the number of SPH decreases slightly whereas the safety of PC and PE largely improved, which can be explained by the change of the chromatographic mobilities of the studied phospholipids (Наук, 1991).

Change of phospholipid spectrum demonstrates corresponding reconstructions of biological membranes where the phospholipids represent 60–70% of the total amount of lipids, which have an affect the functional activity of spermatozoa.

CONCLUSIONS

The researches allow making the following conclusions:

1. Phospholipid spectrum of boar semen is mainly represented by sphingomyelin, phosphatidylethanolamine and phosphatidylcholine, whereas cardiolipin and phosphatidylserine are minor factions.
2. The content of phospholipid fractions is changed under the influence of hypothermal factor.

3. Stabilization of phospholipid is possible through targeted regulatory activity of redox processes at the dilution and storage of boar semen.
4. The ingredients of synthetic mediums can have a positive (or negative) impact on the biochemical status of boar spermatozoa by changing the chromatographic mobility of the phospholipid molecules.

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CORRELATIVE ANALYSIS OF THE EVOLUTION OF GLYCEMIA AND BODY WEIGHT FOR NEWBORN PIGLETS

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Abstract

In a multiplier PIC farm were effectuated complex surveys in order to monitor the health of suckling piglets and of lactating sows, by centering recorded data from the actual situation on field and the correlative analysis of glycemia and body weight level of 18 lots of newborn piglets (n=167) respectively the glycemia of the sows. The testing of glycemia was done using Accu-Chek Active glucometre, utilizing drops of freshly sampled blood, by sectioning the tip of the newborn piglets' tail, respectively by puncturing the pinna of sows. The inquires also included the taking of biometric measurements, consisting of individual weightings of piglets at the moment of parturition and at the moment of weaning. The data obtained on pairs: lot of suckling piglets – lactating sow were statistically analyzed using the Categorial analysis system ANOVA (Unpaired t test for Probability index calculation "P" and linear regression analysis to calculate the Pearson correlation coefficient "r" and for determination of "r^2"). Statistical analysis of recorded data from the sample of newborn piglets revealed high average values of glycemia in first hours after parturition (97.329±10.499 mg/dl), having a rising trend (107.01±7.378 mg/dl) in the next 10-15 hours, in terms of weights between 1.8 and 2.1 kg/piglet. It should also be noted that the piglets have reached an average weight of 7.3 kg at weaning, achieving an average gain of 5.5 kg during the lactation period (21 days). At newborn piglets, glycemia levels increased significantly statistically (p = 0.003) after the first suckling and were positively correlated (r = 0.4945) with the values of their weight at parturition. Noteworthy is the insurance statistics (p = 0.001) of weight gain of the lots of piglets in interval between parturition and weaning (growth spore). According to these correlations, elevated glucose levels can be associated with the weight gain of newborn piglets. Less interesting proved to be the results obtained by monitoring the glycemia of mother sows, who have variations between 50 and 133 mg/dl, with insignificant deviations to physiological ranges of species and category (66-116 mg/dl).

Key words: *piglets, weight, glycemia, mother sows.*

INTRODUCTION

The energetic influx through colostrum and milk is limited in the case of newborn piglets, which affects their immediate survival after parturition and increases the risk of mortality. Thereby, in the first days of life the losses of suckling piglets can reach at 12% being followed also by a reduction in the rate of growth after weaning or even later. At birth, the piglet's glycogen reserve is about 271 kilojoules, and it requires an external source of energy in the first hours of life to prevent hypoglycemia. Such an energy source may be represented only by the colostrum, which contains from 586 to 628 kJ/100 ml. However, it is considered that a newborn piglet needs to consume 250-300 ml colostrum to balance energy (Crenshaw, 1989).

Piglets are born with low organic reserves, thereby energetic influx from milk right away after parturition is essential for surviving. In case of low production of milk during lactation, some long-term effects will exist, which consist in a reduction in the rate of growth after weaning and even the entire period of growing and finishing. Thereby, even in the case of an adequate management, newborn piglets can reach only a part of their growth potential (Halamek et al., 1997). In general, milk production of sows may become a limiting factor for the growth of piglets in about 8-9 days of lactation (Ognean et al., 2015). Moreover, the differences between the needs of

piglets for nutrients from milk and its contents, progressively increase during lactation. In fact, the level of milk production acts as a limiting factor of piglets' optimum growth, whether they are weaned at 14, 21 or 28 days. On the other hand, current strategies of early weaning, about 14 days, only serve to intensify the difficulty of ensuring the necessary for growth and development of the lot of piglets at birth, before weaning. It is believed that fat oxidation for energy purposes is quantitatively lower than the amino acid/ glucose substrate, specific for the oxidation during fetal life, therefore also explaining the ketogenesis low rate of newborn piglets.

MATERIALS AND METHODS

In a PIC multiplication EUROHYB farm were conducted complex surveys to monitor the health of suckling piglets and lactating sows. According to implemented technological standards in this complex, preparturiented sows and those who are lactating with suckling piglets are maintained in maternity compartment, including proper boxes for maintenance and feeding requirements and also for the size of the calving group. For the undertaken surveys we have resorted to using the couple "lot of suckling piglets and lactating sows" as the unit of study. From all assessments conducted, in this paper we intended to make a detailed analysis of the correlative evolution of the glycemia levels, body weight and achieved gain of newborn piglets during the lactation period, respectively glycemia of lactating sows. Recorded data were correlated with high levels of performance indexes made by the investigated farm. From their synthesis appears that those 186 breeding sows, components of livestock jelly, conducted a total of 411 births annually, which resulted in 5238 calving piglets, respectively 4946 weaned piglets, resulting in 27.1 piglets/year/sow.

Monitorization of glycemia and ecometric indices consisted in correlative investigating of glycemia evolution and body weight of suckling piglets (n=176) from 18 "nests", taken randomly, respectively glycemia of those 18 mother sows. Biometric measurements were conducted and focused on weighing piglets from investigated nests and also the determination of individual weight gain and average (per lot and sample), based on the difference between the initial weight values (at birth) and final (at weaning).

Glycemia testing was based on using the quick method with Accu-Check Active glucometre provided with approved kits by Roche Diagnostics GmbH. The adopted method for glycemia testing required several adaptations of technical instructions to the working conditions in maternity and especially to the specific biologically fields accounted by newborn piglets and calving sows. Among these are used as area of election the tip of the tail for piglets, respectively pinna for sows; grooming (washing with soap and warm water), disinfection (with sanitary alcohol) and drying (by swinging) the election area; harvesting of blood drops in small caliber syringes (1 ml), by puncture of a vein pinna of sows, respectively severing the piglets' tip of the tails. The adoption of these collecting procedures allowed us to deposit correctly the drop of blood into the test strip and read the result in the indicated time (90 seconds). Statistical analysis of experimental variables included grouping of primary data, followed by their processing using Operating System Windows 2010 and GraphPad In State Program (functionally integrate in system by categorical analysis ANOVA). From this program, for processing the obtained data, Unpaired "t" test was used. Using this test allowed the evaluation of the probability index value "P", based on comparative analysis of initial (i) and final (f) weight, also by initial (i) and final (f) glycemia. We also appealed to simple linear regression analysis, which allowed evaluating correlations between initial glycemia (i) and the initial (i) and final (f) weight. They were quantified by expressing the Pearson correlation coefficient (r) and the coefficient of determination (r^2), which indicates the validity of the analysis model.

RESULTS AND DISCUSSIONS

The overall evolution of glycemia levels in the newborn piglets was characterized by tight oscillations influenced by the time elapsed after the first suckling. They were positively correlated with weight of piglets at birth, but

without important influences on the achieved gain during lactation. Therefore, according to data presented in Table 1, the results of statistical analyzes revealed the framing of average values of glycemia for the lots of newborn piglets in the range 93.75 - 112.4 mg/dL in the first 5 hours after first suckling respectively within 91.0 to 109.8 mg/dL in the first 15 hours after first suckling.

Calculated on the entire sample of newborn piglets, average values of glycemia reached 97.329±10.499 mg/dL at the first measurement (1-5 hours after parturition) and 107.01±7.378 mg/dL in the second measurement (10-15 hours after parturition), in conditions of weight between 1.8 and 2.1 kg/newborn piglet. We also note that pigs reached an average weight of 7.3 kg at weaning (67.3 kg/lot), achieving an average gain of 5.5 kg during the lactation period (21 days). Less interesting proved to be the obtained results from monitorization of glycemia of mother sows, who presented variations between 50 and 133 mg/dL, with insignificant deviations to physiological ranges of species and category (66-116 mg/dL).

The results of the statistical analysis are relevantly expressed through correlations and comparisons presented in Table 2. They show a significant increase (p= 0.003) of the glycemia level to newborn piglets after first suckling, recording averages of 97.329± 10.499 mg/dL in interval between 1-5 hours and 107.01±7.378 mg/dL in an interval of 10-15 days after parturition. We attributed special relevance to positive correlation (r=0.4945) between average values of glycemia and of piglets' weight at birth, which was statistically ensured by the values of P (0.0370) and of r^2 (0.2445). It also should be noted the statistical insurance (p = 0.001) of weight gain of lots of piglets in interval between parturition and weaning, representing the achieved gain growth during the lactation period, having a duration of 21 days. These correlations show a possible association of elevated glycemia with increased weight of newborn piglets. We attributed special importance and negative correlation (r=-0.0682), without statistical significance, between the initial glycemia and the weight of piglets at weaning.

As the data shown in Figure 1 illustrates, it a pretty tight grouping of glycemia values of the lots of piglets around the sample's averages was noted (97.329 ± 10.499 mg/dL at 1-5 hours, respectively 107.01±7.378 mg/dL at 10-15 hours after parturition).

Table 1. Glycemia values in piglets groups at 1-5 hours (^{i}) and at 10-15 hours after parturition (^{f}) and values of body weight at parturition (^{i}) and at weaning (^{f})

Parameter	Weight^i (kg)	Glycemia^i (mg/dl)	Weight ^{f} (kg)	Glycemia^f (mg/dl)
Lot 1 (n = 13)				
Mean	1.738	98.923	7.333	100.616
St.dev	0.138	14.499	0.359	8.781
Lot 2 (n = 13)				
Mean	1.8	96	7.285	104
St.dev	0.147	17.306	0.791	7.417
Lot 3 (n = 13)				
Mean	1.685	94.231	7.283	95.846
St.dev	0.408	17.838	0.799	19.274
Lot 4 (n = 13)				
Mean	1.833	112.417	6.975	110.333
St.dev	0.227	12.0789	0.691	7.596
Lot 5 (n = 12)				
Mean	1.833	112.417	6.975	110.333
St.dev	0.227	12.0789	0.691	7.596
Lot 6 (n = 13)				
Mean	1.554	86.923	7.292	105.154
St.dev	0.357	17.299	2.158	9.607
Lot 7 (n = 13)				
Mean	1.769	99.385	7.169	108.625
St.dev	0.184	13.593	0.6223	9.078
Lot 8 (n = 12)				
Mean	2.075	102.083	7.792	105.667
St.dev	0.076	6.402	0.405	6.315
Lot 9 (n = 13)				
Mean	1.8	109.923	7.646	115.158
St.dev	0.1224	7.308	0.504	6.780
Lot 10 (n = 13)				
Mean	1.866	104.167	7.272	117.417
St.dev	0.172	9.656	2.120	9.090
Lot 11 (n = 13)				
Mean	1.815	92.769	7.7	109.846
St.dev	0.199	18.226	2.176	17.411
Lot 12 (n = 12)				
Mean	1.883	104.667	7.633	119. 667
St.dev	0.175	16.511	0.556	18.855
Lot 13 (n = 12)				
Mean	1.8	93.75	7.342	110.083
St.dev	0.105	7.534	0.609	7.452
Lot 14 (n = 14)				
Mean	1.736	86.5710	6.992	106.769
St.dev	0.321	15.589	2.624	30.481
Lot 15 (n = 11)				
Mean	2.0	99.272	7.645	111.727
St.dev	0.054	4.859	0.508	6.929
Lot 16 (n = 9)				
Mean	1.733	71.111	7.4	97.555
St.dev	0.123	19.212	0.505	5.854
Lot 17 (n = 9)				
Mean	1.877	102.111	7.489	91
St.dev	0.109	18.811	0.321	21.354
Lot 18 (n = 10)				
Mean	1.72	85.2	7.5	106.4
St.dev	0.130	22.375	0.349	5.834
Samples (n= 167)				
Mean	1.806	97.329	7.373	107.1
St.dev	0.115	10. 499	0.250	7.378

^{i}- initial; ^{f}- final

Table 2. The obtained values at statistical analysis of investigated parameters

Correlations	
Level	r
Glycemia^{i}/ Weight^{i}	0.4945
Glycemia^{i}/ Weight ^{f}	- 0.0682
Level	r^2
Glycemia^{i}/ Weight^{i}	0.2445
Glycemia^{i}/ Weight^{f}	0.0046
Level	P
Glycemia^{i}/ Weight^{i}	0.0370
Level	P
Glycemia^{i}/ Weight^{f}	0.7878
Comparisons	
Level	P
Weight^{i} / Weight ^{f}	0.001
Glycemia^{i}/ Glycemia^{f}	0.003

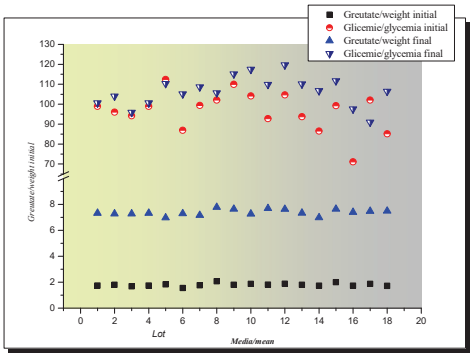

Fig. 1. Comparative evolution of medium values of glycemia and body weight in piglets groups which were investigated

Compared with the values found by us, most researchers in the field report lower levels of piglets' glycemia in the first hours after parturition reported by (Rada et al., 2010). We think that rising trend of glycemia for newborn piglets represents a characteristic feature for PIC breed, respectively for the investigated hybrid line. In same context can also be registered the higher glycemic levels (66-116 mg/dL), and the upward trend in suckling piglets, also recorded by other researchers of the field (Vlasiu et al., 2012). According to other research în field, the increases of glycemia and of weight of suckling piglets were assigned also to high level of milk production in PIC sows (Ognean et al., 2015). However, in our study, the average values of glycemia of mother sows presented less important oscillations in the interval of 50-133 mg/dL, the crossings of the upper limits being registered for only a few cases.

The suckling piglets' metabolism is characterized by intensification of catabolic processes and functional deficiency of the enzymatic equipments which reduces their capacity for synthesis. In these circumstances, piglets' glycemia is lower than that of adult swine, and is located around the value of 83 mg/dL (Rada et al., 2010). To all these it is added the inefficiency of energy metabolism and thermoregulation, which requires increased consumption of carbohydrates. The analysis of glycemic evolution of investigated lots confirms that, for the newborn piglets is essential to maintain this parameter in physiological limits, their limited hepatic glycogen reserve being known.

It is appropriate to notice that since the parturition, the transplacental transfer of glucose ceases, which means that the elapsed period until the first feeding is extremely critical, the stabilizing of the newborn being therefore dependent on their own deposit of hepatic glycogen. In such circumstances, the presence of an appropriate reserve for hepatic glycogen at parturition will increase the survival rate of piglets during this transitional period. It is important to observe that the decline of glycemia appears immediately after parturition and lasts 1-3 hours, because of the quick depletion of hepatic glycogen and compensation of this deficiency through enhanced gluconeogenesis only in the first hours after parturition (Pabst and Rothktter, 1999). It is important to mention that this critical time is amplified also by the metabolic requests of the state of lactation, which are higher in sows than females of other species of mammals, their lactopoesis requiring higher energy and mineral consumption (Acie et al., 1999) . On the other hand, the prevalence of catabolic processes of lactating sows justify why their case should be linked to energy and nutrients requirements to milk production and weight loss (Ognean et al., 2015).

In precarious conditions of feeding, weight losses in lactating sows can up to 40%, which seriously affects their productive performance. As we have already mentioned, after the suspension of maintaining glycemia by transplacentar transferring, the survival of newborn piglet requires adequate hepatic glycogen reserves available until the first sucking (Herpin et al., 2001; Kim et al., 2000). In this regard are relevant the results of studies

conducted on rats, which showed delay in the growth of fetuses with lower hepatic glycogen reserves than normal (Grupposo and Brautigan, 1989). In this context are registered also severel data of research conducted on human models and animals, showing that glucose is the primary energy source of the fetus, while the accumulation of nitrogen and protein are essential components for fetal growth (Hay, 2006; Hiridis et al., 2016). For the human fetus that reached term, hepatic glycogen concentration is 80-180 mg/g of tissue, this concentration being higher than at any other stage of life. In the case of swine the reserves of glycogen body are between 30-38 g/kg body weight (Okai et al., 1978). At newborn piglets, diminution of glycemia leads to depletion of hepatic glycogen and to replacement of gluconeogenesis process with the glycogenolysis. In the context of current developments, it is considered that hypoglycemia, hypothermia and hypoxia of newborn piglets are not pathological conditions themselves, but rather the symptoms of a failure of adaptating during critical postnatal period.

CONCLUSIONS

The high level of glycemia recorded in newborn piglets (n=167) in the first hours after parturition (97.329±10,499mg/dL) with an upward trend in the next 10 to 15 hours (107.01±7.378 mg/dL), reveals that precocity of the first sucking, along with thermoregulation management and oxygen consumption are essential processes for their survival in postnatal period.

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SOME BIOLOGICAL PROPERTIES OF DATE MUSSELS, *LITHOPHAGA LITHOPHAGA* L., 1758 (MYTILIDAE) IN THE GULF OF ANTALYA

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Abstract

This research has been carried out to investigating of some biological aspects of date mussels, Lithophaga lithophaga distributing in Gulf of Antalya, in February 2015 – January 2016. The samples were used to determine length and live weight frequency distributions (nonlinear regression using the allometric function), size (length, breadth, thickness) – size relationship (linear regression). The length – live weight relationships of L. *lithophaga were determined by the general formula W = a x L^b, where "W" is the live weight in grams, "L" the sizes (length, breadth, thickness) in cm, and "a" and "b" are the constants to be calculated. Length – breadth and length-thickness relationship were determined by log W = log a +b log L. The statistical analysis of "r^2" and 95% confidence limits of the parameters "a" and "b" were calculated. Mean size and weight of samples was calculated 75.58 ± 17.65 cm, 6.56 ± 5.27 g, respectively. Positive allometry was found for the breadth (B) on length and length on live weight relationship was displayed negative growth. Negative relative growth was recorded in the breadth on length and thickness on length (P<0.001).*

Key words: *Biological properties, date mussels, Lithophaga lithophaga, gulf of Antalya.*

INTRODUCTION

The Mytilidae is a diverse group of bivalves adapting themselves to various shallow and deep-sea environments. All mytilid bivalves attach their antero-ventral shell margin to hard or soft substrata by a byssus composed of collagen. Three life modes are distinguishable. These are epifaunal, semi–infaunal, and rock boring. Rock-boring bivalves are an important component of rocky marine ecosystems (Guidetti and Boero, 2004; Devescovi et al., 2005).

Lithophaga lithophaga (Linnaeus, 1758) is a bivalve species belong to mytilidae family. Its shell grows up to 12 cm in length and 70 g in weight. This species is distributed throughout the Atlantic Ocean from Portugal down to Senegal and northern coast of Angola. Furthermore, it occurs throughout Mediterranean coasts (Fischer et al., 1987; Gonzalez et al., 2000). It can be found in coastal areas where it inhabits limestone rocks in which it bores holes 10-20 cm long (Gonzalez et al., 2000). The endolithic bivalve *Lithophaga lithophaga* (Linnaeus, 1758) is part of this community, digging into limestone by means of chemical secretions (Morton and Scott, 1980; Mojetta and Ghisotti, 1996; Owada, 2009). In their tunnels, individuals can live for 50 years or more (Katsanevakis et al., 2008). This gonochoristic bivalve is most abundant in the midlittoral and sublittoral zones (Galinou-Mitsoudi and Sinis, 1994).

The date mussel was subject of several studies dealing with its biology, population dynamics, fecundity and habitat (Simunovic´ et al., 1990; Galinou-Mitsoudi and Sinis, 1994, 1995, 1997; Jaafar Kefi et al., 2007; Devescovi, 2009). Some other studies focused on the impact of harvesting and overexploitation on marine ecosystem and Mediterranean rocky coasts (Fanelli et al., 1994; Parravicini et al., 2009).

Allometry is the relation between the size of an organism and the size of any of its parts. Allometric growth is differential growth of body parts (x and y) expressed by the equation "W = aL^b", where "*a*" and "*b*" are fitted constants. Allometric relations can be studied during the growth of a single organism or different organisms. Although, in bivalves allometric growths have been widely studied in many species and used as one parameter to describe the trophic conditions of bivalve species in different habitats (Saxby, 2002; Ross and Lima, 1994; Parky and Oh, 2002). There is no published information available concerning on biological aspects of *L. lithophaga* in the gulf of Antalya, Mediterranean Sea.

The aim of the present study was to investigate some biological properties of *L. lithophaga* were studied in the gulf of Antalya.

MATERIALS AND METHODS

The present work was carried out the Gulf of Antalya, from an anthropogenic ally contaminated area, in February 2015 – January 2016 (Figure 1). Individual of date mussels were collected (extracted) with traditional methods, hammers, chisels and pincers from depths of 0 - 10 m (36.883715°N, 30.679556°E).

Figure 1. Location of studied area in the Gulf of Antalya

A total of 86 various sizes of date mussels were collected by SCUBA diving equipment. After diving all specimens were immediately put inside marine water and kept alive until transferred research laboratory of Akdeniz University, Fisheries Faculty. All live individuals were weighed to the nearest 0.01 g and measured with digital calipers. Length, breadth and thickness of the *L. lithophaga* were measured by digital calliper (Figure 2).

Figure 2. Linear measurements used in *L. lithophaga* for this study. L: length; B: breadth; T: thickness

In order to describe relative growth, relationships were established between Length and breadth (L and B) and weight (W) variable by fitting power functions ($W = aL^b$) for the linear variables and logarithmic functions (log W = log a + b log L) for the volume-related variables, with ''a'' as the intercept and ''log b'' as the coefficient of allometry. To determine whether ''b'' was different from 1 (linear variables) and 3 (volume-related variables), respectively, t tests were performed following Mayrat (1959). In this way, the type of relative growth (negative allometry for b<1 or b<3; isometry for b = 1 or b= 3; and positive allometry for a>1 or a>3) was determined (King, 1995; Pauly, 1983).

RESULTS AND DISCUSSIONS

Length frequency distribution

In this study, a total of 86 specimens of *L. lithophaga* are analysed. Mean size of samples was calculated 75.58 ± 17.65 cm. Other dimensions (breadth and thickness) were used to determine the size of the study by calculating the frequency. However, during the evaluation process, according to the example of studies conducted, only length and live weight frequencies were calculated. The length frequency distribution diagram was given in Figure 3.

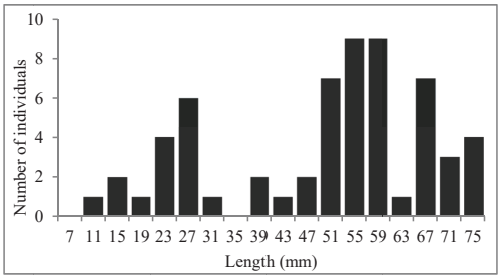

Figure 3. Length frequency distribution

Live weight frequency distribution

Mean weight of samples was calculated 6.56 ± 5.27 g. The live weight frequency distribution diagram was given in Figure 4.

Length – live weight relationship

The length – live weight and breadth – live weight relationships were calculated and shown in Figure 5, 6. From visual inspection, the relationship length on live weight relationship was displayed negative growth and it was

observed positive growth for the breadth on live weight relationship (P<0.001).

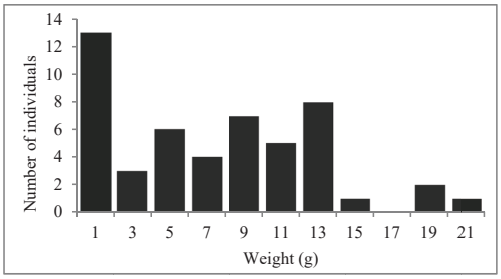

Figure 4. Live weight frequency distribution

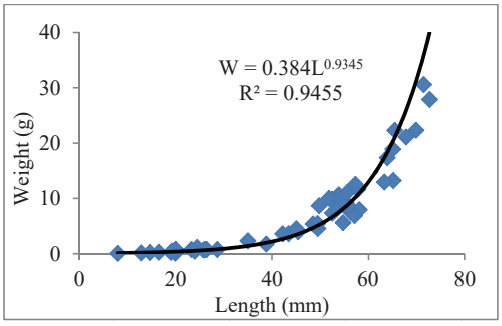

Figure 5. Length – live weight relationship

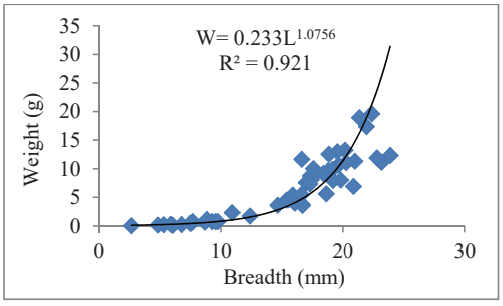

Figure 6. Breadth – live weight relationship

Breadth – length and thickness – length relationship

The regression of log breadth on log length and thickness on length were given a linear relationship expressed by log W = log a + b log L (Figure 7, 8). Concerning the relationships between linear and volume related variables, a negative relative growth was recorded in the breadth on length and thickness on length (P<0.001).

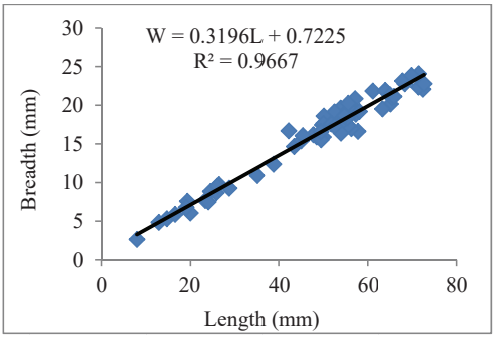

Figure 7. Breadth – length relationship

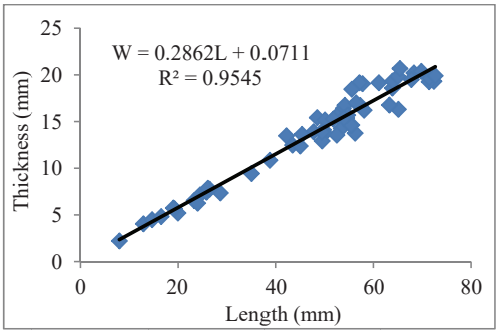

Figure 8. Thickness - length relationship

The present study of growth parameters of *L. lithophaga* collected from gulf of Antalya has demonstrated that a positive allometry was found for the breadth (B) on length and length on live weight relationship was displayed negative growth. Similar results were reported by Kefi et al. (2014) in Bizerte bay, northern Tunusia. According to literature data, *L. lithophaga* has the lowest growth rate of all studied species of the family Mytilidae (Galinou-Mitsoudi and Sinis 1995; Katsanevakis et al., 2008). This slow growth is probably related to the longevity of this species, which can live for more than 54 years (Galinou-Mitsoudi and Sinis, 1995). These authors also showed that individuals of 15–52 mm in length have an age of 6–25 or even up to 36 years.

Negative allometries were recorded in the breadth on length and thickness on length in present study (P<0.001). Although similar results were found in the Bizerte bay (Kefi et al., 2014), Wilbur and Owen (1964) were reported isometric relationships for both breadth and thickness on length. It is considered that these variations are likely

related to environmental and physiological conditions such as the gonad status.

CONCLUSIONS

It is well known, Data mussels*, L. lithophaga* is one of endangered species. It is illegally collected by fishermen and divers from natural habitats of Mediterranean subtidal ecosystem. Knowing biology and understanding their role in the ecosystem is very important. The information may be useful for proposing management measures to protect local wild stocks.

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EFFECT OF MATERNAL AGE ON PLACENTAL CHARACTERISTIC AND KID BIRTH WEIGHT

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Abstract

Optimum placental development influence fetal growth and may hence postnatal mortality of offspring. The aim of this study was to examine the effect of dam age on placental characteristics and kid birth weight in Saanen goats. The experiment were conducted on 10 youth goat singleton bearing Saanen does (ranging from 10 to 12 mounts of age) and 10 mature singleton bearing Saanen does (ranging from 3 to 4 years of age). Birth weight, kid's sex and placental measurements were recorded within 12 h after parturition. Adolescent doe had significantly lower (P<0.05)kid birth weight and placental weight than those of mature doe. Also the total number of placental cotyledons dissected from the chorioallantois in mature does were significantly higher (P<0.05) than those of adolescent doe. There were positive correlation between kid birth weight and placental weight (0.795; P<0.01), kid birth weight and total cotyledons number (0.578; P<0.01) and placental weight and total cotyledons number (0.594; P<0.01).The results suggest that adolescent dams in the first parity may alter placental characteristics and fetal development resulting in a reduced kid birth weight from singleton gestations.

Key words*: maternal age, placenta, cotyledon, fetal development, kid birth weight.*

INTRODUCTION

The placenta is defined as a functional organ which provides nutrients, gases and waste exchange between the maternal and fetal systems (Igwebuike, 2010). Placental characteristics are important indicator of postnatal mortality of offspring in small ruminants (Ocak et al., 2014). Mellor and Stafford (2004) reported that postnatal viability of newborn is associated with placental growth and development during gestation. The caprine have polycotyledonary placenta and placentomes performs exchange between the maternal and foetal circulatory system (Ocak et al., 2014). Thus, exchange capacity of placental between the maternal and fetal systems in the caprine is depend on placental size and number of the placentomes (Ocak et al., 2014). Therefore the size, which is relationship with nutrient transfer capacity of the placenta, play a pivotal role in determining the prenatal growth trajectory of the fetus and hence birth weight and postnatal viability (Sen et al., 2013).

Placental growth and development support consequent fetal development during mid- to late gestation (Redmer et al., 2004; Sen et al., 2013). Previous studies indicated that placental development during gestation is dominantly affected by maternal factors, especially nutrition levels (Owens et al., 1994; Wu et al., 2004; Sen et al., 2013). Also, many studies have demonstrated that there are relationships between weight of the placenta and birth weight of the newborn (Osgerby et al., 2003; Sen et al., 2013). Dwyer et al. (2005) reported that maternal age affected birth weight and placental characteristics. Moreover, Wallace et al. (2001) suggest that nutrient partitioning during gestation was changed to promote growth of the maternal body at the expense of the gradually increasing nutrient requirements of the gravid uterus and mammary gland in young growing females. Thus, adolescent dams have an increased risk of a major restriction in placental mass, and leads to a significant decrease in birth weight with high mortality rates within the first year of life (Wallace et al.,

2001). Therefore, we hypothesized that adolescent dams in the first parity may alter placental development due to a large part of the nutrition intake deliver to continue body mass growth, resulting in change placental characteristics and birth weight. The aim of this study was to examine the effect of dam age on placental characteristics and kid birth weight in Saanen goats.

MATERIALS AND METHODS

The study was conducted on 10 adolescent (ranging from 10 to 12 mounts of age) and 10 mature singleton bearing Saanen does (ranging from 3 to 4 years of age) in normal breeding season. All does were pregnant by naturally mate using mixed multiple sires and housed under the same conditions.

Birth weight (BW) and the sex of kids were recorded within 12 h after parturition. Each doe was left to deliver the placenta naturally and placentas were collected from singleton gestations immediately after delivery; care was taken to ensure that any placental weight (PW) taken were of the total placenta with any fluid being removed before weighting. The total numbers (TCN) of placental cotyledons dissected from the chorioallantois were also counted and determined.

The effects of maternal age on placental characteristics and kid birth weight were analyzed using a completely randomized design by the General Linear Model (GLM) procedure of the SPSS package program. The sex of kids was used as a cofactor in the model to adjust the birth weight and the placental characteristics. Significant differences between means were tested using Duncan's test and results were computed as mean ± s.e.m. Statistical significance was considered at $P<0.05$ and $P<0.01$. Relationships between variable traits for discrete data were determined with Pearson correlation analysis at the 95% confidence interval.

RESULTS AND DISCUSSIONS

Dwyer et al. (2005) reported that increasing maternal parity increased the lamb birth weight carried by ewes and younger ewes have low birth weight than older ewes. Similarly, in the present study adolescent does in the first parity produced kids with low birth weight compared to mature goats (Table 1).

Table 1. Kid birth weight and some placental characteristics of adolescent and mature Saanen doe

	BW (g)	PW (g)	TCN
Adolescent	3098.5 ± 33.8^{b}	412.4 ± 26.3^{b}	118.0 ± 6.0^{b}
Mature	3757.3 ± 45.4^{a}	661.7 ± 30.6^{a}	132.0 ± 5.0^{a}

[a, b.] Different superscript letters in the same column indicate significant difference ($P<0.05$).
BW = kid birth weight, PW = placental weight, TCN = total cotyledon number.

As a general fact, the does may be used as stock breed once they reach to 60-70% of their adult weight. In the present study, although the adolescent does had sufficient live weight for breeding (approximately 30 kg), their some placental traits (placental weight and cotyledon numbers) found to be insufficient and they had low birth weight in their offspring compared to mature goats. Adolescent does relatively having lower body weight, might have caused a delay in feto-placental development during gestation, allowing in lower birth weight in kids. The underlying mechanism of this result can be explained that younger dams utilized dietary nutrients in high level for growth of body when their body weight is lower (Wallace et al., 2001). Otherwise, they might have decreased the transfer of nutrients to the feto-placental growth and development. In the other word, when the does are not reached the optimal breeding body weight, their priority would be their nutritional requirements rather than their fetus. For this reason, breeders should develop strategies fulfilling both maternal nutrient requirements, especially for younger pregnant does, and the feto-placental growth and development.

The results of present study demonstrated that increasing of maternal age increased placental weights and cotyledon numbers. Placenta of adolescent doe was lighter and contained fewer amounts of cotyledons than those of older doe (Table 1). Similarly, Konyali et al. (2007) indicated that the first parity does had lower placental weight and higher cotyledon density, but total numbers of cotyledon in per placenta were greater than higher parity does in contrast to our study. Ocak et al. (2013) also showed

that maternal parity influenced placental traits and ewes in the 1-3 parities had lower placental weight, total cotyledon numbers and total cotyledon weights than those of ewes in the <3 parities, without affecting the cotyledon density. Dwyer et al. (2005) also reported that placenta weight and average cotyledon weight were not changed number of cotyledon, increased with ewe age or parity. Contrast to Ocak and Onder (2011) reported that placental weight was not influenced by parity, but total cotyledon numbers and total cotyledon weights were affected. Previous studies showed that low weight of placenta and reduced numbers of cotyledons associated with growth deficiency of fetus (Jenkinson et al., 1995; Greenwood et al., 2000; Dwyer et al., 2005). Therefore, these differences in placental weight, cotyledon number and total cotyledon weight by parity explained that adolescent doe carried lighter kids than mature goats in the second or third parities. The explanation of this situation is very difficult, but future experiments may be clarified with histological studies. On the other hand, reduced numbers of the cotyledons obtained from placentas of adolescent doe may show evidence of decreased growth of fetus in comparison to those of older does.

Previous studies reported that there was no significant correlation between birth weight and placental weight in sheep (Ocak et al., 2013) and goats (Ocak et al., 2014). However, in the present study pearson coefficient showed a significant positive correlation between birth weight and placental weight (0.795; P<0.01, Figure 1). Echternkamp (1993), Dwyer et al. (2005) and Konyali et al. (2007) reported similar findings for beef cattle, sheep and goats. The positive correlation between kid birth weight and total cotyledons number (0.578; P<0.01, Figure 1)obtained in the present study are in agreement with past studies in beef cattle and sheep (Echternkamp, 1993; Dwyer et al., 2005; Konyali et al., 2007). The positive correlation observed in the present study between placental weight and total cotyledons number (0.594; P<0.01, Figure 1).

This result is support the findings of Ocak and Onder (2011) and Ocak et al. (2014). It was observed that increased placental weight causes an increase in total cotyledon number and kids' birth weights.

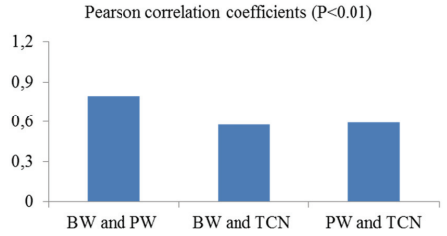

Figure 1. Pearson correlation coefficients of placental characteristics and birth weight

BW = kid birth weight, PW = placental weight, TCN = total cotyledon number.

CONCLUSIONS

In conclusion, the results of the present study imply that maternal age influence placental development and exchange capacity of placenta to fetus, which reflect variations in birth weight of kids. Especially, adolescent dams exhibit different placental morphology cause placental insufficiency.

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THE CONCENTRATION OF LACTOFERRIN AND ITS RELATIONSHIP WITH MINERALS AND AMINO ACIDS IN COWS MILK

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Abstract

Lactoferrin is a biologically active glycoprotein of the transferrin family found mainly in milk and to a lesser extent in other biological fluids as well as the secondary granules of neutrophils. As an iron-binding molecule, Lactoferrin is involved in the transport and excretion of iron but also known to bind to proteins such as IgA, casein, lysozyme and to DNA. This study aimed at establishing the relationships between Lactoferrin in cow milk and the concentration of minerals and amino acids. Ten Holstein Frisian cows in their first lactation were used in this study. Colostrum sample were collected immediately after parturition and milk samples were collected weekly for the first 60 days of lactation. Concentrations of minerals and amino acids in colostrum and milk samples were determined and used to construct the relationships between these variable and Lactoferrin concentration. The concentration of Lactoferrin in the colostrum was higher (P<0.05) than in milk (732.8 vs 350.3mg /L). The concentration of Lactoferrin in milk declines with advancing lactation. The concentrations of some minerals (calcium, phosphorus, magnesium and iron) in colestrum were 130 mg / L, 0.82%, 76.00 mg / L and 16.52 ppm respectively. The correlation coefficient between the lactoferrin and calcium, phosphorus, magnesium were positive and signifecant namely, 0.665, 0.268 and 0.289 respectively. The correlation coefficient between lactoferrin and iron was negative and significant (- 0.614) . Moreover, the correlation coefficient between Lactoferrin and the methionine and Lucien were positive and highly significant (0.18125 and 0.33908 respectively), While with the non-essential amino acids was being positive and significant. It conclusion, lactoferrin has a tight relationship with main milk components, being has an important role for birth calves through its high percentages in colostrum. Also, the repeatability between lactoferrin and minerals has a pronounced importance for birth calves growth.

Key words: *Lactoferrin, component minerals, amino acid, cow milk.*

INTRODUCTION

Both milk and colostrum provide a complete food source for newborn calves. The colostrum is considered as the only source of primary acquired immunity for the newborn. The immune secretions in the breast, will be eliminated by specialized receptors, both colostrum and milk contain viable cells, which include cytokines, proteins and antimicrobial peptides such as lactoferrin, defenses, cathelicidins. The immune system in the colostrum is associated with the ability of the new born's intestine, allowing the passage of large molecules such as alklopeulenat molecules, as the concentrated immunity in the colostrum and intestinal permeability decreases rapidly and gradually in the first 48 hours of the baby's life (Moore et al., 2005), so it is necessary for new born calves to address an adequate amount of the colostrum during the period of his life to gain negative immunity and to be able to survive until the autoimmune system is fully develop. The immune factors, in milk and colostrum play an important role in defending the protected newborn from pathogenic organisms (Boyso – Oviedo et al., 2007.

The innate immune system is the essential line is to protect the body from infectious pathogens before the immune system in the saliva is initiated, as it represents a complex interaction between the cellular and molecular processes, which aims to discover the causes of harmful diseases and eliminating them at a later time, having the innate immune system evolution of the cow's udder to a highly effective defense mechanism in the host (Rainard and Riolled, 2006), it has also been assumed that the udder itself may be an extension for the innate immune system (Vorbach et al., 2006).

One of the most significant immune factors is the lactoferrin protein which is found in colostrum and milk in a number of mammals, as well as physiological fluids, but in different concentrations, so their concentrations in colostrum and milk is higher than the rest of the fluid as well as the concentration of lactoferrin protein, depending on the type of animal, also the different concentrations of immune factors in cow's milk during sickness or healthy cases. In one of the studies milk samples were taken from healthy cows (189 cows), the results showed the presence of more than 20-folds of concentrated lactoferrin. Similarly, many differences occurred in the concentrations of individual Lactoperoxidase in goats and cow's milk (Fonteh et al., 2002). Such a large variation in animals suggests an opportunity to determine where the milk's immune factors would be higher so that we can create herds for commercial exploitation.

Therefore the lactoferrin protein (Lf) is one of the specialized immune proteins and outstanding high energetic effectively through its ability to bind iron, as it is considered one of the proteins sugary basal (Basic glycol-proteins) and a member of a family of proteins Transferrin type. (Jenssen and Hancock, 2009), also this protein has attracted the attention of researchers to study and try to increase the effectiveness of vital during digestion, it emerged as an increase in the effectiveness of the dissolved lactoferrin protein, either by physical or enzymatically by 8-10 times more compared with the natural protein lactoferrin protein (Van der Kraan et al., 2004; Gifford et al., 2005; Flores-Villasenor et al., 2010). This study aims to find the relationship between the concentration of the lactoferrin protein and the minerals, amino acids in colostrum and milk for the usage in increasing the immunity of calves and its better growth.

MATERIALS AND METHODS

This study was conducted at Al-Naser Dairy Cattle Station / Aswera (50 km South of Baghdad) during the period from 15/1/2013 to 1/4/2013. The heifers were inseminated at the age of 16-18 month and not less than 370 kg body weight. Ten cows were randomly selected (at first lactation) to determine the milk lactoferrin concentrations and its contents of amino acid also some mineral in milk (Ca, P, Mg and Fe). Sample of milk were collected fortnightly (after one day of birth) for two month. The calcium, phosphorus and magnesium in milk were estimated according to Richards (1954). Cations were determined according the following equation:

$$\text{ml} - \text{eqw. Ca/L} = \frac{\text{Percent volume x titer}}{\text{Volume of filtrate}} \times 1000$$

The magnesium was not t estimated directly from the filtrate, so it firstly estimated the calcium concentration, and then calcium and magnesium collectively according the equation:

$$\text{ml} - \text{eqw.Ca+ mg/L} = \frac{\text{Percent volume x titer}}{\text{Volume of filtrate}} \times 1000$$

Then from the deference get mg concentration.

P: concentration was calculated according to Olsone et al. (1961) method. The Fe was calculated using atomic absorption from the following method:

Fe	Wave length	Fuel ratio	Slit nm	mA
	248.3	2.0A-Ac	0.5	4.0

Amino acids concentration was determined using HPLC according to the Feng et.al.(2004) and calculated from the following equation:

$$\text{Acid conc. } \mu\text{g/ml} = \frac{\text{F.Bundle area of the sample}}{\text{St.bundle area}} \text{st. conc. XNo of dilution}$$

St.bundle area

HPLC were used to determined lactoferrin concentration according to the following equation :

$$\text{Lactoferrin conc. } \mu\text{g/ml} = \frac{\text{Bundle area of the sample}}{\text{St.bundle area}} \text{st. conc. XNo of dilution}$$

Statistical analysis:

SAS (2010) were used to detect the effect of week and month effects of lactoferrin on some production, traits Duncan test (1955) was used to compare among means.

The correlation coefficient between lactoferrin and each of amino acids and some minerals were calculated under 0.05-0.01 probability.

RESULTS AND DISCUSSIONS

Milk production: daily milk production was recorded rather than monthly milk production. Daily milk production means were 16.95±0.84; 18.50±84 kg respectively while monthly milk production means were 508.50±25.31; 555.0±25.39, respectively for two month of study.

Table 1. Daily and monthly milk production (kg) of cow during experiment

Milk production	Milk amount ±**se**
Daily milk (1)	16.95 ± 0.84
Daily milk (2)	18.50±0.84
Milk production in 1st month	508.50±25.31
Milk production in 2nd month	555.00±25.39

(1) and (2) daily milk in 1st and 2nd month

Milk lactoferrin concentrations:

Table 2 showed that the colostrum lactoferrin concentration is higher than its concentrations in the milk. The results also observed that the lactoferrin concentration decreased with increasing milk production, and this is consistent with those reported by Hiss et al. (2009), Piccinini et al. (2007) that the lactoferrin is higher in the early days of breastfeeding. This increasing may be due to the several reasons, including its contribution in transferring mineral elements, especially iron to the new born calves through the milk, as well as its participating in the growth of bacteria symbiotic in the intestines of the calves, along with its role in the inhibition of the pathogenic bacteria proliferation in the intestine, as well as serving as a counter and others antioxidant the anticlotting through susceptibility of lactoferrin to connect iron ion (Huang et al., 1999) as well as that of the immune proteins specialized to play an important role in the incidence of various infections, it also helps the blood to compose in the spinal cord and helps to reduce the level of iron level in the blood (Wong et al., 2009; Wang and Hurly, 1998) its concentration also increases during infection. It is noted that there is a highly significant relationship ($P < 0.01$) between the lactoferrin and weeks of production.

Table 2 shows the amount of lactoferrin in cow's milk.

Table 2. The amount of lactoferrin in cow's milk

period	Lactoferrin	Sig.
colostrum	732.78±28.03a	**
2 week	604.60±23.94b	**
4 week	541.11± 19.20 c	**
6 week	402.67±17.87d	**
8 week	350.31± 16.24 d	*

Means in the same column with different superscripts were significantly different ($P < 0.05$).

Minerals in milk: as shown in Table 3 the colostrum content of calcium was higher than this content of calcium in normal milk being 130±11.70 mg/L and then this amount was gradually decreased as calcium is very important element for all organism bodies, for example calcium is major constituent of bones and teeth in addition to its role is most of metabolic activities inside the body. However calcium have important role is transformation of neural signs and natural activity of muscle, blood clotting and the activation of certain enzyme and hormones. On other hands there was a decrease in calcium level in coincidence with the increase of milk production.

The content of magnesium in milk has similar trend of calcium (Table 3) but the level of magnesium in colostrum was higher than its content in normal milk being (76.00±3.03 mg/L), and then the level of magnesium in colostrum gradually decreased in coincidence with the increase of the amount of milk production. The level of magnesium at parturition is a reflexion of fetal response to dame status during pregnancy. However the effect of magnesium is very big during first week of life as it reverse. The reverse of independent life of intent in the absence of direct factors that transported by dam blood. Certain studies indicated that magnesium affect on neuron excitation and response it has main role in activating of some enzyme , And that the magnesium element role in protein synthesis digest vitamin As well as it immune function and this explains the high value Magnesium element in the colostrum in the first few weeks of suckling (Spear and Weiss, 2008).

As for the iron element was accounted in colostrum, it is less than the rate of milk as the

percentage (16.52±0.72). The reason may be due to the low percentage of iron in the milk that it is one of the encouraging factors on oxidative stress (Huang et al., 1999). And it is one of the factors that help fat oxidation to be the roots of ion ferrous accelerates oxidation by breaking down peroxides it decomposition of hydrogen peroxide into an effective free radicals (Miller et al., 1996). Also show in the table to iron ratio began increase with the increase in milk production and because it is an important element being included in the composition of and because it is an important element being included in the hemoglobin which enters in the composition of the blood protein also enters in the composition of respiratory enzymes which present in mitochondria. As well as Table 3 shows that the percentage of phosphorus element in colostrum (0.80±0.01) compared with milk, we note that the ratio of phosphorus have declined after two weeks coincides with the increase in the quantity of milk product. It may be due to the high percentage of phosphorus element in colostrum to the importance of this element in the body It is important as a vital element enters with calcium in bone formation, proteins and fats, and enters in the composition of some co-enzymes. There are also some with and some types of sources of the energy stored in the compound energy adenosine tri-phosphate (ATP). Each of calcium, phosphorus plays a vital role in most tissues of the body with roles with structural roles in cell membranes, also participating in cellular processes, bones and teeth (McDowell and Arthington, 2005). Underwood and Suttle (1999) showed the possibilities DNA synthesis, contact between the cells, the cell membrane fluidity, tracks metabolic.

Essential amino acids concentrations in milk

Table 4 explains the absence of significant differences observed for the histidine acid, which shows its concentration in colostrum to be higher than its concentration in milk (239.96 ±62.67). As for the threonine acid, its concentration in colostrum is also higher than the normal milk, but there were no significant differences observed, the results of both (tryptophan, tyrosine) acids show that their concentrations in the colostrums is higher than the normal concentration in milk, reaching proportions in colostrum to (251.80±61.92; 258.80±42.65). Significant differences were observed between the averages of the acid concentrations observed during periods of the study. The valine acid' concentration in colostrum is less than the concentration in breast milk, its concentration in the colostrum is (149.80 ±39.21), this is also observed for the methionine acid which contains a concentration of (223.49± 49.48), with no significant differences observed. The results also showed that the amino acids (isoleucine, leucine, lysine and valine), their concentrations in the colostrum was higher than the normal concentration in milk, reaching their concentration in the colostrum to 451.50+119.04; 551.50+156.20; 591+117.31; 939.80+153.65), respectively.

Non-essential amino acids concentrations in the milk:

The results showed that the concentration of amino acids in cow's milk, varies from period to another during the experiment, due to the nutrition, also the aspartic acid observed to be high at ($P < 0.01$) colostrum (233.04± 33.96), also the results showed no significant differences in the presence of glutamic acid. As for the glycine acid, its amount in the colostrum was higher than the rate in the natural milk, reaching per colostrum to 320.76± 118.84.

It was noted that the presence of high significant differences for this acid in milk, when compared to the averages during the experiment, as for the arginine acid, its concentration in the colostrum was higher than the rate in the normal milk as well as for alanine reaching their percentage in the colostrum to 173.50+34.04; 367.76+86.40, respectively.

The results showed highly significant differences in amino acids concentration in milk. It is possible that the reason behind the fluctuation in the concentration of amino acids, is the nutrition, as well as to the viability of microorganisms to manufacture amino acids and the proportion of acids that reaches the mammary gland, and that the high concentration of certain amino acids in the colostrum of that in breast milk could have caused due to the importance of these acids in growth as interference in the formation of the protein. The presence of the amino acids, arginine and lysine at a high concentration in the colostrum, may be due to

their importance, they are considered one of the significant acids of the lactoferrin polypeptide protein which give it a positive charge of these acids that play an important role in giving adjective positive for peptides and easily chemically linked (Dionysius & Milne chemically bacteria cell wall, 1997), where the amino acids, containing multi-negatively charged fatty sugars such as (aspartic, histidine and tyrosine) in linking iron ion in comprising a strong complex The arginine forms a strong component of a complex CO3-2 linking carbonate ions (Baker, 1994).

The correlations between lactoferrin and some productivity the qualities

The results showed that there is a negative relationship between the lactoferrin and daily as well as monthly milk yield, the value of the correlation coefficient between the total lactoferrin and daily as well as monthly milk yield were 0.065 and 0.652 respectively (Table 6) there were significant differences between lactoferrin and milk yield. The results also showed the presence of correlation between the lactoferrin ratios milk components as well as in relation to the minerals (calcium, phosphorus, magnesium) and there is a correlation between lactoferrin and the iron has been related to the negative as a decline in the concentration of lactoferrin accompanied by a rise in the proportion of iron in the milk, which is linked to the production of milk.

The correlation coefficient between lactoferrin and essential amino acids:

The results in the Table 7 show a significant correlation between the protein lactoferrin and some Essential amino acids found in milk, Informed the correlation coefficient between the lactoferrin and essential amino acids (His, Thre, Trp, Tyro, Val 0.24, 0.006, 0.30, 0.31, and 0.25) respectively.

The table shows a positive and highly significant link between lactoferrin and methionine acid. The same applies to the Lucien and methionine acids the correlation coefficient with lactoferrin, 0.18, 0.33 respectively. It was also shown that the amino acids are associated positive and significant with Lactoferrin, This means that any increase in the concentration of lactoferrin, accompanied by an increase in the concentration of essential amino acids in milk.

The correlation coefficient between lactoferrin and non-essential amino acids in milk:

The results in Table 8 shows that the correlation coefficient between lactoferrin and non-essential amino acids (Glycine, Glutamic acid) found in milk was positive and high significant, the correlation coefficient was 0.36 and 0.35 respectively. The link between lactoferrin and the other non-essential amino acid has been positive and significant as shown in the Table 8. This may be due to the importance of amino acids in the body-building protein to the fact that amino acids are the structural units of protein.

Table 3. Some of the minerals in cow milk during the study

Period	Ca mg/L	Mg mg/L	Fe ppm	K %
Colostrum	130.0±11.70a	76.00±3.05a	16.52±0.72a	0.82±0.10a
2 weeks	78.50±8.88b	58.00±7.27b	18.74±0.27b	0.39±0.01b
4 weeks	64.50±6.43bc	40.50±5.84c	17.70±0.33bc	0.39±0.06c
6 weeks	58.00±3.51c	44.00±7.29cd	19.69±0.53c	0.38±0.06c
8 weeks	49.50±4.5 c	47.00±3.00d	22.44±0.95d	0.32±0.02c

Means in the same row with different superscripts were significantly different (P < 0.05).

Table 4. Essential amino acids concentration of (mg \ liter) in cow's milk during the period of study.

acid	colostrum	2 weeks	4week	6 weeks	8weeks	Sig.
HIS	367.80±86.40[a]	157.50±28.59a	191.20±44.59a	174.35±35.31a	115.10±16.81a	*
Thre	173.50±34.04[a]	90.80±14.95ab	114.20±35.96a	148.00±35.96	105.10±18.37b	*
Trp	251.80±61.92[a]	175.70±39.83[ab]	118.70±32.83[b]	130.80 ± 21.79[b]	121.17±17.13[b]	*
Tyro	149.80±39.21[a]	125.00±23.00[ab]	166.70±46.65[b]	130.80 ±20.79a	119.00±12.09a	*
Val	223.49±49.48[a]	133.90±34.05a	111.00±25.19a	311.30±133.83a	221.50±47.10a	*
Met	982.90±233.24[a]	277.00±117.30a	184.30±47.78a	158.40±34.26a	141.20±27.10a	*
Iso	592.10±117.31[a]	818.60±76.77[ab]	297.20±45.10[b]	806.10±123.49[b]	224.00±40.93[b]	*
Leu	551.50±156.20[a]	395.10±64.17[ab]	209.40±49.87[b]	402.60±138.66[b]	141.10±23.61[b]	*
Ph	451.50±119.14[a]	689.30±159.14[ab]	293.20±29.89[b]	712.50±152.66[b]	195.10±26.95[b]	*
Ly	113.26±21.62a	457±30119.04[ab]	408.80±63.71[b]	318.90±111.81[b]	169.90±26.95[b]	*

Means in the same row with different superscripts were significantly different (P < 0.05).

Table 5. Non-essential amino acid concentrations(mg/liter) in cow's milk during the study.

acids	colostrum	2 weeks	4 weeks	6 weeks	8 weeks	Sign.
ASP	233.04±33.96[a]	278.30±26.89[a]	166.70±42.82[ab]	198.20±36.79[ab]	145.00±21.31[b]	*
GLU	113.26±21.26[a]	162.10±20.02[b]	97.20±15.57[bc]	100.70±26.12[bc]	76.40±10.35[c]	*
SER	320.76±118.84[a]	106.40±17.56[a]	73.70±26.93[a]	92.20±24.12[a]	81.00±13.74[a]	NS
GLY	239.96±62.67a	109±20±13.38[ab]	160.70±36.05[b]	100.90±24.26[b]	83.50±31.44[b]	*
Arg	148.60±33.46a	139.50±25.44[ab]	222.80±44.54[b]	313.60±62.03[b]	206.60±17.93[a]	*
Pro	285.80±42.65a	106.30±18.31[ab]	104.20±27.90[b]	179.80±33.80[bc]	915.91±90.90[c]	*
Cyst	939.80±153.65[a]	517.80±218.11[a]	513.90±123.91[a]	517.80±218.11[a]	410.90±51.20[a]	NS
Ala	204.00±39.94[a]	160.60±22.34[b]	111.70±32.35[b]	160.60±22.34[b]	79.30±13.86[b]	*

Means in the same row with different superscripts were significantly different ($P < 0.05$).

Table 6. Coloration coefficient between lactoferrin, milk production and some minerals

The adjectives	Corrol. coef.	Sign.level
Lactoferrin &daily milk production	-- 0.065	*
Lactoferrin &monthly milk production	-- 0.652	*
Lactoferrin & calcium	0.665	*
Lactoferrin & phosphorus	0.268	*
Lactoferrin &magnesium	0.289	*
Lactoferrin & ferrous	– 0.614	*

** Sign < 0.01 * Sign. P< 0.05

Table 7. Correlation coefficient between lactoferrin and essential amino acids

The adjectives	Correlation coefficient	Sign. level
Lactoferrin & His	0.24694	*
Lactoferrin &Thre.	0.00679	*
Lactoferrin & Trp.	0.30260	*
Lactoferrin &Tyro.	0.31887	*
Lactoferrin &Val.	0.25665	*
Lactoferrin &Met.	0.18125	**
Lactoferrin & EL. acid	0.39654	*
Lactoferrin & Leu.	0.33908	**
Lactoferrin & Phenil.	0.23851	*
Lactoferrin & Lys.	0.35479	*

** Sign < 0.01 * Sign. P< 0.05

Table 8. Coloration coefficient between lactoferrin and non-essential amino acids

The adjectives	Corre. Coeff.	Sign. level
Lactoferrin & ASP acid	0.17	*
Lactoferrin &Glu acid	0.36	**
Lactoferrin &Ser acid	0.17	*
Lactoferrin &Gly acid	0.35	**
Lactoferrin &Arg acid	0.08	*
Lactoferrin &Ala acid	0.17	*
Lactoferrin &Pro acid	0.17	*
Lactoferrin &Cyst acid	0.29	*

** Sign < 0.01 * Sign. P< 0.05

CONCLUSIONS

The results showed that the concentration of amino acids in cow's milk, varies from period to another during the experiment, due to the nutrition, also the aspartic acid observed to be high at ($P < 0.01$) colostrum (233.04 ± 33.96), also the results showed no significant differences in the presence of glutamic acid. There is a correlation between the lactoferrin ratios milk components as well as in relation to the minerals (calcium, phosphorus, magnesium). It was also shown that the amino acids are associated positive and significant with Lactoferrin. This means that any increase in the concentration of lactoferrin, accompanied by an increase in the concentration of essential amino acids in milk . The link between lactoferrin and the other non-essential amino acid has been positive and significant.

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DETERMINATION OF HEATING AND COOLING DEGREE DAYS FOR BROILER BREEDING IN THE TIGRIS BASIN

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Abstract

Nowadays, despite complex and sophisticated methods for the energy analysis in buildings, the degree-day method, which is one of the most important energy forecasting techniques, is still important. During the research, heating and cooling degree-day values were calculated using dry-bulb thermometer temperature values of long years of five cities (Diyarbakır, Batman, Siirt, Sirnak and Mardin) located in the Tigris Basin. Six different base temperature values were used for each province located in the research area in order to calculate the heating and cooling degree-day values in broiler breeding. Within the proposed six base temperature values, the most heating degree-day values were in Sirnak, and the least were in Batman province. Cooling degree-day values were calculated in the same way. The most cooling need was in Mardin province, except the base temperature value proposed for the first week. The least cooling day values were in Sirnak province. Regression coefficients were obtained by correlating heating and cooling degree-day values and six proposed base temperature values. It was determined that the regression coefficients for all provinces between cumulative heating and cooling degree-day values (dependent variable) and average annual heating and cooling degree-day values and independent variable, in other words, proposed base temperature values, varied between 0.993-0.999, and there was a very strong relationship in a positive way. If the numbers of heating and cooling degree-days are low in a region, then it can be said that the region is suitable for agricultural production. In accordance with the data obtained in the study area, it was concluded that Batman was the most suitable province for broiler breeding in terms of both heating and cooling degree-days.

Key words*: broiler, cooling degree day, heating degree day, Tigris Basin.*

INTRODUCTION

Nowadays, despite complex and sophisticated methods for the energy analysis in buildings, the degree-day method, which is one of the most important energy forecasting techniques, is still important.

In the degree-day method, the energy need of a building is basically directly proportional to the difference between the equilibrium temperature which is related to the building's indoor temperature and the outside air temperature of the place where the building is located. If the indoor temperatures of the building and indoor heat gain are stable, the energy required for the heating and cooling needs of the building can be predicted with high precision using the values obtained from the degree-day method (Bulut et al., 2007).

When the efficiency of heating, ventilation and air conditioning systems and the building's usage are stable, the degree-day method is used as the simplest energy analysis method.

Although the energy consumption of a building can be calculated using computer program packages specially developed for this purpose, the degree-day method and balance point temperature, its basic concept, remain important (Buyukalaca et al., 1999).

Variation in space heating and cooling needs is measured in degree-days method. Degree-day calculations are performed by means of long-term analysis of representative meteorological data (Sarak and Satman, 2003).

The degree-day method is commonly used to estimate energy consumption for heating and cooling in residential, commercial, and industrial buildings, as well as in greenhouses,

livestock facilities, storage facilities and warehouses (Yildiz and Sosaoglu, 2007).
The main purpose of this study was to determine the most suitable province in the Tigris Basin for broiler breeding using heating and cooling degree-day values obtained with the dry-bulb thermometer temperature values of long period of the study area.
It was aimed at guiding the manufacturers planning to operate broiler breeding in the region to select the most suitable region in terms of energy consumption.

MATERIALS AND METHODS

The Tigris basin is one of the largest basins not only in Turkey but also the Middle East. The basin has about 5.500 km catchment area within the borders of the country. Therefore, the Tigris Basin was selected as the study area. The Tigris Basin consists of Diyarbakır, Batman, Siirt, Sirnak and Mardin cities. The map showing the Tigris Basin is given in Figure 1.

Figure 1. Map of Tigris Basin

The annual outdoor dry-bulb thermometer temperatures for a long period of five cities in the Tigris Basin were obtained from the Turkish State Meteorological Service. As a production period of broiler breeding takes an average of six weeks, basic temperature values were determined for six-week periods in Table 1 (Lindley and Whitaker, 1996; Atilgan et al., 2012; Anonymous, 2013; Anonymous, 2015). Heating and cooling degree-days were determined for each province in the study area using 6 different base temperature values given in Table 1.

Table 1. Recommended weekly base temperature for broiler chicken

Weeks	Base Temperature (^{o}C)
1	31
2	27
3	25
4	23
5	21
6	18

Heating and cooling degree-days are defined as the sum of the positive differences between a base temperature and the average daily outside dry-bulb temperature for a certain period (weekly, monthly and annual) time (Eto, 1988). The number of heating and cooling degree-days can be determined using following equation 1 and 2; (Satman and Yalcinkaya, 1999; Buyukalaca et al., 2001; Krese et al., 2012; Yucel et al., 2014; Aydin et al., 2015; Erturk et al., 2015)

$$\text{For } (T_O < T_b),\ HDD = \sum_{i=1}^{n} (T_b - T_O) \quad (1)$$

$$\text{For } (T_O > T_b),\ CDD = \sum_{1}^{n} (T_O - T_b) \quad (2)$$

Where HDD and CDD are the cumulative sum of the heating and cooling degree-days for n days, n is the total number of days in the period, T_b is the base temperature recommended for the broiler chicken and T_o is the mean outdoor air temperature, These equation indicates that only positive values are summed.
Total Heating Degree Day Value (HDDV) and Cooling Degree day Value (CDDV) can be determined using following equation 3 and 4; (Buyukalaca et al., 2001; Yucel et al., 2014)

$$HDDV = \sum_{i=1}^{n} HDD \quad (3)$$

$$CDDV = \sum_{i=1}^{n} CDD \quad (4)$$

Where:
- n is the total number of days which were HDD and CDD during the selected period.

RESULTS AND DISCUSSIONS

Heating degree-day values for each city in the study area were prepared for six base temperature values. Outdoor dry-bulb thermometer temperature values of these provinces were used. A production period of broiler breeding takes an average of six weeks in our country.

The total heating and cooling degree-day values for each city were calculated by correlating the proposed base temperature values of these weeks with outdoor temperature values in the study area. Based on the proposed six base temperature values, the highest heating degree-day values were determined in Sirnak province, and the least heating degree-day values were determined in Batman province. Cooling degree-day values were calculated in the same way (Figure 2). The most cooling need was in Mardin province, except the base temperature value proposed for the first week.

The least cooling day values were in Sirnak province. It can be said that the more the balance temperatures in agricultural buildings in any region are, the less the heating and cooling energy needs of that area will be.

Hence, if the number of heating and cooling degree-days is low in a region, then it can be said that the region is the most suitable location or area for agricultural production.

In accordance with the data obtained in the study area, it was concluded that Batman was the most suitable province for broiler breeding in terms of both heating and cooling degree-days.

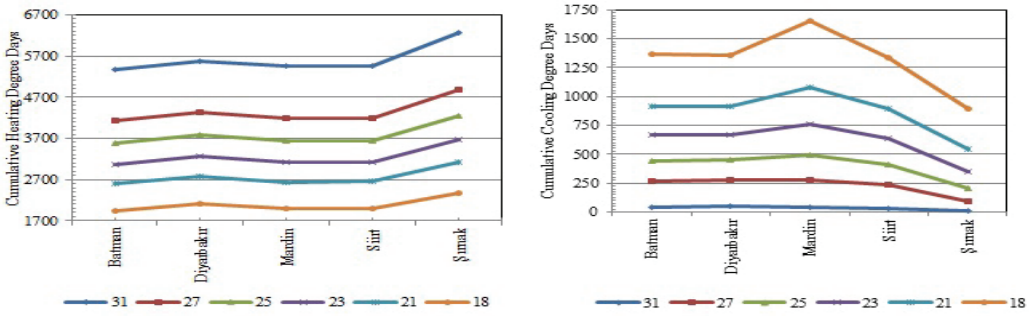

Figure 2. Heating and cooling degree-days according to the base temperature values (°C)

The number of annual average heating and cooling degree-days of the provinces located in the study area were shown in Figure 3. As can be seen from the figure again, the maximum number of average annual heating days was in Sirnak, and the maximum numbers of average annual cooling degree-days were in Mardin province.

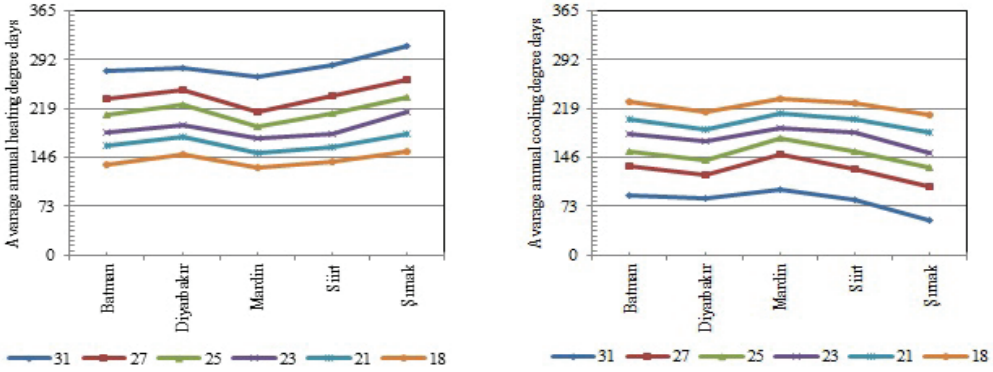

Figure 3. The number of average annual heating degree-days according to the base temperature values (°C)

Regression coefficients were obtained by correlating heating and cooling degree-day values and six proposed base temperature values. It was determined that the regression coefficients for all provinces between cumulative heating and cooling degree-day values (dependent variable) and average annual heating and cooling degree-day values and independent variable, in other words, proposed base temperature values, varied between 0.993-0.999, and there was a very strong relationship in a positive way (Figure 4-5).

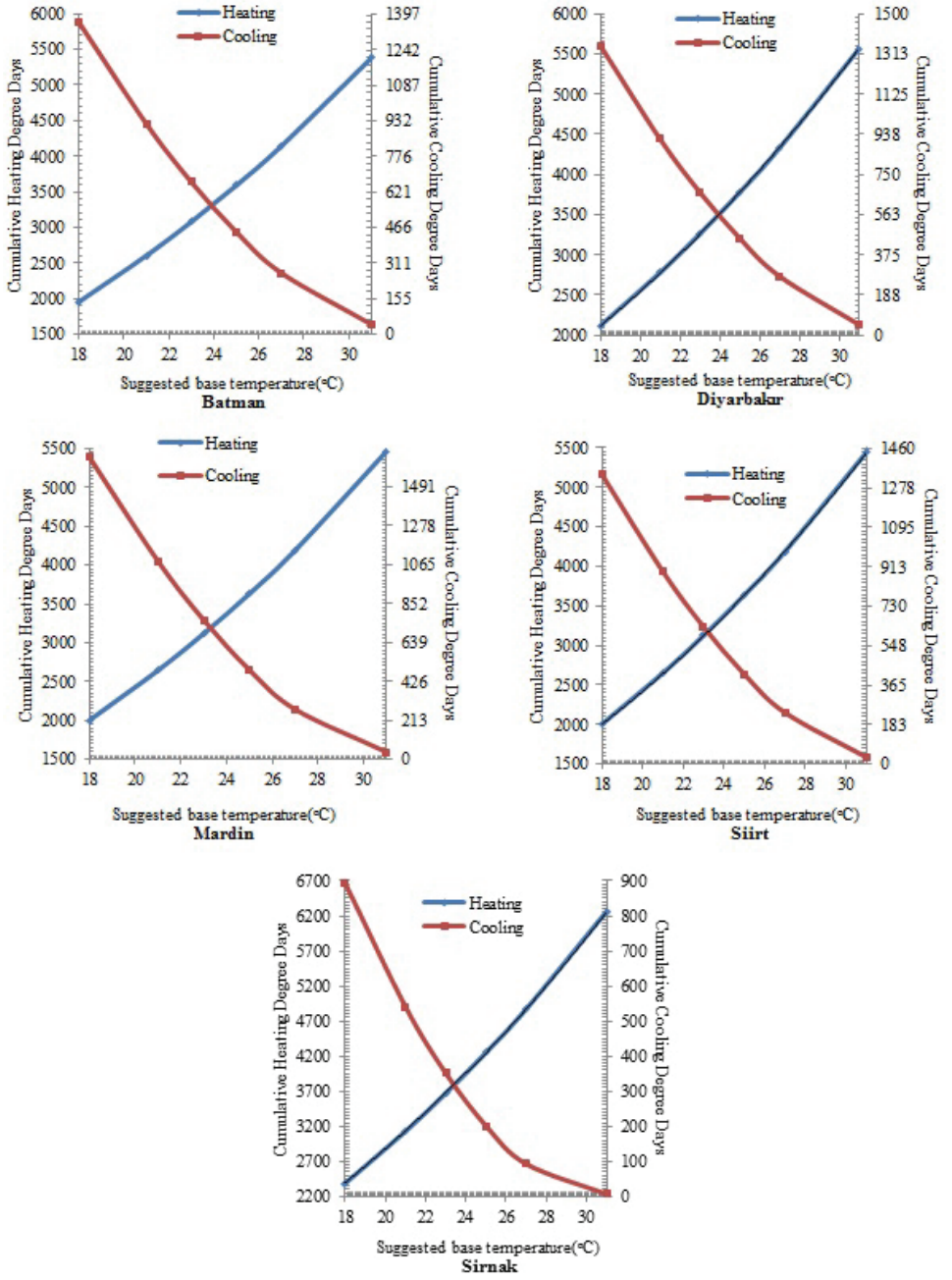

Figure 4. The number of cumulative heating and cooling degree-days according to the base temperature values

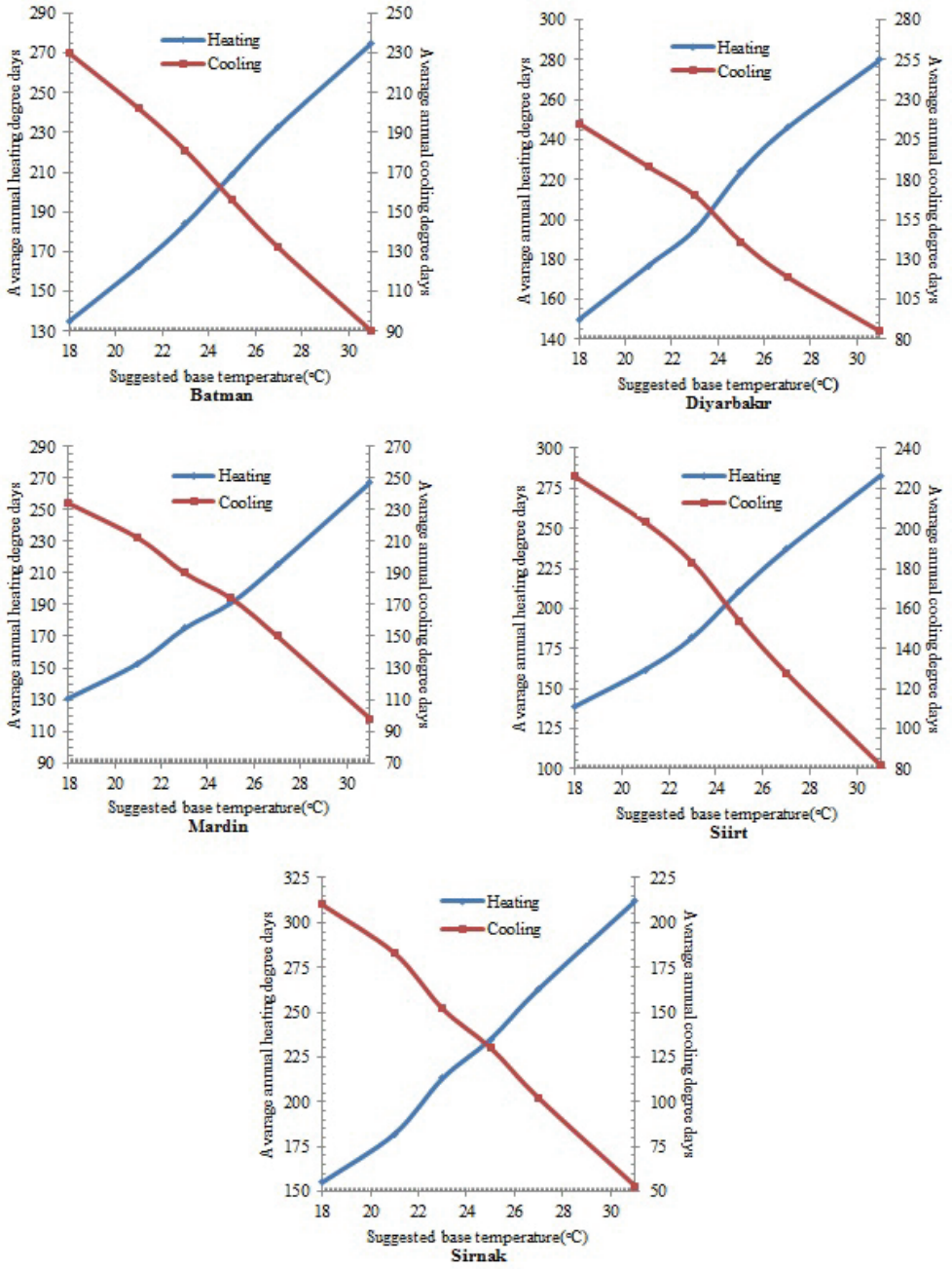

Figure 5. The number of average annual heating and cooling degree-days according to the base temperature values

The researchers reported that prior knowledge on the annual and seasonal heating and cooling energy needs of buildings could be obtained by calculating the degree-day values using the climate data of many years (Duryamaz and Kadioglu, 2003; Erturk et al., 2015).

CONCLUSIONS

The heating and cooling degree-day values based on the six different base temperature values proposed for broiler breeding were determined for five province centers located in

the Tigris Basin based on the daily outdoor air temperature data for the 55-year period. The graphics reflecting the cumulative annual heating and cooling degree-day values and average annual heating and cooling values for six different base temperature points were prepared. The most heating values were in Sirnak Province, and the least heating values were in Batman province. Batman province was determined to be the most suitable province for broiler breeding. In addition, the regression coefficients for all provinces were obtained by correlating heating and cooling degree-day values and proposed six base temperature values, and a very strong relation in a positive way was determined.

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FEED LOSS IN CUP DRINKERS IN GEESE PRODUCTION

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Abstract

The feed losses in drinkers and behavior of geese in two different systems (intensive and free-range) were investigated in this study. Day old age 216 Turkish geese were randomly allocated among 16 pens interspersed within windowed houses, each holding 12-14 goslings. All goslings were reared indoors until the end of 6 weeks of age. After 6 weeks of age, birds in the 'free-range' outdoor system were given 13-hour access. The amount of feed merged into drinkers determined weekly. The effect of production system on feed loss was significant between weeks of 7-18, and the amount of feed loss in free-range system was found higher than indoor (P<0.05). Highest feed lost was determined at 16 weeks of age. Daily mean feed loss in drinkers per goose was calculated between 9.55 and 15.93 g. It has been determined that significant amount of feed loss related to drinkers in geese production.

Key words: *geese, feed loss, drinker, production system, behavior.*

INTRODUCTION

Geese are mainly reared in intensive, free-range and cage systems (Romanov, 1999). Round feed tubs and automatic hanging waterers are the most common used equipment in these systems. Geese pollute water rapidly by playing around rather than drinking (Tilki and Saatci, 2013). Also, geese need water for swallowing feed with small particles and in powder form. Otherwise, choking could occur (Tilki and Saatci, 2013). Next to feed consuming, geese rapidly drink water and some feed particles could merge into water. Drinking water of geese is generally polluted related to these factors and polluted water causes health problems. Water quality is an important parameter on optimum fattening performance (Oviedo, 2006). On the other hand, feed merged into water affects the profitability of producers. There is not enough information about the feed losses-related to drinking or "water". The feed losses in drinkers and behavior of geese in two different systems (intensive and free-range) were investigated in this study.

MATERIALS AND METHODS

The study was conducted at the Ondokuz Mayis University Agricultural Faculty's Experimental Farm between May-September 2014. Day old age 216 Turkish geese were used as animal material in the study. All goslings were then transferred to a production house and randomly allocated among 16 pens interspersed within windowed houses, each holding 12-14 goslings, for a total of 4 replicates for production system (intensive/indoor or 'free-range'/outdoor-access). Pens (3.5 x 3.5 m) were separated by wire mesh. Each pen contained 1 round feeder and 1 round cup drinker. An 8-cm layer of wood shavings was used as litter. Heating was provided by infra-red heaters. Initial temperature was stabilized at 28±1°C; reduced by 3±1°C/per week over the next 3 weeks; and after 21 days, no artificial heating was applied. Natural lighting was applied to naturally hatched goslings during day times and economic white bulbs were used for lighting. All goslings were reared indoors until the end of 6 weeks of age. After 6 weeks of age, birds in the 'free-range' outdoor

system were given 13-hour access (6:30 a.m.-7:30 p.m.) to outdoor pens (14x3.5 m) through a single doorway (50x90 cm). Geese fed with egg-type chicken and chicken growth feed. Feed and water was given *ad libidum* throughout the production period. The amount of feed merged into drinkers determined weekly. The water-feed mixtures of each replicate were filled into bottles, dried and feed were weighed. ANOVA was used to determine the effects of production system. All data analysis was performed using the SPSS software program (Version 20.0, licensed by Ondokuz Mayis University).

Figure 1. The view of water-feed mixture in drinker

RESULTS AND DISCUSSIONS

The amount of feed loss in drinkers was given in Table 1 and weekly losses were showed in Figure 2. The effect of production system on feed loss was significant between weeks of 7-18, and the amount of feed loss in free-range system was found higher than indoor ($P<0.05$). Highest feed lost was determined at 16 weeks of age. Daily mean feed loss in drinkers per goose was calculated between 9.55 and 15.93 g.

Feed consumption behavior of geese was observed during production period. Goose lifts its head after taking the feed from feeder and then swallows. Before or after swallowing, goes towards drinker and lefts a part of feed into drinker. Besides, feed particles around beak fall to drinker.

Feed loss was found higher in free-range system. It is thought that this could be related to foraging and feed searching behavior of geese in free-range system. Correlation between feed and water consumption was reported by Lott et al., (2003). Water consumption was affected by species, activity level of bird, water quality, water temperature, environmental temperature and feed consumption (Lardy et al., 2008; Cemek et al., 2011).

CONCLUSIONS

As a conclusion, it has been determined that significant amount of feed loss related to drinkers in geese production. When considering the long fattening period of geese, the amount of economic loss will be higher, and profitability will decrease. On the other hand; the quality of drinking water decreases by pollution. This causes health and welfare problems. New studies have to be conducted investigating the effects of different drinker types on feed losses.

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Table 1. Feed loss amount in drinkers (g/goose/day)

Production Systems	Feed Loss	
	4-6 weeks	7-18 weeks
Free-range	14.36	14.88 a
Intensive		10.87 b
Standard error of means	1.33	0.75

a,b: Differences in superscript letters within columns represent significant differences between groups ($P<0.05$).

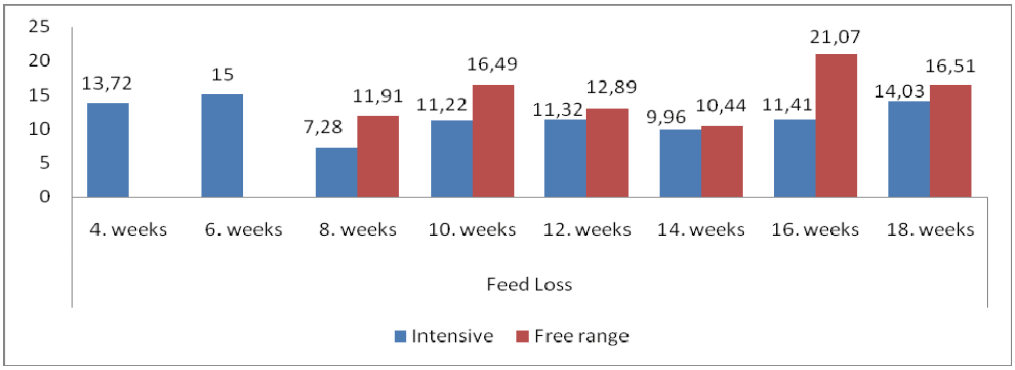

Figure 2. Feed loss amount in drinkers at different ages in free-range and intensive systems (g/goose/day).

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ECONOMIC EFFICIENCY OF GROWTH AND EXPLOITATION OF MOLDAVIAN KARAKUL SHEEP

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Abstract

The aim of this research was to estimate the economic efficiency growth and exploitation a new type of Moldavian Karakul sheep in the Republic of Moldova condition. The research was conducted on Moldavian Karakul sheep at the flock of National Institute of Animal Livestock and Veterinary Medici, Maximovca village, Anenii Noi district, the Republic of Moldova. The skins qualities of Karakul lamb have been evaluated according to the Guidelines of evaluation marks Karakul sheep with improve principles in the Republic of Moldova. Assessing the qualities of salted-dried skins and sorting it was been effected according to standards. The sheep body weight was been determined with technical scales at different times and at different ages of young sheep, as well as, adult sheep - annually, in the autumn before the beginning of campaign mating. The ewes milk production has been determined by control milking twice a month during the whole lactation. Economic efficiency of growth and exploitation of new type of sheep, compared to classic Asian Karakul sheep, as well as and with sheep from local old race Tusca was been calculated, according to the formula:

$$Ee\frac{V \cdot S}{100}K$$

where:

Ee – the economic effect calculated to one animal per year in MD Lei;
V – production value obtained from one sheep per year, expressed in units of measure;
S – the spore of obtained production, as compared with controls, expressed in%;
K – the constant spore coefficient of diminishing production, conditioning by additional costs to obtain this spore.

As a result of the research showed that the new type of Moldavian Karakul sheep has advantages compared to the classical type of the Asian Karakul, the body weight of breeding rams - with 53.1%, at the 18 months rams - with 78.5%, at the 6 months rams -with 42.5%, at the ewes - with 28.4%, at the 18 months young ewes -39.2% at the 6 months young ewes - with 29.2%, to milk production - with 53.2% on the skin surface - with 35.1%, giving way at least with share skin sort I – with 15%. Economic efficiency, resulting from the capitalization of meat production (live weight) to these groups of animals constitutes, respectively, 940.5; 239.2; 357.0; 91.5; 211.5 and 164.2 lei / head / year, the ewes milk production - 199.5 lei / head / year and lower after skin sort - with -16.8 lei / head / year. Overall, after all productive characters, new type of Moldavian Karakul sheep has economic efficiency to the race Asian Karakul equal to 274.2 lei / head / year. The Moldavian Karakul sheep possess and economic advantages to the initial race Tusca after skin quality - by 4.5 times, the rams body weight - with 53.2%, of ewes - by 30.5%, giving way after milk production -with 29 1%. In total, the economic effect of the new type of sheep, compared to race Tusca, constituted 156.3 lei / head / year.

Key words*: economic efficiency, new type, Karakul sheep, skin, milk, meat.*

INTRODUCTION

In the world there are over 600 known race of sheep production specialized in different directions of production, such as for wool, meat, milk, wool-meat, wool- meat- milk, milk-skins etc (Вениаминов А.А., 1984). In different regions of the world were created by humans, grown and spread those race of sheep than, at different stages of development of human society, satisfying the demands of society, they corresponded most appropriate traditions and local pedo-climatic conditions. Throughout the ages, race of sheep have been improved, modernized and specialized. Thus, well-developed European countries (England, France, Holland, Germany), pedo-climatic favorable conditions for intensive technologies, was spread specialized race for meat, milk, meat-wool and prolific. In undeveloped countries (Africa, Asia), with difficult pedo-climatic condition for intensive technologies, were been created and spread a large number of race with mixed ability production, late, with

low performance of milk-meat-fat, wool -milk-meat, wool –milk- skins etc.

Following, in the advanced stages of human society development, to cover the increasing requirements on the world market some specific sheep products, were created and in Asian countries (in extensive condition) performing race, specialized for skins (Karakul), meat-fat (Ghisar), wool (Australian Merinos) and others.

The Karakul sheep were spread around the world, but they acclimatized and exploiting effectively in countries and regions with huge areas of natural pastures, even barren of desert and semi desert (Central Asia, Africa), but extensive climate that allows their maintenance during the entire calendar year without capitals spaces, with minimal expense circulating costly, where sheep are grown not only to ensure the pressing needs of the rural population, but also to obtain an income from the sale of skin at the fur international tender.

In the Republic of Moldova, the sheep breeding is one of the oldest and most traditional sectors in the livestock sector. The sheep ensure the food security of the rural population with dairy products (cheese, curd) and meat, and processing industry – with raw materials (skins, furs, hides, wool). The sheep efficiently use natural grassland in food and plant remains on the stubble fields of harvested crops. Therefore, the sheep breeding is a accessible branch and indispensable for the local population and importance to the national economy.

In the period after World War II (1947-1979), the local race of Tusca sheep was, practically, substituted by Karakul race throu crossbreeding absorption en masse (Богданович Н.И., 1957; Богданович Н.И. и др., 1979, 1982, 1983, 1984; Ильев Ф.В., 1957, 1957а, 1969, 1976, 1984, 1966, 1966а; Ильев Ф.В. и др., 1966, 1981). As a result of these crossings it has been observed that the level of milk production and body weight (meat production) of local Karakul sheep began to decrease.

Research the economic value of the selection character (Buzu I. et. al., 2014) have been demonstrated that while the retail price of skin on domestic and foreign sales have stabilized at 30-40 years ago, and price at the sheep food products (meat, cheese) increased during this period by 5-10 times, growing the Karakul sheep without enhanced skills of meat and milk becomes unprofitable in the Republic of Moldova.

Considering the fact that in recent decades, the sheep in our country is growing not only for the pressing needs of the families of the rural population, but also to obtain profits for farmers, then antique Karakul sheep, with low skilled of meat and milk, no satisfy the economic requirements of society. To increase economic efficiency of the sector in the Republic of Moldova has been created a new type of Karakul sheep with mixed ability of heightened production for skins, milk, meat.

In this context, estimating economic efficiency of growth and exploitation of the new type of Moldavian Karakul sheep in the Republic of Moldova condition presents an actual problem.

MATERIALS AND METHODS

The research has been conducted on the Moldavian Karakul sheep from the flock of National Institute of Animal Livestock and Veterinary Medicine in Maximovca village, Anenii Noi district, the Republic of Moldova.

The skin qualities of Karakul lambs were been evaluated, according to the Guidelines of evaluation marks of Karakul sheep with amelioration principles in the Republic of Moldova (Buzu et al., 1996). Assessing the qualities of salted-dried skin and sorting them was been performed, according to the standards in force in the Republic of Moldova: ГОСТ 8748-70 – *Каракуль чистопородный черный невыделанный, технические условия* (1970); ГОСТ 2865-68 – *Каракуль чистопородный серый невыделанный, технические условия* (1968); ГОСТ 11124-65 – *Каракуль чистопородный сур невыделанный, технические условия* (1965); ГОСТ 11124-77 – *Каракуль чистопородный цветной невыделанный, технические условия* (1977); ГОСТ 10327-75 – *Каракуль-метис всех цветов невыделанный, технические условия* (1975); ГОСТ 10701-84 – *Каракульча чистопородная и метисная невыделанная, технические условия* (1984).

The sheep body weight was been determined by technical scales at different times and at different ages of young sheep, and adult sheep - annually, in the autumn before the beginning of

mating complain, according to the methodology perfected by us (Buzu I., 2012).

The ewes milk production was been determined by the milking control twice a month during the whole lactation by Nica T. method (1937, 1940), improved by us (Buzu I., 2014).

Economic efficiency the growth and exploitation of new type of sheep, compared to classical Asian sheep, as with local ancient race sheep Tusca has been calculated according to the method of academician Лоза Г.М. (1983). The essence of this method consists in determining of production difference obtained from sheep of different type, expressed in cash and additional expenses decreased by multiplying coefficient 0.75. Economic efficiency calculations were been performed using the following formula.

$$Ee\frac{V \cdot S}{100}K$$

where:

Ee – economic effect calculated to one animal per year, in MD lei;

V – production value obtained from 1 sheep per year, expressed in measure units (meat, milk - in kg skins - in pieces);

S – increase obtained production, compared with the control group, expressed as a percentage (%);

K – constant coefficient of diminishing spore production, conditioning by additional costs to obtain this growth.

In determining the value of production has been applied currently trading prices of these products. There have been taken into account meat production, expressed in body weight of different groups of sheep, the milks production and the skins production. The economic efficiency of a new type of sheep has been determined in report to standard the classic Asian Karakul race, as and the local Tusca race.

RESULTS AND DISCUSSIONS

The research results have been demonstrated, that the selection directional progressive carried over generations after the main productive characters, such as body weight, milk production and skins quality, leading to the formation of a new type of sheep Karakul, which differs net of the type classic Asian Karakul through superiority body weight and milk production, giving few after skin quality (share skin sort I). However, we must mention that after some skins qualities (standard usable surface of skin), these skins obtained from Moldavian Karakul lambs are superior to those of Asian Karakul. For example, in 2005 in the flock INZMV have been obtained skins with average surface by 1839±26 $cm^{2,}$ as compared with 1361 ± 32 cm^2 to the sheep skin type Asian Karakul Kazakhstan (Карынбаев А.К. и др., 2008), the firsts surpassing after this character with 478 cm^2 or 35.1% (t_d=11.6; P<0.001).

It was found, that the biggest relative difference production of new type of Moldavian Karakul sheep to the old classic - Asian Karakul there are at the body weight character and milk production (Tab. 1). Regarding the body weight, the most significant difference have 18 months rams (78.5%), reproduction rams (53.1%) and 6 months rams (42.5%). This is not casual and is explained by the fact that males at all stages of age were subjected to more exigent selection. From here, the differential and selection effect of rams is significantly larger, than females. The economic efficiency obtained from meat production (live weight) to these groups of animals is respectively 940.5; 239.2 and 357.0 lei / head / year.

More evident is the difference between females bodies weight. At the sheep, the females body weight of a new type is higher than the Asian type with 28.4%, at 18 months young ewes are - with 39.2% and at 6 months lambs - with 29.2%. The economic efficiency valued of this productivity is at sheep 91.5; at 18 months young ewes - 211.5 and at 6 months young ewes - 164.2 lei / head / year. On average in all groups of animals, obtained economic efficiency from the superiority of body weight is 334.0 lei / head/year. Significant economic efficiency in the exploitation of the new type of Moldavian Karakul sheep has been obtain, also, and at the milk production. Given that the milk production at the Moldavian Karakul sheep is with 53.2% higher, compared to the Asian Karakul sheep, economic effect from this morpho-productive character, highly important, constitued 199.5 lei / head / year.

Unlike the Asian Karakul sheep, the new type of sheep gives less (15%) those after their skin quality, so have a lower economic efficiency after this character.

Table 1. Economic efficiency of new type of Moldavian Karakul sheep, compared to classic Asian Karakul

Title of the production character, in profile on age groups of sheeps	Unit of measure	The productivity of sheep		The difference in production		Price of production, lei/unit	Economic efficiency, lei (col. 5 x col. 7 x 0.75)
		Standard race Asian Karakul	New type mean, 2003-2005	in nature	in %		
1	2	3	4	5	6	7	8
Body weight:							
Rams reprod.	kg	60	91.9	+31.9	53.1	10	239.2
Ewes	kg	43	55.2	+12.2	28.4	10	91.5
Rams 18 months	kg	42	75.0	+33.0	78.5	38	940.5
Ewes 18 months:	kg	36	50.1	+14.1	39.2	20	211.5
Rams 6 months	kg	28	39.9	+11.9	42.5	40	357.0
Ewes 6 months:	kg	25	32.3	+7.3	29.2	30	164.2
Mean at1 head	*lei*	x	x	X	x	X	*334.0*
Milk at 1 ewes	*kg*	50	76.6	+26.6	53.2	10	*199.5*
Skins sort I	*buc*	0.8	0.68	-0.12	-15	140	*-16.8*
Mean at 1 ewes	*lei*	x	x	X	x	X	*274.2*

Furthermore, because the economic value of the skins production character is smaller compared to the production of meat and milk, a decreasing of overall economic efficiency of the new type of sheep is not essential.

It was found that, overall, after all productive characters, new type of Moldavian Karakul sheep, compared to type Asian Karakul has a fairly evident economic efficiency and constituted 274.2 lei in averaging at one sheep per year.

At the same time, new type of sheep Moldavian Karakul has economic superiority compared to aboriginal race Tusca (Tab. 2).

From the data presented is noted that Moldavian Karakul sheep have economic benefits to the local race Tusca after skins quality and body weight. Thus, the share of I sort skins at the new type of sheep is 68%, or 4.5 times higher than at the race Tusca (15%). Body weight of the new type rams is higher with 31.3 kg or 53.2%, of ewes - 12.9 kg or 30.5%. The economic effect obtained from spore body weight of reproduction rams, compared to Tusca race constitued 239.3 lei / head / year and of ewes - 96.8 lei / head / year. From skins quality spore the economic effect constituted 55.7 lei / head / year.

However, we cannot mention, that mass crossing of Tusca sheep with Asian Karakul rams, which took place in the period after World War II, without taking into account the production of milk and meat, have led to loss some important qualities of this race, as milk production. Thus, the new type of Moldavian Karakul ewes gives up after milk production those from Tusca race with 31.4 kg or 29.1%. The economic effect from valorization of this character diminishes at the new type, compared to Tusca race with -235.5 lei/ head/year.

Table 2. The economic efficiency of the new type of Moldavian Karakul sheep compared to local race Tusca

Specification	Unit of measure	The sheeps productivity		Difference		The price of unit of production, lei	The economic efficiency (col.5x7x 0.75), lei
		Tusca (after Ильев Ф., 1969)	Karakul Moldavian	in nature	in %		
1	2	3	4	5	6	7	8
Body weight:							
Rams	kg	60.0	91.9	31.3	53.2	10	239.3
Ewes	kg	42.3	55.2	12.9	30.5	10	96.8
Mean at1 head	*lei*	x	x	X	x	X	*168.1*
Milk at 1 ewes	kg	108	76.6	-31.4	-29.1	10	-235.5
Skins 1 sort	buc	0.15	0.68	0.53	453.3	140	55.7
Total at the 1 sheep	*lei*	x	x	X	x	X	*156.3*

Overall, thanks to the superiority after body weight and skins qualities, the economic effect of the new type of sheep, compared to race Tusca constitutes 156.3 lei/head/year. We should be mentioned, that the work of selection and genetic improvement of Moldavian Karakul sheep populations, especially, in the direction of increasing of milk production, continued to the present.

For the special qualities of production, the new type of Karakul sheep are everywhere required in the northern and central regions of the Republic of Moldova and beyond its borders, being exported to neighboring countries, Romania and Ukraine.

Thus, during the creation of this type from creative households were traded over 1.5 thousand heads of breeding animals. Breeding rams have been supplied almost in all districts of the Northern and Central areas of the Republic, both in the individual and in different households associated.

During 1993-1994 years has been provided 314 head of sheep Moldavian Karakul in Romania (Research Station for sheep farming "Ruşeţu" Buzau).

In 2006 year have been supplied 890 head of Moldavian Karakul sheep in Frumuşica village, Tarutino district, Odessa region, Ukraine, where has been founded a breeding farm quite important with a total of over 5000 heads.

At the international exhibition "Infoinvest", which took place at "Moldexpo", Chisinau city, in 2001, the new type of Moldavian Karakul sheep has been highly appreciated by the visitors and the Committee of Experts of the exhibition, for which realization of selection in animal livestock "New type of Moldavian Karakul sheep" and authors-creators of this type have been awarded with Diploma of the III degree.

CONCLUSIONS

1. New type of Moldavian Karakul sheep has advantages compared to the classical type of Asian Karakul, at the body weight, the milk production and the skins surface, giving up less at skins sort I. The body mass of a new type of sheep is higher, compared with type of Asian Karakul sheep: at the reproductive rams - with 53.1%, at the 18 months rams - with 78.5%, of the 6 months rams - with 42.5%, of the ewes - with 28.4%, at the 18 months young ewes - 39.2% of the 6 months young ewes - with 29.2%.

2. The economic efficiency, obtained from meat production (live weight) at these groups of animals is, respectively, 940.5; 239.2; 357.0; 91.5; 211.5 and 164.2 lei/head/year.

3. The milk production of Moldavian Karakul sheep is 53.2% higher, compared to the Asian Karakul sheep. The economic effect from spore of this morpho-productive character, highly important constitued 199.5 lei/head/year.

4. The new type of sheep gives less (15%) of Asian type after skins quality (skins share of sort I), so, have a lower economic efficiency after this character -16.8 lei/head/year.

5. Overall, after all morpho-productive characters, new type of Moldavian Karakul sheep, compared to with Asian Karakul type, it has an economic efficiency equal to 274.2 lei on average at one sheep per year.

6. The Moldavian Karakul sheep possess economic advantages and as to initial race Tusca after skin quality and body weight. The skins share of sort I at the new type of sheep is 68%, which is 4.5 times higher than in race Tusca (15%). The body weight of the new type of rams is higher with 31.3 kg or 53.2%, of ewes – with 12.9 kg or 30.5%.

7. The ewes of new type Moldavian Karakul gives up after milk production those from Tusca race with 31.4 kg or 29.1%, reducing the economic effect from capitalization of this production with -235.5 lei / head / year.

8. In total, the economic effect of the new type of sheep, compared to race Tusca, constituted 156.3 lei/head/year.

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INFLUENCE OF REARING TECHNOLOGY ON FEED CONSUMPTION OF YOUNG BROILER BREEDERS

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Abstract

Study was performed to observe influence of some environmental factors (light intensity and poultry density) and of litter type on feed consumption of young broiler breeder males ROSS 308 during whole raising period (0-18 weeks). Researches are part of a massive experiment analyzing quality of semen material and breeding efficiency of broiler breeding parents. Three experimental procedures were designed (A – with analyze parameters sub-standard and litter made of chopped straws, B – with analyze parameters above standard and litter made of rice hulls and C – with analyze parameters at the level recommended by the manufacturer of biological material and litter made of wood shavings). Registered values of feed consumption were similar to those recommended by the manufacturer of biological material in all three cases (differences were not assured statistically). Comparison of average values of individuals from the three groups has revealed that those values are not significantly different. So variation of environmental factors and litter type do not affect feed consumption.

Key words: *litter, feed intake, rosters, density, light intensity.*

INTRODUCTION

Modern intensive poultry industry is based on well oiled poultry products production, processing and marketing strategies and politics. Intensive poultry production is allowing high and economical efficient productions of high quality hatchable eggs by surface unit. It is performed on litter and in climate controlled houses with performing feeding and watering systems and automatically nests which is offering a higher usage of eggs. (Appleby et al., 1992).

MATERIALS AND METHODS

Researches have been performed during two years on chicks of ROSS 308 hybrid for studying influence of some environmental factors (light intensity, bird density) feed consumption gain of young broiler breeders (Hocking, P. M. and R. Bernard, 1997).
Three trial series have been performed for this purpose:

- experiment procedure A observed influence of some environmental factors (light intensity and poultry density) at sub-standard values and litter made of chopped straws;
- experiment procedure B observed effect of raising environmental parameters beyond standard and using litter made from rice hulls;
- experiment procedure C observed effect of standard light intensity and poultry density and using litter made from wood shavings.
- Works were performed inside three farms with one farm for each experiment procedure: Avicola Călărași, S.C. Agrafood S.A. and Avicola Focșani.

Experiment procedure A was performed based on results from 4100 ROSS 308 male commercial hybrids during rising period (0-18 weeks).
Environmental parameters considered were:

- litter: chopped straws;
- sub-standard light intensity: 7 lux at 1-6 weeks, 20 lux at 6-9 weeks, 7 lux at 10-20 weeks, and 30 lux over 20 de weeks;
- sub-standard poultry density: 3 males/m2;

Experiment procedure B was performed based on results from 6000 ROSS 308 male

commercial hybrids during rising period (0-18 weeks). Environmental parameters considered were:

- litter: rice hulls;
- over standard light intensity: 30 lux at 1-6 weeks, 60 lux at 6-9 weeks, 30 lux at 10-20 weeks, 70 lux over 20 de weeks;
- over standard poultry density: 5 males/m^2;

Experiment procedure C was performed based on results from 4400 d ROSS 308 male commercial hybrids during rising period (0-18 weeks). Environmental parameters considered were:

- litter: wood shavings;
- standard light intensity: 15 lux at 1-6 weeks, 40 lux at 6-9 weeks, 15 lux at 10-20 weeks, 40 lux over 20 weeks;
- standard poultry density: 4 males/m^2;

Poultry were raised in uniform conditions inside the three units (for the three experiment procedures) on litter bed and in up-to-date houses and with feed and water delivered according to technical book of the hybrid (Aviagen, 2005). Poultry used in the three experiment procedures were fed the same way for results to be compatible.

Live weight was the parameter observed during rising period (0-18 weeks).

Classical statistical methods were used for phonotypical identification of groups as following (Sandu, 1995):

- *Student* test to compare evenness of two samples averages;
- *Fisher* test was used for several samples after a variance analyze. Calculated value F was obtained by referring square averages between samples to samples square average;
- χ^2 test was used to verify evenness of an empirical distribution (of observed frequency O_j) with a theoretical distribution (of frequency T_j).

RESULTS AND DISCUSSIONS

To emphasize the possible influence of environmental factors and litter type on feed consumption during raising period, we are showing average values of analyzed parameter for the three experimental procedures and statistical significance of differences observed between average figures. Observations and records were performed weekly during whole raising period (0-18 weeks) (Mtileni et al., 2007).

Values obtained for feed consumption from individuals in experiment procedure A during raising period are presented in Table 1 and graph from Figure 1. We mention that error of average and variability coefficient in for feed consumption were not established as in farm condition recording consumption for each individual is not possible and the issue is also proper to only pure lines in exceptional situations.

Table 1. Average values for feed consumption in the growth period, for first experience series

Week	n	$\bar{X}$ (g/day)	Standard
1	4100	26	26
2	4100	36	36
3	4100	46	44
4	4100	56	54
5	4100	63	61
6	4100	70	66
7	4100	72	67
8	4100	76	70
9	4100	79	72
10	4100	83	75
11	4100	87	77
12	4100	91	79
13	4100	94	81
14	4100	96	84
15	4100	99	86
16	4100	108	95
17	4100	111	98
18	4100	112	101
Differences significance	$\chi^2 = 16.75^{NS}$ $\chi^2_{17;0.05} = 27.59$; $\chi^2_{17;0.01} = 33.41$		

It is noticed that feed consumption values are overlapped or above hybrids standard curve. Noticed differences were tested for statistical significance between average values of analyzed parameter during the 18 weeks and hybrids technical curve and value of test χ^2 (16,75) pointing to some differences without statistical significance between the two provisions.

The issue is having special practical significance for liveweight. These differences non-significant statistically even at a feed consumption higher than standard point that litter type and environmental parameters under normal values does not affect feed intake but are stress factors not allowing birds to obtain planed dayly gains.

Figure 1. Average values for feed consumption in the growth period, for first experience series

The issue is having special practical significance for liveweight. These differences non-significant statistically even at a feed consumption higher than standard point that litter type and environmental parameters under normal values does not affect feed intake but are stress factors not allowing birds to obtain planed dayly gains.

In ROSS 308 hybrids males from experiment procedure B (Table 2, Figure 2), average feed consumption values are very close to hybrid's standard curve which is beneficial for unit' economical efficiency. Noticed differences between average values of the analyzed character during the 18 weeks and hybrid's technical curve were tested for statistical significance and value of test χ^2 (2.38) point that as in procedure A there are differences without statistical significance between effectively registered values and values recommended by hybrid's standard.

Obtained results apparently are sustaining usage of values over standard of environmental parameters and a litter containing rice hulls as weekly live weights higher than standard were obtained with a feed consumption with little difference over those recommended by hybrid's technical book. So increasing technological parameters and usage of rice hulls positively affects and boosts physiological processes contributing to obtaining the average daily gain without being stress factors for poultry at least in experimental conditions.

Table 2. Average values for feed consumption in the growth period, for first experience series

Week	n	$\bar{X}$ (g/day)	Standard
1	6000	26	26
2	6000	36	36
3	6000	44	44
4	6000	54	54
5	6000	63	61
6	6000	66	66
7	6000	74	67
8	6000	74	70
9	6000	76	72
10	6000	78	75
11	6000	80	77
12	6000	81	79
13	6000	84	81
14	6000	85	84
15	6000	87	86
16	6000	90	95
17	6000	93	98
18	6000	95	101
Differences significance	$\chi^2 = 2.38^{NS}$ $\chi^2_{17;0.05} = 27.59$; $\chi^2_{17;0.01} = 33.41$		

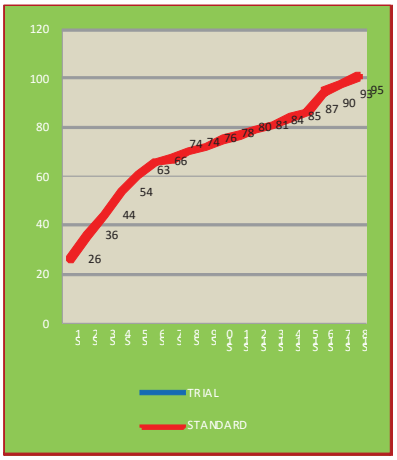

Figure 2. Average values for feed consumption in the growth period, for second experience series

Results obtained in experiment procedure C are shown in Table 3 and Figure 3 and average feed consumption values during the 18 weeks of production period are entirely inside hybrid's standard curve.

Noticed differences between average values of feed consumption during the 18 weeks and hybrid's technical curve were tested for statistical significance and value of χ^2 test (0.11) points that similar to the other experimental procedures there are differences without statistical significance between values

actually registered and those recommended by hybrid's standard.

Results obtained in experiment procedure C and their correlation with results obtained for the live weight are validating the suggestion according to whom sticking to standard environmental technological parameters and usage of a classical wood shavings litter are keeping poultry on the right growth curve with a feed intake similar to that recommended by hybrid's technical book.

Table 3. Average values for feed consumption in the growth period, for third experience series

Week	n	$\bar{X}$ (g/day)	Standard
1	4400	26	26
2	4400	37	36
3	4400	45	44
4	4400	55	54
5	4400	61	61
6	4400	65	66
7	4400	67	67
8	4400	70	70
9	4400	72	72
10	4400	75	75
11	4400	77	77
12	4400	79	79
13	4400	81	81
14	4400	84	84
15	4400	87	86
16	4400	95	95
17	4400	98	98
18	4400	101	101
Differences significance	$\chi^2 = 0.11^{NS}$ $\chi^2_{17;0.05} = 27.59$; $\chi^2_{17;0.01} = 33.41$		

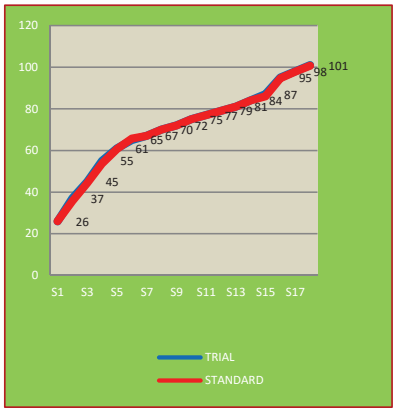

Figure 3. Average values for feed consumption in the growth period, for third experience series

Next would be pointing to and evaluation of differences which are present between average values of feed consumption la males of ROSS 308 hybrid in the 3 experiment procedures and the nature of those differences and testing their statistical significance.

Noticed differences between average values registered in the three experiment procedures for the analyzed character are being shown in Table 4 and Figure 4.

Table 4. Differences between experimental series for feed consumption

Week	Group A $\bar{x} \pm s_{\bar{x}}$ (g)	Group B $\bar{x} \pm s_{\bar{x}}$ (g)	Group C $\bar{x} \pm s_{\bar{x}}$ (g)	Observed differences A-B (g)	A-C (g)	B-C (g)
1	26	26	26	0	0	0
2	36	36	37	0	-1	-1
3	46	44	45	2	1	-1
4	56	54	55	2	1	-1
5	63	63	61	0	2	2
6	70	66	65	4	5	1
7	72	74	67	-2	5	7
8	76	74	70	2	6	4
9	79	76	72	3	7	4
10	83	78	75	5	8	3
11	87	80	77	7	10	3
12	91	81	79	10	12	2
13	94	84	81	10	13	3
14	96	85	84	11	12	1
15	99	87	87	12	12	0
16	108	90	95	18	13	-5
17	111	93	98	18	13	-5
18	112	95	101	17	11	-6
Differences significance	$F=0.60^{NS}$ $F^{51}_{1;0.05} = 19.50$; $F^{51}_{1;0.01} = 99.50$; $F^{51}_{1;0.001} = 999.50$					

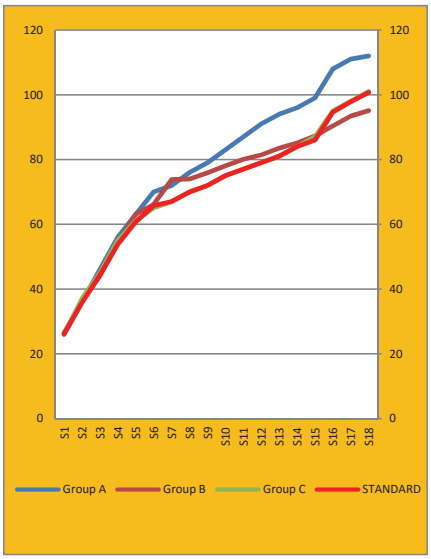

Figure 4. Differences between experimental series for feed consumption

Calculated values of Student test are higher than the presumed values which are revealing the existence of some differences with a high degree of statistical significance between average values of feed consumption for all the 3 experiment procedures. This result is supportive to the hypothesis according to which the variation of environmental parameters and of litter type is having an effect only on growth curve with no effect on feed consumption. Feed consumption is staying unchanged and very close to feed consumption recommended by hybrid's technical book with small individual variations but without statistical significance.

CONCLUSIONS

1. In ROSS 308 hybrids males cluster from grouping A average feed consumption values are being overlapping or are above standard hybrid's curve with differences with no statistical significance.
2. In ROSS 308 hybrids males cluster from grouping B average feed consumption values are being very close to curba standard hybrid's curve which is beneficial for economical efficiency of the unit (differences with no statistical significance).
3. In ROSS 308 hybrids males cluster from grouping C average feed consumption values during the 18 weeks of the rearing period are being entirely on standard hybrid's curve.
4. Calculated value of Fisher test is being smaller than critical (tabular) value which is revealing the existence of some differences with no statistical significance between average feed consumption values for all the 3 experiment procedures. This result is supportive to the hypothesis according to which the variation of environmental parameters and of litter type is having no effect on feed consumption.

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ANALYSIS ON THE EVOLUTION OF ROMANIAN SHEEP AND GOAT SECTOR AFTER EU ACCESSION

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Abstract

The paper intends to identify the main trends of the Romanian sheep and goat sector, after the accession to the EU, based on the analysis of the specific indicators regarding sheep and goat livestock and the production at national and EU level, using the data provided by the Ministry of Agriculture and Rural Development, National Institute for Statistics and European Commission. Sheep and goats are mainly present in Central, North-Eastern and South-Western Romania. About 362 thousand farmers are sheep breeders and 127 thousands are goat breeders. The average farm size is about 28.5 heads for sheep and 10.8 heads for goats. The sheep livestock increased by 29% in the analyzed period accounting for 12.288 thousands heads in 2015 and the goat livestock increased by 45%, accounting for 1.839 thousand heads in 2015. Mutton and goat meat production increased from 134 thousand tonnes in 2007 to 182 thousand tonnes in 2015. Sheep and goat milk production increased from 469 thousands litres in 2007 to 701 thousands litres in 2015. As a conclusion, sheep and goat sectors registered a increase after the accession in terms of livestock and both for meat and milk production.

Key words*: evolution, sheep and goat, European Union, sector, milk.*

INTRODUCTION

Shepherding has represented, in time, one of Romanian folk's personality manifestations, maintaining the concept of stability and cohesion in the Danube-Carpathian space. Sheep and goat breeding represent a symbol of the Romanian folk's existence and its permanent and traditional bound with nature. These have ensured the fundaments for a real domestic industry that is processing milk, wool and hide; with deep economic and social implications over the development of the material and spiritual life.

After the critical livestock decrease from the early 90s, the sheep sector is the only one that recorded an increase and from which Romania exported more than imported. In the period 2007-2015, an increase of the sheep and goats livestock can be observed, especially as regards the total livestock, where a consistent growth can be observed.

One of the reasons of this revival could be represented by the modest conditions, in terms of housing, feed and care, necessary for sheep and goats compared with other species. Both species use feed resources that cannot be put in value at an optimal level by other species, and their growth requires small energy consumption. Thus, investments in sheep and goat breeding are more profitable (Taftă, 2008).

Meat and milk obtained from the two species are nutritious product with high biological value. Sheep meat is tasty, with a high content of calcium and phosphorus but low in iron, when it's compared with beef and pork. Sheep and goat meat have the highest digestibility from all domestic ruminant species (Manole, 2008).

According to recent studies, sheep milk has a higher content of vitamins and minerals than cow's milk that are essential for growth (Ashworth, 2000).

The calcium content in the milk of sheep is between 162-259 mg/100g compared with the 110 mg/100g in cow's milk, and phosphorus, sodium, magnesium, zinc and iron content is also higher. Recent studies have shown that goat milk has many health benefits like the reduction of cholesterol deposits, but is biologically impossible to obtain milk cholesterol. Although cheese products obtained

from sheep and goat milk (i.e. Roquefort, Pecorino Romano or Feta) are consumed frequently, not the same can be said about sheep and goat milk (Ward et al., 1995).

MATERIALS AND METHODS

The study of the sheep and goat sector was made between 2007 and 2014 and intends to identify the main trends of the Romanian sheep and goat sector, after the accession to the EU.

To achieve these goals we analysed specific indicators regarding sheep and goat livestock and the production at national and EU level, using the data provided by the Ministry of Agriculture and Rural Development, National Institute for Statistics and European Commission.

RESULTS AND DISCUSSIONS

The results of the study shows that at EU level, sheep and goat farming in 2007 -2014 period was a minor agricultural activity (3.6% of the total value of livestock production) but occupies a significant portion of agricultural land in some MS (UK, Ireland, Spain, Greece, Romania and Italy) (Figure 1).

Sheep and goat breeding, at EU level, represents a small agricultural activity (3.6% from the total value of the livestock production), but in some MS (i.e. UK, Ireland, Spain, Greece, Romania and Italy it's occupying a significant percent of the agricultural land.

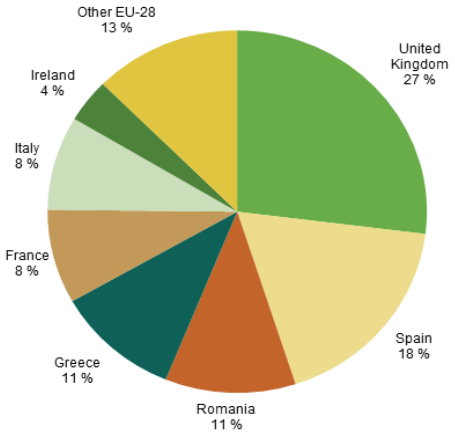

Figure 1. Distribution of sheep in the EU-28

As regards the structure of the agricultural holdings, according to the statistical data (Meat production statistics, 2015), only 7.7% from the EU farms are dealing with sheep livestock and almost half of them are farms with over 500 heads. Goat farms represent only 4.4% from the total EU farms and only 51.1% from the total goat livestock is to be found in herds with more than 200 heads. These average values conceal somehow contrasts between small flocks, which are located in small rural farms and large flocks, which are found mainly in regions with natural or environmental constraints.

Farm structures in the EU have changed little, except for France (increase of holdings), Romania (reduction of holdings number and increase of their size, as a result of EU accession) and Ireland, where an extensification of production can be observed.

As regards flock size at EU level, these are varying considerably between Member States, ranging from large flocks (23.4 million heads in the United Kingdom) to very small ones (12 thousand heads in Luxembourg).

The 14 EU Member States reporting on sheep population can be distinguished depending on the share of dairy ewes in the ewe flock (Table 1).

Table 1. Sheep livestock at the EU level - 2013

	Number of ewes (1000 heads)			% Goat milk
	Total	Milk	Meat	
Northern Group of Countries	15.679	35.0	15.373	0.2
Germany	1,126.5	11.0	1.115.5	1.0
Ireland	2,430.2	0.0	2,430.2	0.0
Hungary	855.0	18.0	838.0	2.1
Holland	600.0	6.0	594.0	1.0
Sweden	271.5	0.0	0.0	0.0
Great Britain	10,396.0	0.0	10,396.0	0.0
Southern Group of Countries	41,084.7	24,525.0	16,559.7	59.7
Bulgaria	1,157.9	1,078.0	80.0	93.1
Spain	6,697.0	6,400.0	297.0	95.6
Greece	11,281.2	2,463.3	8,818.0	21.8
France	5,479.0	1,575.0	3,904.0	28.7
Croatia	498	143.0	355.0	28.7
Italy	6.203	5,142.0	1,051.1	82.9
Portugal	1,607.4	331.2	1,276.1	20.6
Romania	8,161.0	7392.5	765.5	90.6

The 'northern' EU countries have no or limited dairy production whereas dairy ewe sheep farming is significant in the 'southern' countries.
The farming systems for sheep and goats in the 'southern' EU countries have similar levels of complexity in terms of their organisation as those for bovine animals in the EU generally, with both dairy and fattening flocks co-existing. Individual dairy animals are more profitable than meat animals and therefore they are more likely to be supported by technology than meat animals (feeding, genetic progress, farm equipment, farmer education, etc.).
The volume of milk fluctuates between MS and production regions, according to the commercial relations with cheese and the feed price. Milk processing on farms is more frequent, as daily milk volumes are lower than from cows' milk, i.e. milk collection by a dairy is suitable only where dairy farm density within the territory is sufficient (Znaidi, 2001).
Sheep and goat meat represents only 1.4% of total EU agricultural production. Milk obtained from sheep, goats and buffaloes represent 3.2% of total milk production (approx. 158 million tonnes). The profitability of holdings for sheep meat production has decreased in almost all Member States except Ireland.
Between 2007 and 2014 the sheep flocks from the 14 Member States that are reporting to Eurostat, decreased by approx. 11.4 million animals.
The drop reflects the significant fall observed in the main sheep meat producing countries. Spain lost 31% of its flock (7.1 million sheep), Portugal 30%, Ireland 22%, Germany 21% and France 18%.
The Netherlands and Croatia, with a smaller share of the total number of sheep, also decreased their flock (– 38% and – 24% respectively). The United Kingdom, which is by far the most important sheep meat producer (28% of EU-28 production), lost 3 % of its flock. In contrast, the sheep flock grew in three countries: Romania (+ 25 %) and Greece (+ 4%), where more than 90% of ewes are dairy ewes and in Sweden (+ 23%).
Goat livestock, which is mainly exploited for milk, experienced a fluctuating trend, decreasing in Greece and Spain and increasing in France. The total contribution of goat meat production in the EU-28 is modest (0.1%) and concentrated in seven Member States, representing 95.6% of the EU goat meat production. Meat can be perceived as a by-product of milk production. (Massot-Marti, 2008).
Sheep and goat breeding can be especially developed in areas with limited agricultural potential (rough grazing), but only at a low level of return per head (Pollott et Wilson, 2009). Lands with poor pasture can also be used as part of the overall business strategy, when is the case of a diversified farms. The diversity of landscapes and climatic conditions within some EU Member States often helps explain regional specialisations as regards farming (Taftă, 2006).
Sheep and goat breeding represents in Romania a traditional activity. The sheep and goat flock size is placing Romania on 4th in the European Union, after the UK, Spain and Greece, with a flock of 14.1 million heads (Table 2).

Table 2. Evolution of the main indicators between 2007-2015

Indicators	2007	2009	2011	2013	2015
Total livestock (thou. heads.)	**9.878**	**10.755**	**11.331**	**12.809**	**14.127**
Sheep (thou. heads)	8.771	9.641	10.003	11.203	12.287
Goats (thou. heads)	1.015	1.133	1.327	1.605	1.839
Meat production (thou. tonnes)	**134**	**141**	**150**	**170**	**182**
Sheep	122	127	135	152	162
Goats	12	14	15	18	20
Milk production (thou. hl)	**4.691**	**5.099**	**5.263**	**5.608**	**7.013**
Sheep	3.338	3.607	3.795	4.024	5.037
Goats	1.353	1.492	1.467	1.583	1.976

The sheep and goat flock increase, as well as meat production increase that can be observed in recent years was a result of the subsidies policy granted by authorities:

- towards the improvement of sheep and goat population exploited for meat production;

- towards farms with flocks of minimum 50 sheep and 25 goats.

In Romania, the aid allowed an improvement of genetics and technology, which was accompanied by an increased production (ANT International, 2011).

As regards the ratio between the two species (sheep/goats), this has undergone slight modifications from 8.64/1 in 2007 to 6.68/1 in 2015 in the favour of the goat sector, but the potential of these two species is still not used entirely.

In 2014, the total number of sheep and goat farms was 480 thousand, of which 73.95% were sheep farms and 26.05% goat farms. Sheep livestock decreased in farms with less than 10 heads (from 28.83% in 2007 to 15.47% in 2014). The same phenomenon can be observed in farms with a livestock between 11- 100 heads, which also fell from 36.86% in 2007 to 28.02% in 2014.

In the same period the flocks have concentrated in farms between 101-500 heads (from 26.51% in 2007 to 40.28% in 2014) and in farms with more than 500 heads (from 7.80% in 2007 to 16.23% in 2014) (Romanian Statistical Yearbook, 2014). This shows an increase in the number of medium and large size farms at the expense of small farms, as well as a specialization for the type of production (i.e. meat or milk).

The production of sheep and goat meat increased by 27% in analysed period (from 134 thousand tonnes in 2007 to 182 thousand tonnes in 2015). When we analyse separately each species an increase in production by 25% for sheep meat can be observed (162 thou. tonnes in 2015) and by 40% for goat meat (20 thousand tonnes in 2015).

Total milk production increased by 33% from 4691 thousand hl 2007 to 7013 thousand in 2015. Milk production in sheep represents 73% of total production in 2015.

In 2014, the average sheep farm size, nationwide, was 28.57 heads/farm compared to 11.96 heads/farm in 2007 and the average goat farms size was 10.74 goat heads/farm compared to 4.33 heads/farm in 2007. However, the average size of holdings is still small for the implementation of intensive growth technology (Table 3).

Table 3. Structure of sheep and goat farms in 2014

HOLDINGS	NUNMBER OF EWES					
	ANIMALS (THOU. HEADS)		% OUT OF TOTAL		HEADS PER HOLDING	
YEARS	2007	2014	2007	2014	2007	2014
TOTAL	6375	10150	100.00	100.00	11.96	28.57
< 10 HEADS	1838	1569	28.83	15.47	4.32	6.28
11 - 20 HEADS	847	640	13.29	6.31	14.13	15.48
21 - 50 HEADS	671	780	10.53	7.68	28.83	32.62
51 - 100 HEADS	831	1424	13.04	14.03	64.79	72.24
101 - 200 HEADS	797	1639	12.50	16.15	109.51	148.78
201 - 500 HEADS	893	2449	14.01	24.13	283.54	338.09
> 500 HEADS	497	1648	7.80	16.23	661.36	814.15
	NUMBER OF GOATS					
TOTAL	630	1397	100	100.00	4.33	10.74
< 10 HEADS	331	553	52.55	39.58	2.44	4.91
11 - 50 HEADS	167	411	29.43	29.43	20.17	32.13
> 50 HEADS	132	433	30.99	30.99	74.63	94.15

As regards trade balance, the sheep meat sector recorded a positive trend, in recent years, by ensuring the necessary domestic consumption and creating export facilities.

As shown in Figure 2, meat trade balance is positive for sheep and goats, where the imports represent only 2.48% of the meat exported.

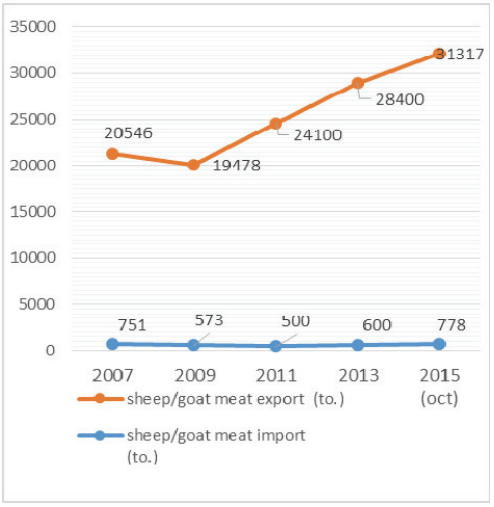

Figure 2. Evolution of sheep/goat meat exports and imports 2007-2015

Romania is the largest exporter of sheep and goat meat to third markets from the EU. In 2014 according to European Commission

statistics, Romania exported 22 thousand tonnes of mutton carcass on third markets.
This quantity represents 62% of EU mutton carcass exports to third countries, purchased in 2014. The main export destinations for sheep and goat meat are the Arab countries. (Meat production statistics, 2015).
Consumption of sheep and goat meat lies at national level on the 4th place after beef, pork and poultry, accounting for approximately 10% of total meat consumption in Romania.
Average sheep meat consumption is around 2.1 kg/capita compared to 2.9 kg/capita in the EU. Domestic consumption of mutton has increased slightly from an average of 1.4 kg/capita in 2007 to 2.1 kg/capita in 2015.

CONCLUSIONS

Sheep and goats livestock, as well as milk and meat production increased in the analysed period, thus Romania is ranked on the fourth place as regards livestock and production in the EU.
Sheep and goat meat consumption is increasing and, at the same time, the interest of other EU countries for organic products obtained from sheep and goat meat has increased.
Romania has a huge potential to produce mutton and goat meat especially for Arab countries, but also for the EU market, where there is a shortage of supply.
Sheep and goat meat production can grow by increasing the livestock and especially by improving the quality of the genetic material used for meat breeds and hybrids. This will lead to effective utilisations of pastures and meadows.
Particular attention should be paid to the size of fattening farms, as well as carcass classification, according to EU standards. In this way, the Arab and the EU market will be regained, and the meat trade will further improve the trade balance. Large export of live rams should be transformed in export of meat carcass.
Structure of holdings should be directed to fatten young animals and farm efficiency should be improved by moving from self-consumption to commercial production.

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"TURKISH REPUBLIC" EGG PRODUCTION RISING BRAND IN EUROPE

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Abstract

The egg is one of the sources of animal protein, which is obtained easily and cheap in all around the world. The potential of egg production in the poultry industry in Turkish Republic (TR) is located in the top rankings. The importance of the poultry industry in TR has increased together with intensely increase of avian influenza especially in European countries. Although TR have unplanned and unbalanced development in the egg production industry, it is one of the largest egg producers among the EU countries. Additionally, the egg production industry is growing rapidly in TR, but use of own parents for hybrid production materials (hybrid of the egg-laying hens) is very few. The aim of present study was to evaluate egg production level in European Union vs. Turkish Republic.
This work was supported by the Ahi Evran University Scientific Research Projects Coordination Unit. Project Number: ZRT.E2.16.006

Key words: *layer hens, egg production, Atabey, ATAK, ATAK-S.*

INTRODUCTION

Since the first times, people consume too intense eggs are as animal protein sources with healthy animal product (Filik, 2009). People daily nutritional needs meet to from animal and vegetable products. Especially, majority protein requirements of people in developing countries have been met to vegetable protein. However, to health growth and development from birth to adult of human need to this vital importance of animal protein sources. Egg is accessible, the cheapest and easiest animal protein source which provide animal protein demand of people. A laying hen in 4.5-5 months gives an egg between 50-65 g per day. The egg contain some component such as the yolk (30-33%), albumen (approximately 60%) and the shell (9-12%) (Ahmadi and Rahimi, 2011). Until nowadays such accessible, the cheapest and easiest another source of animal protein is not produced. They can make quick product of laying hens. Therefore it makes important for egg production forever.

The potential of egg production in the poultry industry in Turkish Republic (TR) is located in the top rankings. The importance of the poultry industry in TR has increased together with intensely increase of avian influenza especially in European Union (EU) countries. Although TR have unplanned and unbalanced development in the egg production industry, it is one of the largest egg producers among the EU countries. Additionally, the egg production industry is growing rapidly in TR, but use of own parents for hybrid production materials (hybrid of the egg-laying hens) is very few. The aim of present study was to evaluate egg production level in EU vs. TR.

MATERIALS AND METHODS

Overview of egg productions

World Egg Production

Increasingly growth population of world has more demand for animal protein. While the world's population was 5.735.123.084 in 1995, in 2013 was 7.181.715.139. Also, egg production had been showed similar progress (FAO, 2016). In order to live a healthy life each person can consume eggs a day. The amount of eggs had been 178.9 pieces/per person in 2013 in the World. This situation is observed that the consumption of one egg every two days. From 1995 to 2013 year the increase approximately 20% of world's total population, the total egg

production 38%. But these situations cannot enough and food product needs per person is increasing every year. Especially, balanced nutrition the amount of animal protein production is well below the requirements. The elimination of these deficiencies can be the easiest and cheapest eggs. As shown in Table 1, while world population increased quickly, egg production was increasing as well. In this case the people can meet need animal protein, but it is not produced in sufficient quantity in most countries.

Table 1. The amount of population and egg production by years (FAO, 2016)

	1995	2000	2005	2010	2011	2012	2013
World	**5.735.123.084**	**6.126.622.121**	**6.519.635.850**	**6.929.725.043**	**7.013.427.052**	**7.097.500.453**	**7.181.715.139**
Growth Rate%	100.00	6.39	6.03	5.92	1.19	1.18	1.17
Eggs, hen, in shell (1.000)	**798.963.046**	**959.227.602**	**1.066.197.652**	**1.206.342.397**	**1.229.055.185**	**1.251.373.092**	**1.284.449.014**
Growth Rate%	100.00	16.71	10.03	11.62	1.85	1.78	2.58
EU	**483.502.026**	**486.649.906**	**494.240.613**	**502.083.739**	**503.032.828**	**503.716.322**	**504.219.112**
Growth Rate%	100.00	0.65	1.54	1.56	0.19	0.14	0.10
Eggs, hen, in shell (1.000)	**114.037.584**	**114.638.008**	**115.291.853**	**113.727.924**	**114.236.447**	**111.797.910**	**116.854.446**
Growth Rate%	100.00	0.52	0.57	-2.01	0.46	-2.10	4.83
TR	**58.522.320**	**63.240.157**	**67.860.617**	**72.310.416**	**73.517.002**	**74.849.187**	**76.223.639**
Growth Rate%	100.00	7.46	6.81	6.15	1.64	1.78	1.80
Eggs, hen, in shell (1.000)	**10.268.668**	**13.508.586**	**12.052.455**	**11.840.396**	**12.954.686**	**14.910.774**	**16.523.180**
Growth Rate%	100.00	23.98	-12.08	-1.79	8.60	13.12	9.76

EU Egg Production

While EU constitutes 14.24% of world population and they produce 10.99% of total egg. Also, yearly egg production is defined as 231.8 pieces/per person in 2013 in EU. Despite being above the world average, the number of eggs for per person is low. This data shows that there isn't a relationship between the consumption of eggs with level of development in Table 2.

Table 2. The total egg productions of TR and EU Countries (FAO, 2016)

Country	2010 (1.000)	2011 (1.000)	2012 (1.000)	2013 (1.000)	2013 EU Countries (%)
Turkish Republic	11.840.396	12.954.686	**14.910.774**	**16.523.180**	-
France	**15.093.962**	**14.087.635**	14.155.132	15.749.808	13.48%
Italy	13.157.100	13.482.000	13.660.700	13.839.000	11.84%
Germany	10.190.800	12.035.000	12.799.500	13.736.000	11.75%
Spain	12.896.400	12.995.363	11.409.116	11.787.412	10.09%
United Kingdom	11.274.000	11.200.680	10.806.200	11.517.000	9.86%
Netherlands	10.177.000	10.485.000	10.182.000	10.651.500	9.12%
Poland	11.124.031	10.373.000	9.536.000	10.041.940	8.59%
Romania	5.950.700	6.085.500	6.234.200	6.158.760	5.27%
Belgium	2.681.098	2.694.083	2.436.752	2.754.480	2.36%
Hungary	2.730.000	2.459.550	2.357.900	2.487.500	2.13%
Portugal	2.382.000	2.233.000	2.190.580	2.280.945	1.95%
Czech Republic	2.125.096	2.168.203	2.001.334	2.159.539	1.85%
Greece	1.996.000	2.000.000	2.040.000	2.060.000	1.76%
Sweden	1.762.000	1.840.000	1.936.500	2.047.620	1.75%
Austria	1.550.000	1.683.200	1.728.100	1.744.200	1.49%
Denmark	1.279.300	1.309.400	1.336.300	1.367.150	1.17%
Slovakia	1.176.000	1.244.103	1.242.487	1.261.036	1.08%
Bulgaria	1.430.951	1.178.471	1.168.997	1.187.823	1.02%
Finland	976.200	996.800	987.300	1.061.900	0.91%
Ireland	789.500	793.000	801.000	810.000	0.69%
Lithuania	774.000	729.000	725.000	716.000	0.61%
Latvia	714.133	664.914	671.123	629.175	0.54%
Slovenia	357.086	357.086	369.204	322.704	0.28%
Cyprus	156.600	172.450	174.320	197.500	0.17%
Estonia	176.163	176.877	171.955	181.500	0.16%
Malta	82.100	72.255	63.530	75.200	0.06%
Luxembourg	21.585	28.277	27.723	28.754	0.02%

As shown in Table 2, according to the data of 2013 France is ranked first with 13.48% rate in 28 countries member of the EU. Second and third place, in most countries the volume of production was Italy and Germany, 11.84% and 11.75% respectively.

TR Egg Production

While TR was constitute 1.06% rate of the world's population, it was constitutes 1.29% rate in egg production. When analyzed in terms of population, TR was 15.11% of the population of the EU. When analyzed in terms of total egg production, TR seems to be more of the production member countries with 14.14% rate (Table 2). But TR is 216.8 pieces/per person in 2013.

This value is above the world average but it has emerged a value below the EU. In recent years, despite the increase the amount of egg production in TR, it was not desired value (Figure 1).

TR is located in the top rankings in terms of egg production in the poultry industry. Additionally, the egg production industry is growing rapidly in TR. TR begin to use Atabay (white), ATAK and ATAK-S (brown) hybrid the egg-laying hens, which is developed by Poultry Research Institute, Food Agriculture and Livestock Ministry and increasing use of these animals to be reduce amount of imported laying hens in TR.

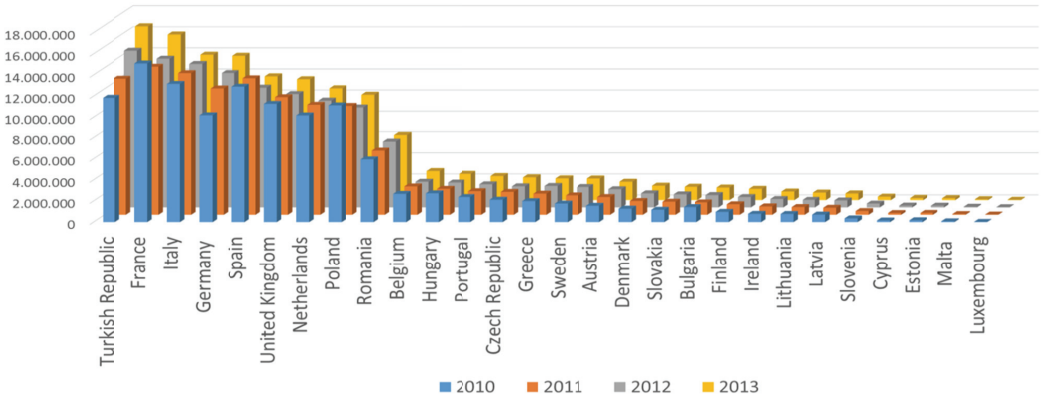

Figure 1. The total egg productions of Turkish Republic and EU Countries (FAO, 2016)

RESULTS AND DISCUSSIONS

New Domestic Hybrid Laying Hens

In recent years, poultry sector made significant improvements in TR that continues to grow rapidly. Especially, TR was imported breeding laying hens and broilers. Food Agriculture and Livestock Ministry has made forward-looking investments for TR that have currently national breeding broiler production project and improved hybrid breeding laying hens developed by Poultry Research Institute. Import substitution in the country is provided significantly. Thus, national genetic resources that instead of chicken imported at high prices have been developed. Thus, producers are provided to reach easier and cheaper the layer hen breeding eggs. Therefore, it is trying to increase the amount of production the characteristics of laying hens domestic hybrid in TR is given Table 3. According to TAEM (2015), it is seen that good at 72 weeks of age performance data of hybrid in the brown and white egg layers breeding.

Table 3. Domestic hybrid in TR and commercial laying hens (TAEM, 2015; ISA, 2016)

Egg Color	**BROWN**			**WHITE**	
Origin	**TR**		**Commercial**	**TR**	**Commercial**
Hybrid Name	**ATAK-S**	**ATAK**	**ISA**	**ATABEY**	**ISA**
Egg Production					
Age at 50% Production (day)	144	147	144	150-155	142
Peak Percentage (%)	96	95	96	96-97	96
Over 90% of the time (week)	18-20	16-18	-	23-25	-
Over 80% of the time (week)	42-47	34-38	-	41-45	-
Hen-Day Egg Number			**18-90 Weeks**		**18-90 Weeks**
72 Weeks	314	307	-	317-320	-

Egg Number Hen Housed			**18-90 Weeks**		**18-90 Weeks**
72 Weeks	312	304	409	313-315	413
Egg Mass Hen Housed			**18-90 Weeks**		**18-90 Weeks**
72 Weeks (g)	20.000	18.750	25.700	19.200	26.100
Average Egg Weight			**18-90 Weeks**		**18-90 Weeks**
72 Weeks (g)	64.7	62	62.9	61-62	63.1
Feed Intake					
1-18 Weeks (kg)	6.9	6.5	2.14	5.5	2.11
Average Feed Consumption per day (g)	115-118	105-110	109	101-105	109
Body Weight					
20. Weeks (g)	1680	1538	1975	1350	1720
End of Term (g)	2410	2000		1680	
Liveability					
Rearing (%)	97-98	96-97	93.9	97-98	95.3
Laying Period (%)	96-97	96-97		95-96	

CONCLUSIONS

TR increases the potential production using existing hybrid that developed by Poultry Research Institute, Food Agriculture and Livestock Ministry. Therefore, TR is trying to brand to become a major supplier of egg market in EU.

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COMPARATIVE STUDY ON FITNESS TRAITS AND REPRODUCTIVE EFFICIENCY IN TURCANA AND TSIGAI EWES

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Abstract

Aim of the current comparative study was to evaluate health, reproductive rates and fitness indicators in Tsigai and Turcana sheep breeds reared under semi-intensive production system conditions. The project herd is consisted of 203 Tsigai (Szombor ecotype) and 226 Turcana (Sibiu ecotype) breeding ewes. The Tsigai ewes were more affected ($p \leq 0.01$) by clinical mastitis compared to Turcana ewes, with an incidence of 10.8±0.21% and 3.11±1.16%, respectively. The Tsigai ewes produced significantly higher litters ($p \leq 0.001$) compared to Turcana breed, 1.45±0.04% compared to 1.17±0.02%. Lambs weaning rates were not influenced by the genotype ($p > 0.05$). This comparative study was the first attempt to provide information on the reproductive efficiency and health traits in Tsigai and Turcana breeds under temperate climate conditions found in Eastern Europe. For the selected specialized Sombor Tsigai ecotype it would be advisable to include fitness traits into the breeding selection schemes in order to improve animal welfare and overall productivity.

Key words*: Tsigai, Turcana, sheep welfare, reproductive efficiency*.

INTRODUCTION

Zackel and Tsigai sheep are widely dispersed throughout Central, Eastern and Southern Europe, being considered as the two main indigenous breed groups found in this area (Papageorgiou 2011; Draganescu and Grosu 2010; Kusza et al., 2008). Moreover, Lawson-Handley et al. (2007) have recently stated that the Tsigai group is strongly influenced by Zackel, and merged the two groups in their studies regarding the genetic structure of European sheep breeds. Zackel (mountain) and Tsigai (lowland) sheep are usually dual-purpose breeds, being reared for meat and milk. Romania has a national flock of 9.508.293 sheep and 1.282.011 goats, with numbers increasing by 5 to 8% each year for both species in the last 5 years (Padeanu, 2014). Also, our country has a pasture surface of 4.9 million ha, which could sustain a flock of up to 16 million small ruminants (Dragomir, 2009).

Over 85% of sheep and goats are being reared under extensive ,low-input' production systems (Belibasaki et al., 2012), with the breed structure being dominated by indigenous rustic breeds, such as the dual-purpose Turcana (Zackel group) and Tsigai sheep (Ilisiu et al., 2013), representing 52.4% and 24.3% from the breed structure, respectively.

Selection schemes practiced in Romania for sheep are focused solely on production levels (milk yield, growth rates and prolificacy), with no selection traits for fitness.

Moreover, the incidence and implications of technopaties such as mastitis and lameness have not been studied in the Romanian flocks. To the best of our knowledge, no other comparative study concerning the reproduction and health traits of Tsigai and Turcana sheep breeds under semi-intensive European rearing conditions exists up to this moment. Furthermore, this is the first attempt to define fitness traits in the indigenous Turcana breed.

Aim of the current comparative study was to evaluate health, reproductive rates and fitness indicators in Tsigai and Turcana sheep breeds reared under semi-intensive production system conditions.

MATERIALS AND METHODS

Location and flock management

The study was carried out in a commercial farm from Sanpetru Mare - Timis county, western Romania (46°3′N/20°40′E). Tsigai and Turcana purebred ewes were included in the study and were managed semi-intensively under identical rearing conditions for a period of one production year (Table 1).

Ewes were between 1.5 and 8 years old, with age and parity balanced across breeds and representing a diverse sampling of genetic lines within each breed. The project herd in consisted of 203 Tsigai (Szombor ecotype) and 226 Turcana (Sibiu ecotype) breeding ewes. No external ewes were included in the experimental herd. All replacement ewes for the two breeds were produced and added to the breeding herd at the age of 1.5 years. The production year started at 1st of September 2014, when the ewes were put to ram (for 3 consecutive oestrous cycles, roughly 55 days) and ended in late August 2015.

Sanpetru Mare region has a typical Central European humid continental climate, with the farm being located at an elevation of 85 m above sea level and a total annual precipitation of 536.3 mm, with a mean annual temperature of 10.8°C.

Table 1. Number of ewes in the study-herd and data on the general rearing conditions

Tsigai ewes	203	Turcana ewes	226
Location	46°3′N/20°40′E	Annual temp.	10.8°C
Altitude	85 m	Precipitation	536.3 mm
Housing	90 days/year	System	Semi-intensive
Weaning	60 days	Creep feeding	*ad libitum*

Both ewes and their lambs were kept confined on deep straw bedding for a period of 90 days during winter (mid December - mid March), with a space allowance of 1.2 m^2 and 1 m^2, respectively. Ewes received high-quality alfalfa and lolium hays *ad libitum*, with an additional 300 g concentrate in late gestation and during lactation period. Lambs were creep fed (*ad libitum*, 16% crude protein) and weaned at 60 days of age. Nutritional flushing was not practiced. And grazed on a natural fenced pastures for the rest of the year (stoking rates of 5-7 sheep/ha). In addition, all animals had free access to potable water twice per day and mineral blocks year around.

The research activities were performed in accordance with the European Union's Directive for animal experimentation (Directive 2010/63/EU).

Data and statistical analysis

Occurrence rates of the following health disorders were recorded by the veterinarian: mastitis, lameness, pneumonia and abortion. Annual attrition rates were determined by identifying ewes in the herd at the start of the production year not present in the herd at the end of the production year. Death and culling because of all reasons were included when evaluating attrition rates. Data on the reproductive performance of ewes (conception rate, litter size and survival rates of un-weaned lambs) were recorded for both breeds.

In order to assess the effect of the genotype (breed) on the above-mentioned health disorders, as well as on the reproduction performance of the ewes, the STATISTICA software was used (Hill and Lewicki, 2007). The Main Effect ANOVA analysis of variance was applied. The model used for statistical analysis is presented below:

$$y_{ijk} = \mu + g_j + e_{ijk}$$

where y_{ijk} is the studied reproduction or health trait; μ is the overall mean; g_j represents the random effect of the genotype with two levels: Tsigai and Turcana; and e_{ijk} is the residual effect. When significant effects of the genotype were observed, the comparison between breeds was tested by performing contrast analysis, using Tukey test.

RESULTS AND DISCUSSIONS

Taking into account the clinical mastitis occurrence rate, the Tsigai ewes were more affected ($p \leq 0.01$) compared to Turcana ewes, with an incidence of 10.8% and 3.11%, respectively (Table 2). Results for the Tsigai breed are consistent with estimates of Giadinis et al. (2011), which report occurrence rates for mastitis of over 10% in commercial sheep flocks. Mastitis has a negative impact on ewe stay ability, milk yield, lambs growth and survival, veterinary costs and represents a major concern for animal welfare. Moreover, the importance of mastitis in Europe was

outlined by Ligda et al. (2003), who found mastitis to be the main cause for culling in the Greek dairy sheep industry, accounting for 46% of the attrition cases.

Table 2. Means (±SE) for occurrence rates of mastitis, lameness, pneumonia and abortions in Tsigai and Turcana ewes

Genotype	Mastitis (%)	Lameness (%)	Pneumonia (%)	Abortion (%)
Tsigai	10.8±0.21	6.4±1.72	5.4±1.59	3.4±1.28
Turcana	3.11±1.16	2.6±1.08	1.3±0.76	1.7±0.88
Differences	**	*	*	*

$^{NS}p>0.05$; $^{*}p\leq0.05$; $^{**}p\leq0.01$; $^{***}p\leq0.001$

Current study highlights the low resistance to clinical mastitis of the Sombor Tsigai. As a result, measures such as including the genetic resistance to mastitis as a trait into the breed's selection scheme should be undertaken. The incidence of clinical mastitis in small ruminants is generally lower than 5% (Arranz and Gutierrez, 2012), whereas the prevalence of sub-clinical mastitis ranges from 10% to 50% in dairy flocks (Sechi et al., 2009).

Lameness occurrence rates were of 6.4% and 2.6% in Tsigai and Turcana ewes, respectively. Significant differences ($p\leq0.05$) were observed between the two breeds.

Pneumonia incidence in sheep flocks is of concern to both breeders and veterinarians, especially in Tsigai sheep, a lowland semi-fine wool breed. Differences ($p\leq0.05$) being registered between the Tsigai and Turcana breeds. Higher pneumonia incidence in the Sombor Tsigai (5.4%) could be attributed to the upgrading of the breed with Italian Mediterranean Bergamasca breed (Cinkulov et al., 2008), which is not adapted to the lower climate during winter found in Central and Eastern Europe.

Occurrence rate of the abortions was influenced by genotype ($p\leq0.05$) in the two studied breeds. According to previous reports, in healthy flocks abortion accounts for less than 2%, with 5% occurrence rate being considered as an alarm threshold (Menzies, 2011).

Tsigai and Turcana ewes expressed similar ($p>0.05$) conception rates (Table 3). Conception rates for the Tsigai ewes are consistent with those estimated by Krupova et al. (2009) for the breed, and those for Turcana are in agreement with reports of Padeanu (2014).

The Tsigai ewes produced significantly higher litters ($p\leq0.001$) compared to Turcana breed. Considerable lower values for litter size in Tsigai are reported by Krupova et al. (2009) and Padeanu et al. (2012). Higher litter size in the Tsigai ewes during current trial might be attributed to the good feeding and management conditions, this aspects might have led to a better body condition of ewes during mating season and thus to higher ovulation rates. For the Turcana breed, the litter size is similar to reports of Budai et al. (2013).

Table 3. Means (±SE) for reproductive performance and attrition rates in Tsigai and Turcana ewes

Genotype	Conception rate (%)	Litter size (lambs)	Weaning rates (%)	Attrition rate (%)
Tsigai	96.0±1.37	1.45±0.04	95.5±1.20	21.1±2.87
Turcana	97.7±0.98	1.17±0.02	96.9±1.06	14.2±2.33
Differences	*NS*	***	*NS*	*

Lambs weaning rates were not influenced by ewes genotype ($p>0.05$). These findings are consistent with reports of Padeanu et al. (2012) and Gavojdian et al. (2013) for Tsigai and Turcana lambs, reared under European temperate conditions.

Differences in attrition rates between Tsigai and Turcana breeds were not significant ($p>0.05$), although there is a statistical tendency for significance ($p=0.0590$). The genotype disparities in attrition rates (21.1% compared to 14.2%) could be explained by the considerable lower selection pressure applied for the Turcana ewes, and as a result, decisions on voluntary culling of ewes were made only in extreme cases for the genotype. While in Tsigai flock the voluntary culling of ewes occurred especially based on traits such as milk yield, fertility, weaning ability, age, body condition and health disorders. Data on attrition rates for the Tsigai and Turcana breeds are consistent with estimates reported by Mekkawy et al. (2009) and Gavojdian et al. (2015).

CONCLUSIONS

This comparative study was the first attempt to provide information on the reproductive efficiency and health traits in Tsigai and

Turcana breeds under temperate climate conditions found in Eastern Europe.
For the selected specialized Sombor Tsigai ecotype it would be advisable to include fitness traits into the breeding selection schemes (with special focus on mastitis genetic resistance and ewe stayability) in order to improve animal welfare and overall productivity.

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THE INCUBATION PERFORMANCES OF DIFFERENT BREEDS OF DOMESTIC DUCKS

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Abstract

Biodiversity domestic livestock species that produce various products is very important in the context of human population growth, but equally for meeting the demand for certain types of special foods especially appreciated by gourmets. Thus, the ducks can provide a wide range of products with high organoleptic and nutritional quality that can meet different consumer categories.This requires analysis of different indices of breeding duck breeds in order to improve them through various means, such as controlling the storage conditions of the eggs (the term, the microclimate in storage room) and incubation parameters.The study was done at the web-footed farm in Moara Domneasca, the biobase of the University of Agriculture Science and Veterinary Medicine from Bucharest, on the collection of duck breeds that consisted of: Barbarie, Pekin, Indian Running and Campbell.We observed six incubation series at an interval of seven days each. Incubation was done using a Smart Performer volume incubator with a capacity of 10.000 eggs.

Key words: *breeds, ducks, incubation, performance, products.*

INTRODUCTION

Ducks are birds that can get top quality food, but also a number of by-products such as lint, which is also of superior quality, or foa gras, considered a delicacy worldwide. Also, the breast provided by this species is highly appreciated, and as well the meat. To obtain and improve these yields it should be continued the research on the artificial incubation. By obtaining superior results in incubation, such as a low percentage of clear eggs, smaller number of ducklings dead in egg or greater number of viable ducklings hatched, the yields will also be higher.

Because of the great variability of domestic breeds of ducks, the incubation period of the eggs it's different as follows: Barbarie 34-35 days, Pekin 28 days and 27 days forCampbell and Indian Running. This implies that the conditions of incubation should be differentiated also. (Popescu - Micloșanu, 2007).

The hatching percent and embryo viability depend of the incubation factors;so if just one of these factors is not properly secured during incubation or regularly inspected, incubation will be affected and the production will decrease.

MATERIALS AND METHODS

To analyze and compare the results obtained in incubation, we used the livestock from the didactic farm owned by the University of Agriculture Science and Veterinary Medicine from Bucharest that is located in Ilfov county.In this farm, the Faculty of Animal Husbandry keeps the collection of several breeds and lines of ducks and geese.

The analyzed livestock were: Barbarie (40 females and 10 males), and Pekin, Indian Runner and Campbell (50 females and 15 males each).In the experiment were incubated 100 eggs from Barbarie and 100 eggs from Pekin, in 6 consecutive series, at a distance of 7 days each, the eggs being between 4-7 days old.For Indian Runner and Campbell breeds were incubated 150 eggs each per series, the age of the egg and the number of series beeing the same as in the first two cases.

The temperature, humidity and turning of the eggs are the most important factors of incubation. Thus, the optimal incubation temperature has been ensured at the level of 37.7° to 37.8°C and the humidity was between 65-70%. The turning of the eggs was made at an interval of two hours until the tenth day of

incubation with 45°, then the turning of the eggs was gradually slowed until the day the eggs were moved in the hatchery.In the hatchery, the temperature was 37.6 and the humidity 80% in order to facilitate the hatching of ducklings.
We followed for each breed the number of clear eggs, the number of dead ducklings, and hatching percent, including fertility and main statistical indicators.

RESULTS AND DISCUSSIONS

After incubation of the six series, there were obtained the results shown below.

Table 1. Results obtaind for Barbarie duck (for year 2015)

Barbarie	Eggs	Duck lings	Clear eggs	Ded in shell	% E[1]	% F[2]	%M[3]
Series1	100	50	28	22	50	72	28
Series2	100	51	26	23	51	74	26
Series3	100	47	32	21	47	68	32
Series4	100	56	25	19	56	75	25
Series5	100	53	27	20	53	73	27
Series6	100	49	30	21	49	70	30
Total	600	306	168	126			
Average	100	51	28	21	51.00	72	28
StDev	0	3.16	2.61	1.41	3.16	2.61	2.61
Variab Coef (%)		6.20	9.31	6.73	6.2	3.62	9.31

Table 1 presents the results obtained for Barbarie in 2015, it can be observed that from the 600 eggs placed in incubation, 306 produced viable ducklings, 168 eggs were clear and the other 126 were dead in shell.
In Table 2 are presented the results optained from Pekin breed recorded in 2015: 450 viable ducklings, 92 clear eggs and 58 dead in shell.

Table 2. Results obtaind for Pekin duck (for year 2015)

Pekin	Eggs	Ducklings	Clear eggs	Ded in shell	% E	% F	%M
Series1	100	77	14	9	77	86	14
Series2	100	80	12	8	80	88	12
Series3	100	76	13	11	76	87	13
Series4	100	74	15	11	74	85	15
Series5	100	73	17	10	73	83	17
Series6	100	70	21	9	70	79	21
Total	600	450	92	58			
Average	100	75	15.33	9.67	75.00	84.67	15.33
StDev	0	3.46	3.27	1.21	3.46	3.27	3.27
Variab Coef (%)		4.62	21.30	12.53	4.62	3.86	21.30

[1]hatching percent
[2]fecundity
[3]percentage of dead duckling in shell

Table 3 shows the performance achieved by the Running Indian by incubation of 900 eggs: 530 viable ducklings, and from the other 370 eggs remaining, 222 were clear, and 148 were recorded as eggs from which ducklings have failed to hatch.

Table 3. Results obtaind for Indian Running duck (for year 2015)

Indian Running	Eggs	Ducklings	Clear eggs	Ded in shell	% E	% F	% M
Seria1	150	86	34	30	57.33	77.33	22.67
Seria2	150	90	37	23	60.00	75.33	24.67
Seria3	150	93	39	18	62.00	74.00	26.00
Seria4	150	92	40	18	61.33	73.33	26.67
Seria5	150	83	35	32	55.33	76.67	23.33
Seria6	150	86	37	27	57.33	75.33	24.67
Total	900	530	222	148			
Average	150	88.33	37.00	24.67	58.89	75.33	24.67
StDev	0	3.93	2.28	5.99	2.62	1.52	1.52
Variab Coef (%)		4.45	6.16	24.28	4.45	2.02	6.16

Table 4. Results obtaind for Campbell duck (for year 2015)

Campbell	Eggs	Ducklings	Clear eggs	Ded in shell	% E	% F	% M
Seria1	150	116	19	15	77.33	87.33	12.67
Seria2	150	113	26	11	75.33	82.67	17.33
Seria3	150	118	24	8	78.67	84.00	16.00
Seria4	150	120	115	15	80.00	90.00	8.67
Seria5	150	115	20	15	76.67	86.67	13.33
Seria6	150	110	26	14	73.33	82.67	17.33
Total	900	692	130	78			
Average	150	115.33	21.33	13.00	76.89	85.56	14.22
StDev	0	3.56	5.05	2.90	2.37	2.94	3.36
Variab Coef (%)		3.09	23.66	22.29	3.09	3.44	23.66

In the fourth table the results obtained are presented for the Campbell breed.
For this breed, from the incubation of 900 eggs, were obtained 692 ducklings, 130 clear eggs and 78 eggs with dead ducklings in shell.
From Table 5 and Figure 1 it can seen that the eggs with the highest percentage of fecundity were obtained from Campbell (85.56%) and Pekin (84.67%), in opposite pol beeing Barbarie (72%).The lowest percentage of dead in shell was found at Campbell (14.22%) followed by Pekin (15.33%) and highest at

Barbarie (28%). For the percentage of hatching the best breed is also Campbell (76.89%), followed closely by Pekin (75%).The breed with the lowest percentage of hatching was Barbarie (51%). The coefficient of variation is greater for dead in shell compared with the other two incubation parameters studied, and based on race, Campbell (23.66%) and Pekin (21.30%).

Table 5. Statistical results obtained from breeds of ducks studied in the year 2015

Breed	Specification	% E	% F	% M
Barbarie	Average	51.00	72	28
	StDev	3.16	2.61	2.61
	Variab Coef	6.2	3.62	9.31
Pekin	Average	75.00	84.67	15.33
	StDev	3.46	3.27	3.27
	Variab Coef	4.62	3.86	21.30
Indian Running	Average	58.89	75.33	24.67
	StDev	2.62	1.52	1.52
	Variab Coef	4.45	2.02	6.16
Campbell	Average	76.89	85.56	14.22
	StDev	2.37	2.94	3.36
	Variab Coef	3.09	3.44	23.66

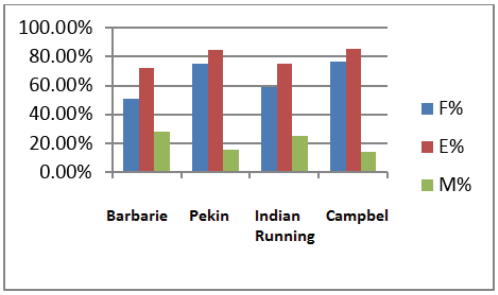

Figure 1. Fecundity, hatching percentage and the mortality for each breed

CONCLUSIONS

From the analysis undertaken one can draw some conclusions.

1. The differences between the results obtained from the four breeds were quite large.
2. The worst results were obtained from the Barbarie breed, this due largely to a very pronounced dimorphism which complicates the process of mating, so the fecundity is quite low, average percentage of hatching of this breed beeing 51%.
3. The best hatching percentage was reached by Campbell (76.89%) and Pekin (75%) breeds, who have a fairly high percentage of fecundity of 85.56% (Campbell) and 84.67% (Pekin).

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EFFECTS OF SUPPLEMENTATION WITHANIA SOMNIFERA L. ROOTS ON SOME EGG PRODUCTION AND QUALITY TRAITS OF HEAT STRESSED JAPANESE QUAILS

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Abstract
This study was carried out to investigate the anti-stress properties of local Withania somnifera roots, (WSR) in alleviating heat stress and improve egg production and quality in Iraq during summer season. The roots were used as ethanolic extract or crude powder. Ethanolic extract was done by using 70% ethanol. Three hundred Quail hens, 6 weeks (wks) old were used which were fed with standard basal diet containing 20% crude protein and 2903Kcal/Kg metabolizable energy and reared under high environmental temperature(27-37-27°C)and relative humidity (40-50%) and were randomly allocated to five groups as follows: Treatment (T_0): control group without any supplementation; Treatment 1and 2(T_1,T_2) quails supplemented orally with a dose of 50 or 100 mg/kg body weight(b.wt)/day ethanolic extract of Withania somnifera roots (WSRE; Treatment 3, 4(T_3,T_4) quails received Withania somnifera roots as powder (WSRP) mixed with the diet at the rate of 1or 2g/kg diet respectively. At 7,9,11,13 weeks of age and the total average of these weeks egg production and quality traits were calculated. Quails supplemented with roots powder at the rate of 1g/kg diet. (T_3) were significantly higher ($p\leq0.05$) in total average of egg production (%) than those received ethanolic extract ($T_1$and T_2). Also T_3 was the best treatment in egg weight, feed conversion ratio, egg mass and albumin height. The supplemented groups T_1, T_2, T_3 and T_4did not differ significantly from control in yolk index and Haugh unit. Egg tests during experimental period showed that there were no appearances of blood spot and meat segments, and no significant differences between treatments concerning with egg flavour test. We can concluded that little benefit in using (WSR) under heat stress to improve egg production and quality and the result favourite T_3.

Key words: *Withania somnifera roots, egg production, quails, ethanolic extract and powder, Heat stress.*

INTRODUCTION

In many countries including, Iraq poultry production suffers from high environmental temperatures in summer for more than six months. High ambient temperature is one of the major factors affecting poultry industry (Al-Hassani and Al-Jebouri,1988). Thus, several researches have been done to investigate the role of supplementing certain medicinal plant in improving birds performance because, its cheap and safety instead of using chemical drug. *Withania somnifera* (WS), also known as Ashwahandha, and Indian ginseng, is mentioned in the ancient Ayurvedic literature (Ghadha, 1976).

The plant grows widely in all dry parts of subtropical India. It is also found in the Mediterranean region, the Middle East and South Africa; and in Iraq. WS is an important medicinal plant widely used as a home remedy for several diseases in India as well as other parts of the world (Owais et al., 2005). The chemistry of WS, as a rich source of bioactive compounds (Padmavathi et al., 2005) has been extensively studied. Twelve alkaloids, 35 with anolides and several sitoindosides from WS have been isolated (Matsuda et al., 2001; Ganzera et al., 2003; Jayaprakasam et al., 2003; Kaure et al., 2003).

The pharmacological and therapeutic efficacy of this plant was well established (Dhuley, 2000). It has multifaceted medicinal properties-inducing antioxidant, adaptogen, aphrodisiac, liver tonic, anti-inflammatory, antibacterial (Sandaram et al., 2011)

There were no previous studies that examine the antistress and adaptogenic efficacy of indigenous *Withania somnifera* roots (WSR) as ethanolic extract, or powder to alleviate heat stress on egg production and quality of heat stressed Japanese quail.

MATERIALS AND METHODS

Experiments were carried out at Poultry Farm, Department of Animal Resource, College of Agriculture, University of Baghdad. The

experimental quails were brought from the Poultry Farm of Agriculture Foundation Research, Ministry of Agriculture, Baghdad, Iraq.
Three hundred Hens quails 6 weeks (wks) old, 190-200g body weight (b.wt.) were fed with standard basal diet containing 20% crude protein and 2903Kcal/Kg ME (Table 1) All birds were in healthy conditions. The birds reared under environmental temperature (27-37-27°C) and relative humidity (40-50%). Diet and water were supplemented *ad libitum*. All birds were acclimatized to experimental condition for 14 day.

Table 1.Composition and calculated analysis of experimental diet

Ingredient	%
Yellow corn	56.1
Soybean meal	31.1
Protein concentrate*	5.0
Vegetable oil	2.0
Limestone	4.9
Dicalcium phosphate	0.6
Food salt	0.3
*Calculated composition	
%Crude Protein	20.0
ME(Kcal /Kg)	2903
%Lysine	1.11
% Mehionine	0.77
%Calcium	2.54
%Available phosphorus	0.35

*Type "Holdmix" manufactured in Jordan, every 1 Kg contain 40% crude protein, 7.5% fat, 2.5% crude fiber, 8% calcium, 2100 kcal ME, 2.30% phosphorus, 2.60% salt, 2.4% lysine, 1.7% methionine and cystine, 2500 IU vitamin D3, 300 mg vitamin B3, 10 mg vitamin B6, 200 mg vitamin E, 200 mg niacin, 500 mg iron, 600 mg zinc, 10 mg cobalt, 100000 IU vitamin A,10 mg vitamin B1, 100 mg vitamin B12, 20 mg vitamin K3, 0.5 mg Biotin, 80 mg pantothenic acid, 50 mg copper, 700 mg manganese, 2 mg Selenium and 5 mg folic acid.
**calculated composition according to NRC, (1994).

Fresh healthy plants of WS, 2-3 years old were collected from several places in Baghdad. The herb was identified and authenticated at the Iraqi National Herbarium, Abu Ghariab. The roots were separated, cleaned, washed, air dried in shades, crushed and became a powder by an electric grinder.
The fresh powdered root of WS was extracted with 70% ethanol according to (Harborne et al., 1975). Quails were randomly distributed into five equal groups with three replicates for each group. The experimental treatments were as follows:
Treatment (T_0): control group without any additions to diet;
Treatments 1,2 (T_1,T_2): quails supplemented orally with a dose of 50 or 100 mg/kg b.wt. of ethanolic extract *Withania somnifera* roots (WSRE);
Treatment 3,4 (T_3,T_4): quails supplemented with *Withania somnifera* roots as powder WSRP mixed with the diet at the rate of 1 or 2 g/kg diet. For ethanolic extract treatments (T_1 and T_2), a certain weight of the extract was suspended in a convenience amount of distilled water in order to prepare the dose of 50 mg/kg b.wt. for T_1 and 100mg/kg b.wt for T_2. These doses were administrated daily at 12.00 PM for every bird during entire experiment period, by using stomach tube which inserts the substance into the crop. For crude powder treatments, 1 and 2 g of fresh powder were mixed for every kg of T_3 and T_4 diet respectively. These diets were presented to hens daily, until the end of experiment.
Eggs were collected daily at 9.00AM. Egg production (EP) was determined on daily- basis as the number of eggs laid by birds as hen's day (HD) production according to Naji and Hanna (1999).
Ten freshly laid eggs were collected once weekly from each replicate. The eggs were weighed for average egg weight (EW) by using electronic scale with high precision strain gauge sensor system (400-SF). Egg mass (EM) was calculated according to Al-Zubaidy (1986)
The egg shell was broken at the middle portion with the help of blunt end of knife; its contents were poured on perfectly levelled glass plate. The height of thick albumin was taken between the yolk and the outer border of thick albumen. Albumin height (AH) was measured with the help of micrometer with a least count of 0.001 mm after adjusting for the zero error on the plain glass plate. Yolk height (YH) was measured with the help of micrometer.
Yolk diameter (YD) was measured with the help of digital Verne caliber (0.01 - 150 mm).
Yolk index (YI) was calculated according to Card and Nesheim (1973).
Haugh unit (HU) was measured according to the equation mentioned by Card and Nesheim (1973).
The eggs were tested to determine the presence or absence of blood spot and meat segments.

Egg flavor test (FT) was evaluated by presenting boiled eggs randomly to eleven judges that had high qualification in evaluating the flavor of alimentary product and answering the questions presented in special application which include five grades for flavor test (Table 2) according to Ibrahim and Hill (1980).

Table 2. Grade of egg flavor test

Grade	Flavor Test
1	No flavor
2	Few flavor
3	Medium flavor
4	Strong flavor
5	Very strong flavor

According to Ibrahim and Hill (1980).

A completely randomized design -CRD within the Statistical Analysis System- SAS 2010 was used to analysis the data for the effect of difference factors in the studied parameters. Duncans (1955) multiple range tests was used to significant comparison between means.

RESULTS AND DISCUSSIONS

The mean values of egg production (EP) percentage (Hen Day (H.D %) are shown in Table 3. There were no significant differences between treatments at 9 and 11 wks of age on EP.At 7 wks of ages, EP was significantly decreased ($p \leq 0.05$) in T_1 and T_4 group as compared with T_0, while T_2 and T_3 didn't differ significantly fromT_0. At 13 wks of age, T_3 andT_4 had significance increased ($p \leq 0.01$) in (EP) compared toT_1, T_2 and T_0, which did not differ significantly among each other. Quails supplemented with root powder at the rate of 1g/kg diet (T_3) were significantly higher ($p \leq 0.05$) in total average of (EP) than those received ethanolic extract ($T_1$and T_2).

Statistical analyses of Egg weight (EW) are presented in Table 3. No significant differences were found between treatments at 7 wk of age and the total average of EW. The values of EW was reduced significantly ($p \leq 0.01$) in T_1, T_2 and T_3 as compared with control group at 9 wks of age.

At 11 wk of age there were a significant increased T_3,T_4 as compared with T_0 and T_1. At 13 wk of age treating quails with root ethanolic extract ($T_1$and T_2) and with crude powder(T_3) at the rate of 1g/kg diet significantly ($p \leq 0.05$) increased EW as compared to control group, which did not differ significantly from T_4.

Table 3.Effect of supplementing *Withania somnifera* roots as ethanolic extract or crude powder on egg production and egg weight of Japanese quail reared under high environmental temperature

	Age (wks)	Treatment					Significant Level
		T_0	T_1	T_2	T_3	T_4	
Egg Production (HD %)	7	a 32.144 ±4.382	b 22.400±6.00	ab 25.400±1.37	ab 28.010±1.870	b 21.333±0.49	*
	9	34.033±5.095	32.680±1,33	33.333±5.49	36.383±1.971	34.713±2.16	NS
	11	61.570±7.892	58.940±4.91	68.373±5.89	61.903±5.499	73.230±4.20	NS
	13	b 69.130±3.389	b 69.800±1.48	b 64.753±1.09	a 82.513±1.157	a 76.423±2.06	**
	Total average	ab 49.219±2.473	b 45.955±2.24	b 47.964±3.92	a 52.202±2.449	ab 51.424±0.13	*
Egg Weight (g)	7	11.00±0.00	10.30±0.17	10.33±0.00	11.33±0.18	10.98±0.76	NS
	9	a 11.49±0.09	b 10.83±0.28	b 10.66±0.19	b 10.49±0.09	ab 11.00±0.00	**
	11	c 10.83±0.09	c 10.83±0.09	bc 10.99±0.19	a 11.49±0.09	ab 11.33±0.19	*
	13	b 11.49±0.00	a 12.00±0.00	a 12.16±0.09	a 12.16±0.288	ab 11.83±0.09	*
	Total average	11.13±0.10	10.99±0.06	11.035±0.03	11.367±0.05	11.280±0.24	NS

T_0 = control, T_1 = 50 mg/Kg b.wt WSRE, T_2 = 100 mg/Kg b.wt WSRE, T_3= 1g/Kg diet WSRP, T_4= 2g/Kg diet WSRP, *Significant differences between treatments at ($p \leq 0.05$) in the same raw, **Significant differences between treatments at ($p \leq 0.01$) in the same raw), NS= No Significant differences between treatments, Values are expressed as mean ± SE.

Table 4 shows that the ratio of feed conversion to egg (FCE) which did not differ significantly due to experimental treatments at 9 wk of age, while, at the rest of experimental periods (7,11,13 wks), there were an improvement in groups supplemented with roots as crude powder in comparison with ethanolic extract and with control. At 7 and 13 wks of age, T_3 improved significantly ($p \leq 0.01$) in (FCE) comparison with T1.Treatments T3 and T_4 were significantly ($p \leq 0.01$) better than T1 at 7, 11 and 13 wks of age and in total average, there were significant improvement ($p \leq 0.05$) in T_3 compared to T_1 and T_2.

As shown in Table 4, there were significant differences in egg mass (EM) between treatments during experimental periods except at 9 wks of age. Hens supplemented with root as extract (T_1 and T_2) or as powder (T_4) had significantly ($p \leq 0.05$) lower EM than control group and T_3 at 7 wk of age, while there were no significant differences between hens in T_3 and hens in T_0.

Hens supplemented with WSRP (T_3andT_4) had significance ($p \leq 0.05$) increased EM compared to WSRE and control groups at 11 wk of age. At 13 wk of age, T_3 had the higher EM value than others and differed significantly ($p \leq 0.01$) from T_1, T_2,T_3 and T_0 .

Total average of EM showed that supplemented hens with 1g/kg diet WSRP (T_3) gave higher values ($p \leq 0.05$) of EM compared to other treatments(T_1, T_2, T_4 and T_0).

Table 4.Effect of supplementing *Withania somnifera* roots as ethanolic extract or crude powder on feed conversion ratio and egg mass of Japanese quails reared under high environmental temperature.

	Age (wks)	Treatment					Significant level
		T_0	T_1	T_2	T_3	T_4	
Feed Conversion Ratio	7	dc 3573±0.260	a 5.551±0.333	ab 4.844±0.371	c 3.218±0.104	bc 4.247±0.092	**
	9	3069±0.032	3.153±0.333	3.025±0.378	3.154±0.350	2.965±0.169	NS
	11	ab 3071±0.112	a 3.204±0.131	ab 3.017±0.200	bc 2.668±0.104	c 2.472±0.101	**
	13	a 2535±0.135	a 2.397±0.079	b 1.551±0.089	b 1.375±0.119	b 1.468±0.075	**
	Total average	ab 3062±0.058	a 3.576±0.041	a 3.109±0.246	b 2.603±0.196	ab 2.788±0.049	*
Egg Mass (g/hen/week)	7	a 42.14±1.46	c 26.17±2.51	bc 28.69±2.65	a 46.04±1.18	b 32.99±0.54	**
	9	50.84±2.28	50.86±6.40	53.30±7.43	49.45±5.42	53.59±1.84	NS
	11	b 51.03±3.63	b 51.22±1.98	b 46.94±2.93	a 64.68±0.32	a 61.19±0.65	**
	13	bc 55.97±3.67	bc 57.49±2.49	c 52.40±1.80	a 69.30±0.63	ab 62.16±2.90	**
	Total average	b 49.995±0.21	c 46.435±0.86	c 45.332±1.55	a 57.367±0.48	b 52.482±0.50	*

T_0 = control, T_1 = 50 mg/Kg b.wt WSRE, T_2 = 100 mg/Kg b.wt WSRE, T_3= 1g/Kg diet WSRP, T_4= 2g/Kg diet WSRP, *Significant differences between treatments at ($p \leq 0.05$) in the same raw, **Significant differences between treatments at ($p \leq 0.01$) in the same raw), NS= No Significant differences between treatments, Values are expressed as mean ± SE

From Table 5 it can be observed that at 7 and 9 wks of age, no significant differences between treatments were found in egg albumin height (AH). At 11 wk of age, T_3 group had higher AH ($p \leq 0.01$) than T_1. At 13 wk of age, T_3,T_4 and T_0 had significantly ($p \leq 0.05$) increased AH as compared withT_1 group. Total average showed no significant difference in all treated groups compared with control. Concerning yolk height (YH), no significant differences were found between treatments throughout the experimental periods (Table 5).

Statistical analysis of yolk diameter (YD) presented in Table (6) shows that there was conflict in differences among treatments through experimental periods. At 7 wk of age T_1 reduced significantly as compared with other treatments while at 9 wk T_3 decreased significantly with T_2 meanwhile at 11 wk T_3reduced significantly compared with T_0, $T_2$and T_4also at 13 wk T_1 increased signify-cantly as compared with T_0, T_3,T_4.

Total average of YD revealed that T_4 had significantly ($p \leq 0.05$) higher values than T_1 and T_3. On the other hand, all supplemented quails (T_1, T_2, T_3 and T_4) did not differ significantly from control.

Yolk index (YI) parameters that presented in Table 6 showed no significant differences between treatments at 9, and 13 wks of age and the total average.

Table 5.Effect of supplementing *Withania somnifera* roots as ethanolic extract or crude powder on albumin height and egg yolk height of Japanese quails reared under high environmental temperature.

	Age (wks)	Treatment					Significant level
		T_0	T_1	T_2	T_3	T_4	
Albumin Height (mm)	7	5.16±0.27	5.21±0.03	5.44±0.23	4.79±0.10	5.18±0.47	NS
	9	4.60±0.29	4.67±0.30	5.21±0.07	5.24±0.42	4.81±0.42	NS
	11	ab 4.59±0.12	b 4.18±0.02	ab 4.51±0.17	a 4.70±0.21	ab 4.58±0.06	**
	13	a 5.59±0.64	b 3.97±0.21	ab 4.74±0.03	a 5.31±0.30	a 5.50±0.31	*
	Total average	4.98±0.01	4.50±0.04	4.97±0.04	5.01±0.01	5.01±0.08	NS
Egg Yolk Height (mm)	7	11.47±0.44	11.67±0.10	11.37±0.14	10.96±0.07	11.00±0.20	NS
	9	12.05±0.05	12.41±0.47	12.34±0.33	12.00±0.23	11.87±0.19	NS
	11	12.62±0.28	12.45±0.01	12.29±0.27	12.73±0.12	12.24±0.20	NS
	13	13.19±0.04	13.11±0.16	12.92±0.09	12.94±0.03	13.18±0.12	NS
	Total average	12.33±0.09	12.41±0.00	12.25±0.03	12.15±0.04	12.07±0.05	NS

T_0 = control, T_1 = 50 mg/Kg b.wt WSRE, T_2 = 100 mg/Kg b.wt WSRE, T_3= 1g/Kg diet WSRP, T_4= 2g/Kg diet WSRP, *Significant differences between treatments at ($p \leq 0.05$) in the same raw, **Significant differences between treatments at ($p \leq 0.01$) in the same raw), NS= No Significant differences between treatments, Values are expressed as mean ± SE

Table 6.Effect of supplementing *Withania somnifera* roots as ethanolic extract or crude powder on egg yolk diameter and index of Japanese quails reared under high environmental temperature

	Age (wks)	Treatment					Significant level
		T_0	T_1	T_2	T_3	T_4	
Egg Yolk Diameter (mm)	7	a 24.26±0.08	b 21.43±0.21	a 23.44±0.92	a 24.52±0.30	a 25.26±0.935	**
	9	ab 24.38±0.26	ab 23.92±0.02	a 24.72±0.02	b 23.46±0.53	ab 24.13±0.41	*
	11	a 25.20±0.00	ab 25.11±0.35	a 25.10±0.28	b 23.57±0.23	a 25.21±0.47	**
	13	b 24.08±0.02	a 25.33±0.50	ab 24.77±0.06	b 24.42±0.04	b 24.45±0.24	*
	Total average	ab 24.48±0.09	b 23.94±0.36	ab 24.50±0.12	b 23.99±0.05	a 24.76±0.30	*
Egg Yolk Index	7	bc 0.47±0.01	a 0.54±0.00	b 0.48±0.02	bc 0.44±0.00	c 0.43±0.00	*
	9	0.49±0.00	0.51±0.01	0.49±0.01	0.51±0.02	0.49±0.00	NS
	11	b 0.50±0.01	ab 0.51±0.00	b 0.48±0.01	a 0.54±0.00	b 0.48±0.00	*
	13	0.54±0.02	0.51±0.01	0.52±0.00	0.52±0.00	0.53±0.01	NS
	Total average	0.49±0.00	0.51±0.01	0.49±0.00	0.50±0.00	0.48±0.00	NS

T_0 = control, T_1 = 50 mg/Kg b.wt WSRE, T_2 = 100 mg/Kg b.wt WSRE, T_3= 1g/Kg diet WSRP, T_4= 2g/Kg diet WSRP, *Significant differences between treatments at ($p \leq 0.05$) in the same raw, **Significant differences between treatments at ($p \leq 0.01$) in the same raw), NS= No Significant differences between treatments, Values are expressed as mean ± SE.

Quial hens supplemented WSRE at the rate of 50 mg/kg b.wt (T_1) had increased YI significantly ($p \leq 0.05$) compared with T_0, T_2, and T_3 and T_4 at 7 wk. At 11wk of age, T_3 had significantly ($p \leq 0.05$) increased YI compared with T_0, T_2 and T_4, which did not differ significantly between each others.

Regarding Haugh unit values, no significant differences were obtained between treatments throughout the study wks, and in total average (Table 7).

Egg tests during experimental periods showed that there were no appearances of blood spot and meat segments.

Statistical analysis presented in Table 8 shows no significant differences between treatments concerning with egg flavor test (EF).

Table 7.Effect of supplementing *Withania somnifera* roots as ethanolic extract or crude powder on haugh unit of Japanese quails reared under high environmental temperature

Age (wks)	Treatment					Significant level
	T_0	T_1	T_2	T_3	T_4	
7	71.13±1.77	72.37±0.65	70.51±0.91	67.95±0.465	68.22±0.46	NS
9	74.71±0.35	76.94±2.97	76.52±2.08	74.40±1.43	73.61±1.43	NS
11	78.24±1.78	77.21±0.09	76.19±1.68	78.94±0.80	75.26±0.80	NS
13	81.77±0.25	81.28±0.03	80.12±0.59	80.22±0.21	81.71±0.21	NS
Total average	76.46±0.70	59.33±0.02	75.83±0.12	75.37±0.29	74.70±0.46	NS

T0 = control, T1 = 50 mg/Kg b.wt WSRE, T2 = 100 mg/Kg b.wt WSRE, T3= 1g/Kg diet WSRP, T4= 2g/Kg diet WSRP, NS= No Significant differences between treatments, Values are expressed as mean ± SE.

Table 8.Sensory evaluations for egg flavor of heat stressed Japanese quails supplemented with *Withania somnifera* roots as ethanolic extract or powder

Treatments	Evaluation degree*
T_0	3
T_1	3
T_2	3
T_3	3
T_4	3
Significant level	NS

T_0 = control, T_1 = 50 mg/Kg b.wt WSRE, T_2 = 100 mg/Kg b.wt WSRE, T_3= 1g/Kg diet WSRP,
T_4= 2g/Kg diet WSRP, NS = No significant differences between treatments.
*Ibrahim and Hill (1980), Flavor: 1=No flavor, 2=little flavor, 3=intermediate flavor, 4=strong
Flavor, 5=very strong flavour.

The results of EP(H.D%) indicated that the usage of WSR did not significantly affect egg production of Japanese quail reared under heat stress conditions in comparison with unsupplemented group. Yet, it was found an improvement in EP when the roots were added as powder at the rate of 1g/kg diet. This effect could be happened as a result of the significant increased in the amount of feed consumption in this group under heat stress conditions; this means that females body made use of bioactive components available in the roots powder which in –turn may improve the exportation of egg yolk precursors from the liver (Asli et al.,2007), perhaps through mediated protection of the liver during heat stress (Harikrishnan et al.,2008), and therefore increased egg production, whereas daily oral administration with ethanolic extract for several wks under high environmental temperatures may reduce the advantage usage of WS.

The significant reduction in average egg weight of T_2 group could be attributed to the reduction in feed intake and FCE in this treatment.

The reduction of FCE, which was obtained in groups supplemented with ethanolic extract, could result from their reduction in feed intake and egg weight in comparison with groups received root as crude powder.

Regarding EM, the possible explanation for significant increase in T_3 group as compared with both extracts and unsupplemented groups may relate to; T_3superiority in EP percentage and the inequality in EW, as, EM consequential EP x EW.

The absence of significant differences among supplemented groups and control in HU may correlate with the disimprovement in EW and AH due to treatments. This result indicated that treated of heat stressed quails with WSR whether as powder or ethanolic extract did not had any effect on HU values.

The absence of blood spots and meat segments in eggs of quails reared under heat stress condition could be attributed to the possible role of WSR in normalizing blood pressure inside ovarian follicle vessels.

Results of EF revealed that supplementing WSR as ethanolic extract or powder did not affect this parameters in Japanese quail eggs and this could be a good indicator for its safety use in layer hens' nutrition with a good possibility for marketing.

CONCLUSIONS

Regarding egg production performance of the hens, root powder 1g/kg diet resulted in a significant improvement in egg mass and feed conversion ratio; whereas egg production, egg weight, yolk diameter, yolk index, Haugh unit and egg flavor were not affected by experimental treatments.

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THE EVOLUTION OF AGRICULTURAL LAND AND LIVESTOCK EXPLOITED IN ORGANIC FARMING SYSTEM IN ROMANIA BETWEEN 2010-2014

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Abstract

In the last years and especially after the country's integration into the European Union the number of organic producers and the organic area grew at a rapid rate in Romania. The driving force for the developing of organic sector in our country is represented by the export market and the payments for organic farming system which are an important foundation for the financial performance of organic farms.The aim of this study was to analyze the evolution of organic farming system in Romania, with special reference to the area of organic land, the number of organic operators, the use of organic land and the organic livestock. The results of study showed that the total area of organic land in our country in 2014 it was 289.252 ha which is higher with about 58% as compared with that existing in 2010 which represent 2.22% of the total utilised agricultural area. As regards the use of organic agricultural land, it was found that in 2014 the largest part of them, namely 63.6% were occupied with arable land, 33.1% with permanent grassland and 3.1% with permanent crops. This study shows that in 2014 there were 14,470 operators in organic farming system and the main activities in the organic sector at farm level are the organic crops and rearing of organic animals.

Key words: *organic area, livestock, organic farming, organic producers.*

INTRODUCTION

Organic farming is a sector of agriculture which has registered a constant growth in our country in recent years and especially after the country's integration into the European Union (Răducuţă, 2011; Răducuţă et al. 2014).

The driving force for the developing of organic sector in our country is represented by the export market and the payments for organic farming which are important foundations for the financial performance of organic farms, since they compensate them for additional costs or income foregone due to their organic commitments (Willer et al., 2014).

Organic farming is a way of agricultural production which uses organic production methods and places the highest emphasis on environmental and wildlife protection and, with regard to livestock production, on animal welfare considerations.

The respect for the environment is an intrinsic part of organic agriculture. Organic farming is a method of food production that combines best environmental practices, a high level of biodiversity and the preservation of natural resources.

Our country's potential for organic farming system is very high because in recent decades were used small amounts of fertilizers and pesticides and as a consequence we have a reduced pollution of water and soil compared to other countries (Răducuţă, 2012). In addition, in the last years, the farmers have adopted technologies for crop production and livestock which are friendly for the environment.

Organic farming system has a great future in Romania, both in the plant cultivation and in the animal sector, confirmed by the evolution of the number of operators which registered a high trend in recent years, increasing organic land area and number of animals raised organically (MARD, 2016).

The aim of this study was to analyze the current state of development in organic farming sector in Romania.

MATERIALS AND METHODS

The analyze of organic farming system evolution was made in 2010-2014 period with special reference to the area of organic land, the number of organic operators, the way of use of organic agricultural land and the organic livestock number.
To achieve this goals we analyzed the data of official statistics provided by different institutions (Eurostat, FAOSTAT, Ministry of Agriculture and Rural Development from Romania), after that was calculated the percentage difference between reference years to interpret the obtained data and finally were issued the conclusions arising from this study.

RESULTS AND DISCUSSIONS

The results of this study showed that in 2010-2014 period the total organic land area increased with 58.3% (Table 1). The area of organic agricultural land in 2014 was 289.252 ha from which 190.430 ha are fully converted to organic farming and 98.822 ha are under conversion (MARD, 2016; Eurostat, 2016). Also the area of organic agricultural land in 2014 is with about 0.6 times higher as compared with the existing area in 2010 and represents 2.22% of the total utilised agricultural area from our country.

Table 1. Surface dynamics and number of operators in organic farming

Specification	2010	2012	2014	Change 2010-2014 (%)
Organic agricultural land (ha)	182.706	288.261	289.252	58.3
Share of total utilised agricultural area (%)	1.37	2.17	2.22	-
Number of organic operators	3.155	15.544	14.470	358.6
Organic arable land crops (ha)	148.034	174.644	184.128	24.4
Organic permanent grassland (ha)	31.579	105.836	95.685	203.0
Organic permanent crops (ha)	3.093	7.781	9.439	205.2

The size of the organic area differs considerably from one EU Member State to another. In terms of the total organic area of each EU Member State as a share of the total EU-28 organic area, four countries together accounted for around 51% in 2014: Spain (16.6 %), Italy (13.5%), France (10.8%) and Germany (10.0%).
Organic production comes from fully converted areas. Before an area can be considered as organic, however, it must undergo a conversion process. The total organic area is the sum of the area under conversion and the fully converted area (Eurostat Statistical books, 2016).
The area under conversion as a percentage of the total organic area can give an indication of the potential growth in the organic sector in the years to come (Rohner-Thielen, 2010).
From this point of view, our country has a high potential, the area under conversion occupied 34.2% from total organic area (Table 2). In 2014, seven EU Member States had shares of more than 20% land area under conversion, Romania being placed on fifth position, after Bulgaria, Croatia, Malta and Cyprus.

Table 2. Share of area under conversion, 2014 (% of total organic area — fully converted and under conversion)

Specification	Total Organic Area (ha)	Share of total Organic Area (%)	
		Fully converted area	Area under conversion
Romania	289.252	65.8	34.2

As regards the use of organic agricultural land, it was found that in 2014 the largest part of these, namely 63.6% were occupied with arable land crops, 33.1% with permanent grassland and 3.3% with permanent crops (vineyards and orchards) (Figure 1).

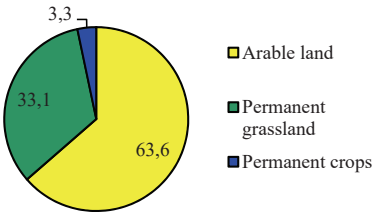

Figure 1. The use of organic agricultural land in Romania in 2014

The analysis of the main types of organic agricultural land in the period 2010-2014 reveals that the area of organic arable land

crops increased with 24.4%, the area occupied by organic pastures and meadowland increased by 2.0 times, and the area occupied by organic permanent crops (vineyards and orchards) increased by 2.1 times (Table 1).

The three main crop types grown organically are arable land crops (mainly cereals, industrial crops, green fodder, fresh vegetables), permanent crops (mainly orchards and vineyards) and permanent grassland. Among the arable crops, cereals and industrial crops occupied the biggest area from total (85.1%), respectively 55.7% for cereals and 29.4% for industrial crops.

In our country currently operate 14 inspection and certification bodies approved by MARD for the inspection and certification of organic products throughout Romania in accordance with article 4 of Ministerial Order no. 181/2012 and article 27 of Regulation (EC) 834/2007.

Also this study shows that in 2014 there were 14,470 organic operators from which over 99% were organic producers and remainder were processors and traders. The number of organic operators in 2014 is with about 3.6 times higher as compared with the existing number in 2010.

In 2014, there were close to 257.100 organic producers in the EU-28 from which Romania had a share at about 5.6%.

This increase was due, in particular to existing support measures for the conversion period granted under article 68 of Regulation (EC) no. 73/2009 establishing common rules for direct support schemes for farmers under the common agricultural policy and establishing certain support schemes for farmers (MARD, 2014).

Activities within the organic sector include the food chain from production at farm level right through to industrial processing. Imports, exports and other activities, such as wholesale and retail trade, are also included. The production of organic crops and the rearing of organic animals are the main activities in the organic sector at farm level, but the processing of goods is also important.

Animal breeding in the organic farming system has grown in Romania in recent years. It is focused on producing animals from a predominantly forage-based system, with an emphasis on maintaining animal health through improved of animal welfare and a reduction in the use of conventional veterinary treatments. Feeding, accommodation and care of animals are important factors for health and welfare of farm animals in organic systems. Also in organic farms, the native breeds seem to be used more than in conventional farms (Răducuţă, 2012).

The livestock sector in 2014 (under conversion and fully converted to organic farming) recorded an increasing of number of animals reared through the organic production methods, especially at cattle, sheep and goats (Table 3). According with these data the situation of certified organic livestock in 2014 was as follows: 33.782 heads of cattle, 114.483 heads of sheep, 6.440 heads of goats, 126 heads of pigs and 57.797 heads of poultry.

Table 3. Dynamics of organic livestock (heads)

Specification	2010	2012	2014	Change 2010-2014 (%)
Cattle	5.358	7.044	33.782	530.5
Sheep	18.883	51.722	114.483	506.3
Goats	1.093	1.212	6.440	489.2
Pigs	320	344	126	-60.6
Poultry	21.580	60.121	57.797	167.8

In the analyzed period (2010-2014) the number of organic cattle increased by 5.3 times, the organic sheep number by 5.1 times and the organic goats number by 4.9 times.

Regarding the organic poultry sector their number during the analyzed period it increased by 1.7 times, whereas the number of organic pigs decreased by 60.6%.

The statistic data showed also that cattle and sheep (with a share of 1.67% and respectively 1.25% of total livestock) are the most popular species reared using organic production methods in our country (FAOSTAT, 2016) (Table 4).

Table 4. Organic livestock 2014 in Romania and their share from total livestock (heads)

Specification	Total livestock	Organic livestock	% of total livestock
Bovines	2.022.408	33.782	1.67
Sheep	9.135.678	114.483	1.25
Goat	1.312.967	6.440	0.49
Pigs	5.180.173	126	0.002
Poultry	79.440.000	57.797	0.07

Not surprisingly it is for the pork sector that the sector has the lowest weight. This stems partly from the difficulties posed by the provision of organic animal feed (compound feed).
The agricultural land area of an ecological holding varies according with the type of production. The average size of organic agricultural holdings was estimated at 36.7 ha for the EU-28 as a whole, compared with 16.1 ha for all agricultural holdings (Eurostat Statistical books, 2016). In Romania the average size of organic agricultural holdings is about 25 hectares.
The Romanian organic sector is highly export-oriented. The main export products are cereals, and collected wild mushrooms and berries. The main import products are processed food (Willer H. et al., 2014).
The demand for certified organic products is continuously increasing and the price representing an important factor in the purchasing decision. Currently, the domestic market for organic products is continuously expanding and the organic products can be sold directly from the farm gate, home deliveries, sales via the Internet, farmers' markets, seasonal sales markets or fairs, specialized stores and supermarkets.
Domestic consumption of organic products is extremely low (1% from the total foodstuff and mainly in urban area).

CONCLUSIONS

The total organic land area increased with 58.3% in 2010-2014 period reaching to 289.252 ha, which represent 2.22% from total utilised agricultural area from our country.
Regarding the use of organic agricultural land, it was found that the largest part of these, namely 63.6% were occupied with arable land crops, 33.1% with permanent grassland and 3.3% with permanent crops.
The number of organic operators in 2014 is with about 3.6 times higher as compared with the existing number in 2010.
The production of organic crops and the rearing of organic animals are the main activities in the organic sector at farm level, but the processing of goods is also important.
The livestock sector in analyzed period recorded an increasing of number of animals reared through the organic production methods, especially at cattle, sheep and goats.
Cattle and sheep (with a share of 1.67% and respectively 1.25% of total livestock) are the most popular species reared using organic production methods in our country.

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PRACTICAL AND THEORETICAL ASPECTS REGARDING THE PRECISION DAIRY FARMING CONCEPT IN ROMANIA

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Abstract

This paper aims to present in a systematic way the practical and theoretical aspects of the Precision Dairy Farming Concept. This relatively new concept has emerged around the 2000s and for 10 years it referred to the feeding technology of dairy cows (Precision Feeding). Then, in 2010, a new trend appeared, that was to extend the concept of precision feeding to all the dairy farming technologies, and this includes optimizing the production processes by treating individually the cows, with help from automatic sensors. The data presented in this article were collected using the internet and the processing was done in order to create a theoretical basis to facilitate understanding of this concept in the first place, by the dairy specialists, and then by other professionals involved in dairy farming.

Key words*: dairy specialists, new concept, precision dairy farming, precision feeding, theoretical basis.*

INTRODUCTION

Dairy cows are one category of livestock of real interest due to at least two practical aspects: the share occupied by their productions in the total animal production and nutritional and organoleptic quality of their main production, milk. The first is of statistical nature and can be evidenced by the data presented below.

The main productions of dairy cows are milk, meat, hides and manure. Of these, milk represents 85.12% of the total milk production (Figure 1), and the meat, 20.60% of the total meat production (Figure 2).

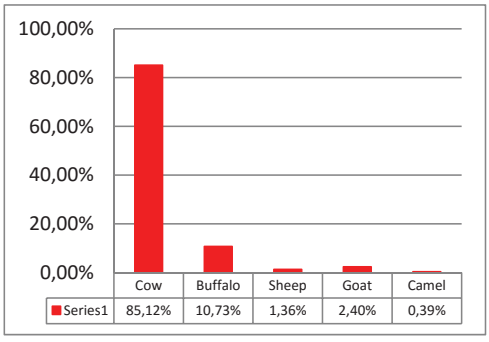

Figure 1. Share of cow milk production in total milk production worldwide
(FAOSTAT 2015 for year 2013)

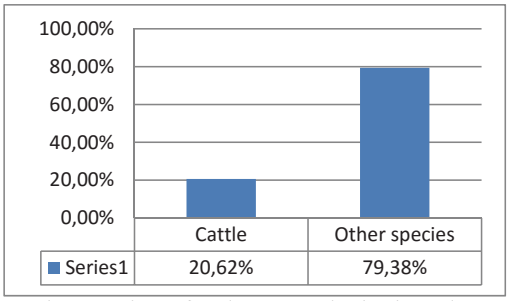

Figure 2. Share of cattle meat production in total meat production worldwide
(FAOSTAT 2015 for year 2013)

In 2013 there were, worldwide, about 1.47 billion heads of cattle and by analyzing the evolution of the livestock of the last 10 years, we can observed a tendency of increasing their number by about 0.90% per year, which totals an increase of 9.00% for this period.

This trend is observed in the case of milk production too, but the growth is more important, of approx. 2.00% per year and 20.00% for the decade to which we refer. In absolute figures, in 2013, it was registered a global production of 636 million tonnes of milk. The more accelerated pace of increase in milk production compared with the livestock number, is due to genetic progress achieved for this species by applying genetic improvement

programs, and due to increase of dairy technologies efficiency.

In Romania, the total cow milk production and livestock decreased during the analyzed period (2004 - 2013) with 12.00% for milk production (from 5.053.100 tons to 4.384.354 tones) and with 37.00% for livestock (from 2.897.000 heads to 2.009.135 heads) while milk production per cow increased by about 21.00% (FAOSTAT 2015 for year 2013).

The major desideratum for the cow milk production is the optimal growth of quality milk production per cow. Total milk production can be determined by the following formula:

Total milk production / unit of time = number of cows * production / cow / unit of time

From the above relation one can deduce that the increase in milk production can be obtained by increasing the livestock and/or increasing milk production / cow. It is generally accepted that increasing the production of milk in an optimum and sustainable manner can be made primarily by increasing production / cow and not by increasing the livestock. This can be achieved through genetic improvement of dairy livestock and by a continue process of modernizing of dairy technologies. In the last 10 years, a new concept appeared in dairy farming, called "Precision dairy farming". Broadly speaking, this modern concept refers to treating the cows individually at all levels and in an abstract mode can be defined as a perfect overlap between what must be done in dairy farming and what is practical done.

MATERIALS AND METHODS

This article is a study, based primarily on information available on websites specialized in Precision Dairy Farming (PDF) field, and also on the results obtained so far and published in various scientific papers. The practical necessity of such article is proven by the results of the survey done among specialists in the field. The results of this survey revealed that respondents do not have a clear definition of the concept of PDF, primarily because of the novelty of the concept and secondly due to the fact that in Romania this topic has not yet been studied thoroughly.

The first step consisted in identifying sites of real interest for the this paper, and then, using the available information, we tried to create a theoretical base on PDF meant to provide answers to questions such as: what is PDF?; which are the means of implementing the PDF in dairy farms?; which are the objectives of implementing the PDF?; what are the advantages of PDF and how they can be quantified at the dairy farm level?; which are the developing directions of PDF?; etc.

RESULTS AND DISCUSSIONS

The survey. The survey was conducted in January 2016, among Romanian specialists in dairy farming, in order to determine the level of knowledge and the opinion of respondents on PDF. The data were collected using a questionnaire which contained eight questions of which, the first two were designed to identify the respondents and the other six remaining questions role was to determine the respondent's link with the dairy farming sector and her/his knowledge about PDF.

27 people responded to the questionnaire. Figure 3 shows the age structure of the respondents, and table 1, their link to dairy farming sector.

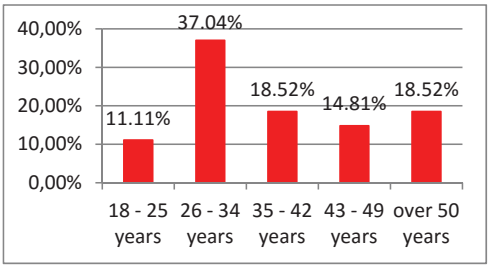

Figure 3. Age structure of the respondents

Table 1. Link to dairy farming sector

Link	% of respondents
New graduate[1]	7.41
Student (Master, PhD)[1]	40.74
Manager in dairy farm	7.41
Dairy farming specialist	44.44

It should be noted that this survey should be continued in the need to increase the representativeness of dairy farm managers.

[1] Specialized college (animal husbandry, veterinary medicine, agriculture, etc.)

Also, by "experts in the field", we will understand researchers, academics and advisors in various fields such as nutrition, reproduction, genetic improvement, etc.
74.07% of respondents say that they know at least one dairy farm that uses sensors.
Data are centralized in Figure 4.

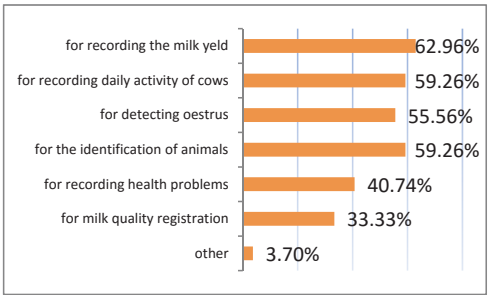

Figure 4. Types of sensors and their popularity among respondents

The respondents were asked to give a mark from 1 to 5, where 1 = not at all useful and 5 = very useful, depending on the utility they consider that different types of sensors have. The results are shown in Table 2.

Table 2. The usefulness of sensors based on respondents opinion

Type of sensor	Mark	SE[2]
for recording the milk yield	4.70	0.14
for detecting oestrus	4.67	0.14
for milk quality registration	4.67	0.13
for the identification of the animals	4.63	0.17
for recording daily activity of cows	4.52	0.15
for reporting health problems	4.48	0.19

To determine whether the respondents consider that the use of sensors in dairy farms is an important component of the PDF, we've asked them if they heard about this concept. 33.33% of those surveyed said they had not heard about the PDF concept although, 74.07% of them said they know at least one dairy farm that uses sensors. This can be explained by the fact that some respondents (at least 7.00%) did not consider the simple use of sensors as being PDF, or simply do not know the significance of the concept.
The last question in the questionnaire was designed to reveal how respondents define the notion of PDF. Those who responded to the previous question that they heard about PDF were asked to choose between four definitions of the concept on the one they considered to be most accurate. The definitions used were as follows:

Definition a: *a method that reduce workload and streamline the management process by improving the productivity and the economic outturn, all this obtained by treating individual the dairy cows with help from sensors that collect information automatically from each cow and then process the data with the computer, in order to increase the degree of automation in dairy farms*;

Definition b: *the use of at least one sensor in dairy farms to retrieve various information directly from the cow's body and storing them in a computer*;

Definition c: *the use of information and communication technologies to improve the control that the farmer has on each individual in the herd of dairy cows in order to optimize economic, social and environmental protecttion performance of the farm (Estwood et al., 2012, cited by Borchers and Bewley, 2015)*;

Definition d: *a new technology that determines the increasing of automation in dairy farms*.

The results for this question are shown in Figure 5.

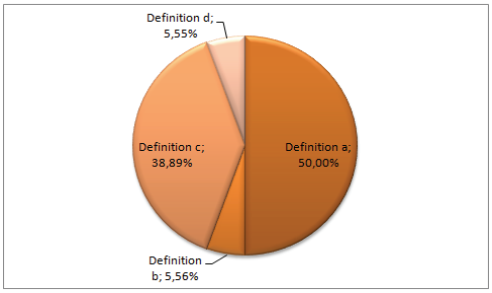

Figure 5. The way respondents define the notion of PDF

Given the results of this survey, one can conclude that persons involved in the dairy farming do not have a unified vision of the concept of PDF because, in Romania, this issue

[2] Standard Error

has not been yet addressed by researchers in the field of dairy. Also, worldwide, although there are concrete results on this subject, they are still not integrated as a whole, a situation that hampers understanding of the concept by specialists.

Definition. To understand the concept of PDF, one must start from the premise that the dairy farm is a productive unit that exists in the context of market economy and can be defined, from this point of view, as an economic system that has the particularity that the optimization process is strongly influenced by biological restrictions (Kelemen et al., 2015). For this reason, the main objective of the dairy farm is to maximize the profit because profit gives the possibility of dairy farm development and, at the same time the sector development and, finally, the livestock industry development.
In this context, PDF is an integrated subsystem whose role is enhancing the efficiency of management by individualizing dairy cows with help from sensors that collect information from each cow and computer processing the data in order to increase automation of the dairy technologies, so, the farmer's control over every individual in the herd significantly increases and makes it possible to optimize the economic, social and environmental protection performance of the farm (Estwood et al., 2012, cited by Borchers and Bewley, 2015).
PDF's main objectives are to (http://www.precisiondairyfarming.com/): 1) maximize animal performance, 2) detect diseases in individual cows early, 3) detect herd level health and production problems early and 4) minimize the use of medication through preventive health measures. In addition we can mention: optimizing the size of the dairy farm, reduce the expenses, streamline the feeding technology, improvement of animal welfare, etc.
At EU level there is ongoing the EU - PLF project which aims to bring the results of research in practice. The main objectives of the EU - PLF are (http://www.eu-plf.eu): 1) determine key animal welfare, productivity and health indicators (KIs) and gold standards that can be related to automated and continuous data stream, 2) perform extensive field tests of a few tools currently available at laboratory level or as prototypes, 3) analyse the data obtained and derive integrated solutions to make PLF a service to the farmer, 4) define the value created by the use of the PLF tools, 5) facilitate PLF-related innovation through High-Tech SMEs, 6) create the innovation blueprint and 7) disseminate the project results.

The sensors. The extrapolation of the precision concept from the feeding technology to the entire dairy farming technology was made about 10 years ago (Muller and Schrader, 2003; Munksgaard et al., 2006), by the introduction of specialized sensors used to measure various parameters like cows activity or their physiological state. To this end, a number of scientific papers have been published to validate the performance of these sensors or for developing various models through the data collected can be exploited in an optimum manner. One of the first articles that discussed this topic was conducted by Trenel et al. in 2009. The objectives were: recording the motion behaviour of the animal (walking, body position) and developing a filtering procedure that detect the position with greater accuracy. IceTag sensor (icerobotics.com) was created to measure the intensity of the upright or lying posture of the calves and daily activity as a percentage of time that the animal spends lying down or in an ortostatic position. The authors have developed a 3-step procedure that was intended to improve the precision and accuracy of data provided by the sensor. The experiment has shown that the procedure improves the quantifying of the number and the duration of positions taken by the calves, but the locomotion measurements should be used with extreme care.
Andre et al. in 2011 published the results of an experiment that they conducted with the objective of quantifying the effect of stress caused by high temperatures on average daily milk production. To collect the data for the experiment, they used database from 2003-2006 period, made through automation techniques of the process in the dairy farms. The authors conclude that estimating these effects is helpful in identifying those factors of management (grazing, technology maintenance, feeding technology) that have an impact on how the animals are feeling the heat stress, and

by manipulating these factors the dairy farmer can reduce stress.

Liang et al. conducted in 2013 a study on the influence of race, milk production, season and the ambient temperature on the body temperature of dairy cows measured at reticulo-rumen level. The authors concluded that the results may be useful for interpreting data obtained with the use of automatic recording systems of the temperature and also for heat stress management and genetic selection for cows with high tolerance to extreme temperatures.

In 2013 Rutten et al. made a bibliographic study regarding automated systems based on sensors used for health management in dairy farms. The purpose of the researchers was the systematization of these technologies into four levels depending on the complexity of the outcome: (I) techniques for measuring the parameters of the cow's body; (II) the interpretation of registered values to get information about the cows; (III) integration of information obtained with other information to create management strategies; (IV) systems that facilitate the management process and/or it automates it. Based on 126 scientific papers published in the period of 2002-2012, that analysed 139 sensors, the authors determined that 25.00% of them have been made in order to identify mastitis, 33.00% for fertility problems, 30.00% for measurement of the cows activity, and only 16.00% for detecting metabolic disorders. The sensors used in practice up to that point were of level I and II, and those of levels III and IV, did not exist. Another important observation revealed that the sensors are influenced by the performance of the algorithm used for data processing and by the size of the sample. The most advanced automated systems based on sensors are designed for diagnosing the mastitis and the oestrus. The authors did not identify works that discuss the issue of integrated decision models based on data collected.

A recent paper with a very pronounced practicality was published in 2015 by Borchers and Bewley. The study was conducted in March 2013 using an online questionnaire, and in May 2013, data collected from 109 respondents have been statistically processed. Dairy farmers were asked to choose from a predetermined list what kind of sensors they have in to the farm. Among the surveyed farmers, 68.8% use such technologies as follows: average milk yield (52.3%), cows daily activity (41.3%) and mastitis (25.7%). Farmers also indicated the usefulness of these systems on a scale from 1 (not at all useful) to 5 (very useful): mastitis (4.77 +/- 0.47), estrus (4.75 +/- 0.55) and the average milk yield (4.72 +/- 0.62). This result is similar with the result from the survey presented earlier in this paper. Another objective of the study was to determine the importance that farmers assign to certain aspects before deciding to buy a technology for implementing PDF concept in their farms. The results revealed that the most important indicators are cost / benefit ratio (4.57 +/- 0.66), the cost of investment (4.28 +/- 0.83) and ease of use (+/- 4.26 0.75).

PDF, animal welfare and costs. The study and implementation of the PDF have started from the premise that between this concept and the various aspects of dairy farming there is a significant correlation, which, once determined and understood, can be the basis for an integrated optimization of the economy of milk production, having in regard the context which involves acerbic competitive environment, cyclical imbalances of the milk market, availability of resources, bio-nutritional value of cow milk, etc. The two aspects mentioned in the title[3] are crucial for the economic efficiency of the farm and the dairy sector. How they interact is complex, derived from dairy technologies and genetic quality of animals and integrated at a macroeconomic level and food security of mankind, so we can say that the precision with which they are managed is a current topic of real importance!

Animal welfare can be defined as a complex concept involving scientific, ethical, economic and political dimensions of a real importance (Lund et al., 2006), and it represents the quality of animals life. In addition to this definition, welfare supports many other definitions more or less complete, accurate and sometimes contradictory. For this reason, measuring this parameter in the dairy farm can be difficult.

However, from the objective perspective of PDF, welfare of dairy cows can be seen as

[3] Animal welfare and costs.

being the degree in which the cow's necessities are satisfied in relation with availability of resources. So, from the point of view of optimising dairy technologies, welfare is a restriction in dosing the production factors in the sense that imposes the minimum level of the allocated resources , so that, the dairy farm will achieve the maximum economic efficiency with a minimum cost and the animals will benefit from an enhanced quality of life. The maximum limit of animal welfare is, in turn, imposed by the need of the production units to generate maximum profit. Graphical representtation of interrelations between welfare and resource availability and welfare or resource availability and profit may look like in Figure 6.

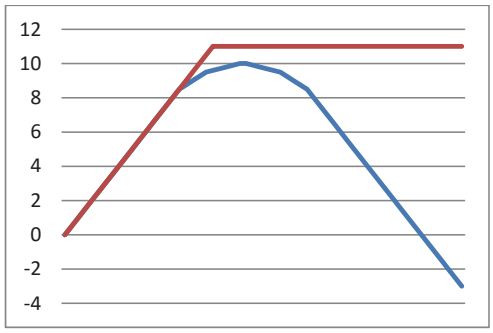

Figure 6. The possible interaction between welfare and resource availability (red) and the possible interaction between profit and resource availability / welfare (blue)

Chart 5 highlights that increasing the availability of resources, namely the degree of welfare, can have a huge positive correlation with the profit as long as the increased availability of resources generates increased profits. Functions appear to be rather nonlinear. So far, the relationship between welfare and economic efficiency was not clearly determined, but past studies indicate that the application of this concept has a major impact on the productivity of animals.

Another important aspect of the link between welfare and PDF is the fact that by using automatic sensors and processing the data collected by them with the computer, we can estimate how animal welfare at farm level evolve over time and in relation to other aspects of the dairy technologies, such as milk production, production quality, health, natality, fertility, economic efficiency, etc Regarding costs, the hypothesis is that by applying the concept of PDF, although there are generated relatively high investment cost, long-term impact is to reduce production costs, and consequently, increase the economic efficiency of dairy farms.

Given the share of feeding costs in total costs, the objective of one of the published studies was to evaluate the economic impact of the use of precision feeding technologies in dairy farms. The economic analysis was done in order to predict revenue, taking into account the cost of feed and labour (White and Capper, 2014), given that precision feeding plays a role in optimizing the economical and ecological performances by achieving a production of quality milk that is accepted by most consumers (Spilka and Fahr, 2003). Precision feeding has a major impact in the management activity of the dairy farm because it increases efficiency, reduces costs, improves milk quality and the health and welfare of the herd (Bewley, 2010). A number of other studies argue the positive impact of PDF on the economic outturn because it helps to balance the nutrients in the farm by improving the productivity due to matching of the administered ration to the nutritional requirements of an individual or group of animals (Wang et al., 2006b; Cerosaletti et al., 2004; Ghebremichael et al., 2007; Gehman, 2011). Experiments performed under practical farm conditions confirms to the benefits of precision feeding, so, by monitoring the feed intake of dairy cows, the DM intake can be optimized (Halachmi et al., 1998), and by implementing decisions based on the information analysis systems developed by PDF, milk production and the revenue can increase (Andre et al., 2007).

In 2014, White and Capper have published a economic analysis of formulating rations with increased frequency. For this they considered the following: 1) the costs were represented by the cost of feed and labour and 2) the income from milk was the only source of income used. The conclusion of the study for the economic analysis, was that the implementation of precision feeding at dairy farm level improves economic efficiency in general, since any increase in costs is attenuated by increased profitability due to increases recorded in the production of milk.

Romania and PDF. Milk is an essential aliment, at least for the harmonious development of children, having a functional role in human nutrition. Therefore, we can expect an increase in demand for milk in the coming years, growth that must keep pace with demographic evolution. In the next 35 years, FAOSTAT expects that in 2050, the human population will be approximately 30.00% higher than the current situation. Livia Vidu, in 2002, argues that an important indicator for assessing the standard of living is milk consumption / capita. According to data published on the website EUROMONITOR, milk consumption in Romania has an upward trend, which demonstrates the need to increase average milk production / cow. Currently, Romania is disadvantaged by the average milk yield / cow (3.447 liters) compared to that in EU (6.085 liters); (Average for the period 2004-2013) (FAOSTAT 2015 to 2013). To this is added the low efficiency in the exploitation of dairy cows in our country, due to the lack of coherent and sustainable politics for this strategic sector. Same with the ratio between imports and exports of raw milk, that is unbalanced in our detriment. In 2012, the value of imports was approximately 11 times greater than that of exports. There are still premises for the installing of an equilibrium regarding import / export relation, due to lower growth rate of imports (average for the period under review, 150.00%) than that of exports (average for the period under review, 188.00%).

Although Romania has never achieved the limit imposed by the allocated milk quota, the elimination of it represents a real threat to the Romanian dairy sector, in the context of weak productive efficiency and the mediatic scandals. The vehiculated scenario supposes that the import of milk in Romania, from countries such as Hungary or Poland, will increase. The specialized press highlights the tendency of dairy farmers, from countries with great performance in this area, to increase the livestock. In addition, milk processors from Romania prefer the imported milk due to the following reasons: 1) the possibility to supply at regular intervals and with optimal amounts, in terms of transport and flux of production; 2) hygienic and sanitary quality of milk easier to control because of the possibility of cooperation with a smaller number of optimally sized dairy farms and 3) easiness in supply. In Romania, in mountain and hill areas (about 66% of the country) there are areas inaccessible to the cars that transport the milk.

In this context, it can be concluded that Romanian dairy farms are vulnerable in the face of near future, and one of the methods handiest for farmers to ensure increased productivity and economic outturn is efficient management.

CONCLUSIONS

In Romania, the PDF concept has not yet been thoroughly studied and therefore, specialists and farmers do not have a clear vision of this modern notion.

PDF is a modern and opportune solution for optimizing the dairy farming technologies, including in Romania, given the global context of the sector that implies the increasing of demand for quality food due to acceleration of demographic growth.

PDF is the concept of integrated optimization of all the dairy technologies, but also enable the optimization of the whole sector of milk production because it takes into account the effects that management decisions may have on the environment in which the dairy farm exists (market economy).

PDF concept is relatively new, appearing for the first time around 2000s in dairy feeding, and then, was extended to the entire dairy farming during 2005-2010 period.

Studies published so far prove the existence of an interrelationship between the PDF and the various aspects of the economic efficiency of dairy farming (animal health, welfare, costs, etc.).

It is necessary to deepen the studies if we want to determine more accurate the impact that precision technologies have on economic efficiency of dairy farms, both worldwide and in Romania.

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RESEARCH REGARDING THE QUALITY OF PELTS FROM KARAKUL LAMBS OF BOTOŞANI AS EFFECT OF CROSSINGS BETWEEN BREEDERS BELONGING TO THE VARIETY OF BROWN COLOR

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Abstract

Interest of sheep breeders of Karakul of Botoşani breed led to the pursuit of activities of technical evaluation and management of quality of pelts to a variety of colour approved in Romania in the year 2012. The entire batch of lambs subjected to assessments in each generation analysed come from crossings between genitors who belonged to the same variety of black colour. The aim was to study the mode of transmission of the characteristics that influence directly the quality of pelts when applying homogeneous crossings. Breeding activity was based on matching-pairs and was made on the basis of a number of different couples, taking into account, however, the origin, the home line and the goal of improvement. Appraisal method of transmission of the characters which depends on the quality of pelts was realised on the first day after birth, taking account of the technical instructions and specifications of the certificate of origin and productive value. Classification of lambs in relation to the productive performance was done on the basis of final total average score. The study was conducted on successive generations of lambs obtained in the calving season between 2010 and 2015. From the analysis of the way in which descends in descent the waited characters is found that the process of improvement must be based, in particular, on an intensification of selection and on retention for breeding only the rams which show an improvement effect. Character analysis represented by the curl shape indicates that the desired types show a considerable improvement, as the last generation of assessed lambs, the valuable curl forms to be identified in over 70% of the total number of lambs subjected to research. Also, the effect of the selection applied it notes that at the apprizing of the curls after size, the desired type has rising values which confirms that the process of genetic improvement is one way. Instead, variations observed in ranking lambs in livestock classes after the total score value indicates a decrease from 33.3% to only 9% of lambs that meet the minimum requirements for the record. This suggests that it is necessary to measure the immediate reassessment of the reproductive couples and on the medium and long term a stronger selection for base characters and the application of more effective testing of males.

Key words: *lamb, pelts, Karakul of Botoşani, Romanian sheep.*

INTRODUCTION

Interest of sheep breeders of Karakul of Botoşani breed led to the pursuit of activities of technical evaluation and management of quality of pelts to a variety of colour approved in Romania in the year 2012.

The entire batch of lambs subjected to assessments in each generation analysed come from crossings between genitors who belonged to the same variety of black colour. The aim was to study the mode of transmission of the characteristics that influence directly the quality of pelts when applying homogeneous crossings.

Breeding activity was based on matching-pairs and was made on the basis of a number of different couples, taking into account, however, the origin, the home line and the goal of improvement.

Appraisal method of transmission of the characters which depends on the quality of pelts was realised on the first day after birth, taking account of the technical instructions and specifications of the certificate of origin and productive value. Classification of lambs in relation to the productive performance was done on the basis of final total average score.

The study was conducted on successive generations of lambs obtained in the calving season between 2010 and 2015. From the analysis of the way in which descends in descent the waited characters is found that the process of improvement must be based, in particular, on an intensification of selection and

on retention for breeding only the rams which show an improvement effect.
Character analysis represented by the curl shape indicates that the desired types show a considerable improvement, as the last generation of assessed lambs, the valuable curl forms to be identified in over 70% of the total number of lambs subjected to research. Also, the effect of the selection applied it notes that at the apprizing of the curls after size, the desired type has rising values which confirms that the process of genetic improvement is one way.
Instead, variations observed in ranking lambs in livestock classes after the total score value indicates a decrease from 33.3% to only 9% of lambs that meet the minimum requirements for the record. This suggests that it is necessary to measure the immediate reassessment of the reproductive couples and on the medium and long term a stronger selection for base characters and the application of more effective testing of males.

MATERIALS AND METHODS

All researches, studies and analyses conducted on the biological material Karakul of Botoşani of the brown variety and which is entered in the Genealogical Register of the breed. To increase the accuracy of the data range of the data collection was extended on five successive generations of lambs obtained in seasons of breeding and calving, between 2010 and 2015.
At the whole reviewed herd the applied reproduction was based on the crossing of genitors belonging to the dark brown variety, and for each breeding season the mating was made in accordance with the list of directing mating.
This document has been previously prepared and has been drawn up on the basis of a careful analysis of the particularities and specific characteristics of the paternalist forms, so that, the effect on the properties and quality of pelts from new generations of lambs to be quantifiable and easy to reveal.
Quality evaluation of character traits of which depends the quality of pelts was performed in the first 24 hours after birth, and to have the same level of efficiency the appreciation requirements of the work was performed by a single person.

RESULTS AND DISCUSSIONS

The brown variety within the breed Karakul of Botoşani is a biological creation of a more recent date, having been approved in 2012. This colour is a mixture of fibers which contain melanocytes yellow-orange, brown-black and dark-chestnut and resulted from mating the Black Karakul with the Gray Karakul, through the recombination of melanocytes in different colours (Taftă et al. 1977; Pascal 2015). Given the existence of a gene-fund proper to the colours brown and grey, these mutations have been fixed and strengthened by new breeding of homogenous type. Studies on the breed Karakul of Botoşani (Pipernea, 1976; Pascal 2011;Nechifor et al., 2014) emphasizes that the black sheep Karakul variety are heterozygous, also in its hereditary substrate, possess genes for brown colour in hypostatically state and which are designed to inhibit the activity of certain non-allelic genes.
In relation to the distribution and intensity of melanocytes contained in the chemical content of covering fibers, can be distinguished within the brown variety some shades from dark tones up to light, even beige. Promoting or producing the required shades depends upon the ability of the breeder to improve the reproductive activity between individuals which make the base batch.
Qualitative characteristics are numerous, complex and contributes greatly to the aesthetic and commercial value expression of pelts. That is why in matching pairs are of great help the information resulting from the evaluations on the quality of the curls at each breeder, information obtained since the first days of life and stored in certificates of origin and productive value.
According to the official technical regulations, assessing the quality of pelts in Romania is applied in the first 24 hours after birth and is carried out by specialized personnel authorized to do so.
Overall, each pelt depends on the expression of the character on which the curling depends, respectively the form of the curls, the size of the curls, fiber quality, the shine of curls. All these characters are favored in the expression of the way in which is every couple of reproduction of female and male is consisting.

The proportion of valuable forms increases significantly when the reproduction is based on homogeneous mating within each varieties of colour. This is confirmed by other studies conducted both in Romania (Taftă et al.1997; Pascal, 2011; Pascal, 2015) as well as in other countries where growing sheep for pelts (Buzu, 2012; Clote et al. 2014; Bravenboer, 2007).

The research activity has been carried out on different generations of lambs resulting from seasons of breeding and calving that had place between 2010 and 2015. The total number of lambs which were subject to direct assessments was 227, all belonging to the dark brown variety, resulting from the genitors breed variety belonging to the same colour.

All evaluations regarding the quality of the curling is determined by subjective methods, which is why the person authorized in such assessments must have experience in this field and know well the wanted kind of expression. In table 1 are presented and centralized, a result of all the planned assessments through the research plan approved.

Curl shape represents an extremely important character for the quality of the curling in overall, and through the activities of improving the objectives are shaping and fixing some valuable form of curls to be as close as possible to the requirements and demands of existing operators on the market.

Table 1. Result of assessment of main characters

Specific characters of pelts		Appraisal period					
		2010-2011		2012-2013		2014-2015	
		n = 65	%	n = 72	%	n = 90	%
Curl shape	tube	20	30.8	37	51.5	6	6.7
	tube + kernel	2	3.1	7	9.7	11	12.3
	flattened	23	35.4	14	19.4	61	67.7
	other shapes	20	30.7	14	19.4	12	13.3
The size of the curls	medium	41	63.1	33	45.8	55	61
	medium-small	10	15.4	30	41.6	31	34.5
	small	-	-	1	1.4	-	-
	big	14	21.5	8	11.1	4	4.5
The quality of the fibers	silky smooth	16	24.6	32	44.5	66	73.3
	normal	34	52.3	39	54.1	23	25.5
	rough	-	-	-	-	1	1.2
	soft	1	1.6	1	1.4	-	-
The shine of the curls	intense	9	13.8	13	18	20	22.3
	very good	38	58.5	41	57	22	24.5
	good	18	27.7	17	23.6	41	45.5
	satisfactory	-	-	1	1.4	1	1.2

Considering the fact that this variety has been approved relatively recent, in the year 2010, the first generation of valued lambs was obtained during the parturition season in the coming year. If the appreciation of this character on this generation may find that cylindrical curls, it will be represented by tube shapes and bob represented 33.9% of the overall analysed population.

Although for the cylindrical shape of the curls is found a variation in expression, the proportion being higher, respectively of 61.2%, of the total number of lambs assessed during the parturition season in 2013, through better control of the breeding, the wanted curl shape, meaning those flattened, increases the proportion above 65%. The preference towards fixing the curls with a flattened shape is due to the fact that these curls have the longitudinal axis parallel to the dermal layer willing and have the ability to better reflect the natural light, having a lustre and a silky smooth better expressed.

Fig. 1. Pelt brown with lustre good

Fig. 2. Karakul of Botoșani– pelts with brown colours

The size of the curls represents a very important character and therefore represents a selection criterion applied to sheep bred for their pelts. This character is favoured in the expression by length, height and width of the curl. Ideally, on the surface of the pelts to retrieve curls which have values close to the specified size, thing that seems impossible to reach. That is why, to improve this character, the main objective is the fixing of the curls as being uniform.

Assessing the size of the curling at lambs from the brown variety of the breed Karakul of Botoşani indicates a favourable expression for the wanted type of curls represented with small or medium size. Values greater than 95% of the proportion of lambs with such a size shows that the choice of the breeders and the nomination of the crossings were done better in the last season of breeding-parturition researched.

The gloss and silky smooth directly participates in the aesthetic value of pelts, and if you take into account the tendencies, desired type of curling is the one that is associated with a high well expressed gloss. Overall, this character is determined by the size, shape and arrangement of cells at the level of the cuticle layer of fibers.

Because the smoothness of the fibers from average curl exceeds 45 μm, the cuticle layer cells have an arrangement on a single row of round, irregular edges, they exhibit a mostly oval shape and are larger compared to the cells of the same histological layer, being present thin fibers and intermediates. Studies conducted for the evaluation of the basic characteristics of which depend the gloss expression specific to fibers forming the curls at the lambs from Karakul of Botoşani, indicate that this character is positively correlated with average thickness and silky smoothness (Pascal, 2011; Buzu, 2012;Taftă, 1997;Nechifor, 2015). Therefore, in the selection applied for the Karakul of Botoşani breed it shall be taken into account the quality of the fibers. This character is well expressed, when there is a decrease in the average thickness of the fibers components to values smaller than 40 μm. The quality will improve because this type of fiber will have a layout of cuticular flatter cells, and by standardizing their size, both the gloss and smoothness will be externalized.

Taking into account all these data and information, during the research period has been given an increased attention to the routing plan of the mating and as a direct consequence is the increased proportion of lambs that were the desired type for both the quality of the fibers and the gloss of the curls. If in the assessment of the first generation of lambs, the proportion of those who had silky fibers was just 24.6%, their share increased to 44.5% respectively 73.3%. This situation has contributed to the improvement of gloss, character which has improved obviously, increasing the proportion of lambs that had an intense gloss of the curling from 13.8% to lambs assessed in the action conducted in 2011, to 18% respectively and 22.3% in the other two generations be subject to assessments.

Flock hierarchy analysed in relation to the performance of which depends the quality of curling represent the technical activity which conclude the whole process of assessment and evaluation of the quality of pelts. Based on the data contained in the certificate of origin and productive value, at the end, is determine the class after the self performance. This result from the summation of points awarded due to an appreciation of how each character externalizes, taking however into account also the scale of general minimum total score, for enrolment in the partial class after productive performance. The data resulting from the hierarchy of lambs Karakul of Botoşani breed, belonging to the brown variety, are presented in table 2.

Table 2. Ranking of lambs from Karakul of Botoşani breed, of brown variety, after the production performance

Specific characters of pelts		Appraisal period					
		2010-2011		2012-2013		2014-2015	
		n = 65	%	n = 72	%	n = 90	%
Framing after production performance	Record	14	21.5	24	33.3	61	68
	Elite	27	41.5	25	34.7	21	23
	Class I	24	37	23	32	8	9
	Class II	-	-	-	-	-	-

Analysing the final results we can see that the lambs which presented a total score that was achieved with the minimum requirement for

enrolment in higher classes, as record and elite, has grown constantly. If at the first generation of lambs subjected to assessments their share was only 63%, the proportion of subsequent generation increases at 68% and 81%. This aspect is due to the correct nomination of the mating between the breeders, and this positive direction of lambs which constitute a valuable curling, show that many of the quality of characters of the curling have a high degree of transmission and can be easily modified and fixed in future generations by intensifying a controlled reproduction and selection. As regards to the biological quality of breeders, Pipernea (1974) states that the promotion of breeding must be a result of testing how they convey the desired characters, which represent the basis of the genetically win, starting from the assumption that other improving breeders are able to pass on to their valuable qualities to offspring.

CONCLUSIONS

In the case of assessments relating to the form of the curls, the desired types record an increase in the wanted proportion, reaching the last generation of lambs assessed the valuable curl forms, to be identified in over 70% of the total number of lambs subjected to research.

As an effect of the applied selection, following the conducted research, it notes that at the appreciation of curls after size, is found that the desired type, which is associated with small to medium sized curls registers rising values which confirms that the process of genetic improvement is on the efficient way.

The gloss participates in the expression of the beauty of pelts, also representing an important criterion of market requests in relation to this product. By the fact that the appreciation of this character at the lambs from the last assessed generation, the proportion of individuals with good lustre exceeding 40% indicates that the selection should be intensified and the promotion of breeders should be applied a more efficient management.

The fluctuations determined in lamb ranking in performance classes of production shall certify that the basis for reducing the proportion of lambs that meet the minimum requirements for class record, from 33.3% to just 9% in the last generation, that the establishment of criteria for breeding couples to be reassessed.

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THE IMPACT OF A LIGHT PROGRAM WITH ASYMMETRICAL HOUR INTERVALS IN THE PERIOD AFTER THE PEAK OF LAYING TO QUAILS HENS OF "BALOTEŞTI" POPULATION

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Abstract

In order to study the effect of the intermittent light at adult quails after the peak of laying was organized an experiment on a total of 450 quails divided into two batches: a lot has been subjected to continuous illumination with photoperiod duration of 16 hours daily (control group), and the other lot has undergone a lighting program divided according to the following scheme: 10 hours light (natural), 2 hours darkness, 6 hours light (artificial) and 6 hours darkness. The duration of the experiment was 16 weeks and at the beginning of the experiment quails had 29 weeks of laying.
From the research, it was found that the quails from the experimental group registered an average laying percentage high with 16.16% and average production per head with 18.89% higher compared to the control group. The average live weight was higher with 19.61%, and average egg weight was 18.89% higher compared with the control group. Weekly average mortality was 1.11% higher in the control group. Also, the average daily consumption of compound fodder has been about 12.32% higher; in exchange specific consumption was lower with 6.96% at the experimental group compared with the control group.
Given the superior performance recorded in case of experimental batch, it is recommended to use asymmetric fractionated lighting system at quails in the period after laying peak.

Key words: *quail, egg, divided light, performance.*

INTRODUCTION

Raising quails for the production of eggs or meat has spread quite a lot in recent years, particularly in the context of small farms. But considerable difficulties appear as regards the keeping of poultry in these holdings due to use of the wrong or at least not at the optimum level of environmental factors which may exercise a beneficial effect or not in the performance of quails production. Such environmental factor is light, which has more effect on quails through the duration of illumination and its variation than by intensity of light. Rizzoni and Lucceti, relying on June photoperiod, the reproduction period of quails in the wild, recommends a lighting duration of 16 hours and a period of obscurity of 8 hours a day, yet using waking lamps. (Rizzoni and Lucceti, 1963, quote by Velcea M., 1997). Small farmers in our country still often use at laying quails 24 hours light per day (Ioniță et al., 2015).
Recommendations for using of a particular illumination program, either at the youth of quail, either on adult quails, are still quite controversial, especially in the light of new researches of some authors, in which are used divided programmes even from youth period, after that adult quails are subject either for a programme of continuous light with 14-18 hours a day (Popescu-Micloșanu, 2007; Chelmonska et al., 2008; Vali, 2009), either on a divided illumination programme (Ioniță et al., 2015).

Zahoor et al. (2011) studied effects of intermittent light on production of Japanese quail and found that properly designed intermittent lighting (8L:6D:2L:8D) could increase egg production (57.3%), improve feed conversion and reduce mortality (8.33%) compared to conversional (16L:8D) (50.5% and 1.04%).

MATERIALS AND METHODS

Research was conducted on an initial number of 450 adult quail hens from Balotești population, which during the first 28 weeks of laying were subjected to a daily program of 16 hours of light and 8 hours of dark (16L + 8N). Starting with week 29 of laying, quails were split into two groups, in this manner: a batch of 200 quails (control group) were subjected to the same illumination program (16 h light per day) and a batch of 200 quails (the experimental group) have undergone a divided program by the duration of lighting with 16 hours per day (10 h L + 2 h N + 6 h L + 6 h N).

The research was conducted at the quail farm Ionita T. Lucian, individual enterprise located in the Gherghiţa village, Prahova County, Romania. The other environmental conditions in which the experiment was conducted were within the limits set by the specialty literature.

During 29-50 laying weeks, were recorded egg production, mortality, and was determined daily fodder consumption, living body weight and egg weight for each group.

Data were processed using Microsoft Excel 2010 and for testing the differences between averages Student test was used. The average and average error have been calculated for percentages of laying and mortality, daily consumption of combined fodder, specific consumption, living body weight and the egg weight for each batch.

RESULTS AND DISCUSSIONS

1. The average production performances of the initial 1 to 28 weeks of laying

During the period from 1 to 28 weeks of laying (tab. 1), quails from the initial batch that were maintained at the daylight duration of 16 hours, was recorded an average percentage of laying of 65.10%, a production of 4.56 eggs/day per head of and an average percentage of mortality of 0.42%.

Table 1. The average performances of the production at the initial quails batch from 1 to 28 weeks of laying

Specification	% laying	Prod/head/ day	% death rate	Cons c.f. (g)	Specific cons.	Live body weight (g)	Egg weight (g)
X ± s_x	65.10 ± 2.82	4.56 ± 0.19	0.42 ± 0.05	32.78 ± 0.52	54.83 ± 4.23	188.89 ± 2.88	10.28 ± 0.05

The average daily consumption of compound fodder was 32.78 g/head/day, while specific consumption was 54.83 /head/day. The average live weight was 188.89 g, and average egg weight was 10.28 g/egg.

2. The evolution of production performances in laying quails from the two batches analysed in the period from 29 to 50 weeks of laying

Between 29-50 weeks (Table 2, Figure 1), quails from the control group experienced a downward curve in relation to the average percentage of laying (58.55% in the 28th week laying at 48.65% in the 50th week of laying) and average egg production per head and per day (from 4.10 eggs in the 28th week of laying to 3.40 eggs in the 50th week of laying), and the average mortality recorded an increasing trend.

Table 2. The evolution of the average production performances in laying quails from the two batches analysed in the period from 29 to 50 weeks of laying

Batch	Control group			Experimental group		
Week lay	% lay	Prod/head	% death rate	% lay	Prod/head	% death rate
29	58.55	4.10	1.00	58	4.06	0.5
30	58	4.06	1.51	58.67	4.10	0
31	57.78	4.04	1.02	60.35	4.22	0
32	58.5	4.09	1.55	65.67	4.59	0
33	57.5	4.02	1.05	70.45	4.93	0.50
34	56.55	3.95	0.53	75.65	5.29	0
35	57.55	4.02	1.06	80.45	5.63	0
36	55.15	3.86	0	78.96	5.52	0
37	57.55	4.02	1.08	77.55	5.42	0.51
38	56.75	3.97	0.54	78.23	5.47	0
39	55.15	3.86	1.09	79.45	5.56	0
40	57.15	4.00	1.67	77.35	5.41	0
41	58.15	4.07	2.25	75.45	5.28	0.51
42	57.55	4.02	1.15	74.55	5.21	0
43	56.78	3.97	2.33	72.45	5.07	0.51
44	55.45	3.88	1.19	72.4	5.06	0
45	54.15	3.79	0.61	71.55	5.00	0.51
46	54.55	3.81	1.83	71	4.97	0
47	52.15	3.65	1.24	72.34	5.06	0
48	51.75	3.62	0.62	70.34	4.92	0
49	50.75	3.55	1.89	70.67	4.95	0
50	48.65	3.40	2.58	70.15	4.91	0.52
X ± $S_{\bar{x}}$	55.73 ± 0.51 aaa	3.90 ± 0.04 bbb	1.27 ± 0.12 ccc	71.89 ± 1.21 aaa	5.03 ± 0.08 bbb	0.16 ± 0.05 ccc

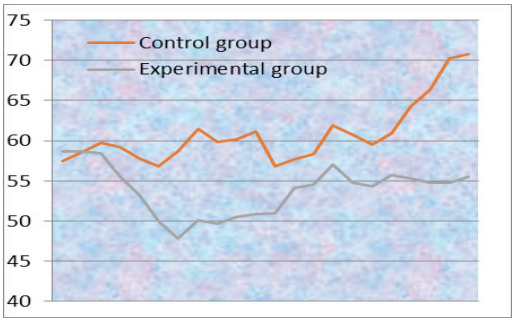

Figure 1. Evolution of the average percentage of laying on 29-50 weeks in the two batches of laying quails

Instead, the quails from experimental group (Table 2, Figure 1) once with changing lighting programme (continuous lighting to divided lighting made up for 16 hours) there has been a situation like a new ascendant curve of laying, meaning that the average percentage of laying and average production per head and per day began to increase (up to 80.45% and 5.63 eggs in 35 laying week) reaching the 50th laying week at an 70.15% average percentage of laying of and an average production of 5.03 eggs.

Also the average percentage of mortality was significantly reduced as effect of changing the illumination programme, this recording significantly lower percentage compared to the average mortality in the control group.

Table 3. The average live body weight, egg weight, daily fodder consumption and specific consumption at quails from the two batches during the period from 29 to 50 weeks of laying

Batch	Control batch				Experimental batch			
Week lay	Body weight (g)	Egg weight (g)	Fodder cons. (g)	Specific consump-tion	Body weight (g)	Egg weight (g)	Fodder cons. (g)	Specific consump tion
29	185.15	10	33.65	57.47	187.55	10.23	34.00	58.62
30	186.25	10.5	33.97	58.56	188.95	10.33	34.44	58.70
31	187.56	10.15	34.55	59.79	195.65	10.55	35.25	58.40
32	189.45	9.90	34.65	59.23	205.5	10.76	36.44	55.48
33	188.67	10.24	33.23	57.79	210.45	10.88	37.55	53.30
34	189.43	10.11	32.15	56.85	220.54	11.1	37.86	50.04
35	186.56	10.00	33.76	58.66	230.56	11.24	38.45	47.79
36	185.43	10.23	33.88	61.43	233	11.88	39.55	50.08
37	186.78	10.33	34.45	59.86	235	12.13	38.56	49.72
38	187.34	10.11	34.15	60.17	237.45	12.11	39.57	50.58
39	185.56	10.00	33.75	61.19	238.55	12.23	40.45	50.91
40	185.00	9.90	32.45	56.78	240.67	12.5	39.45	51.00
41	186.65	9.95	33.56	57.71	240.95	12.54	40.85	54.14
42	187.55	10.05	33.55	58.29	242.25	12.55	40.66	54.54
43	188.56	10.23	35.15	61.90	243	12.65	41.34	57.06
44	186.75	10.11	33.65	60.68	244	12.55	39.65	54.76
45	185.56	10.12	32.24	59.53	245	12.45	38.87	54.32
46	184.67	10.34	33.25	60.95	245.75	12.25	39.55	55.70
47	185.75	10.23	33.54	64.31	255.45	11.85	40.00	55.29
48	186.76	10.43	34.35	66.37	255	11.95	38.55	54.80
49	185.15	10.11	35.65	70.24	257.55	11.87	38.67	54.71
50	185.00	9.67	34.45	70.81	255	11.5	38.95	55.52
$X \pm s_{\bar{x}}$	186.62 ± 0.27 aaa	10.12 ± 0.04 bbb	33.82 ± 0.16 ccc	60.85 ± 0.74 ddd	232.17 ± 4.05 aaa	11.73 ± 0.15 bbb	38.57 ± 0.37 ccc	53.89 ± 0.58 ddd

For quails from the control group, average live weight was maintained at a roughly constant level, while in the case of the quails from experimental group live body weight began to grow with changing lighting program (187.55 g in the 29th week of laying to 232.17 g/head in the 50th week of laying), the differences between the two batches being very significant.

The same trend was apparent in the case of the average egg weight, it remaining constant for quails in the control group and increasing significantly in the case of quails in the experimental group (from 10.23 g at week 29 of laying at 11.50 g/egg in the 50th week lay). Average daily consumption of compound fodder has risen to quails in the experimental group (from 34 g in week 29 of laying at 38.95 g/head/day at week 50 of laying) compared to quails in the control group, in which the consumption remained at a relatively constant level.

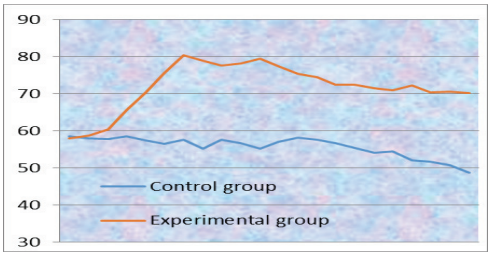

Figure 2. Specific egg consumption trends during the 29-50 weeks of laying quails in the two batches

Between 29-50 weeks of laying, the specific consumption (tab. 3 fig. 2) was significantly lower in the experimental group compared with quails in the control group. In a study conducted in Pakistan by a team led by Jatoi A.S (2013) on a flock of quails that are in the period after the

laying peak, but for a shorter duration (12 weeks during weeks 22-34 of laying), the authors mention the following performances from quails maintained at a programme of 16 hours continuous light per day: 268.81 g/head body weight, 79.49% average percentage of laying, 30.70 g of fodder compound consumption and weight of 11.34 g/egg. The same authors mention higher performance when a programme divided into intervals of asymmetrical lighting of 16 hours a day (8 hours light, 6 hours darkness, 2 hours light, 8 hours darkness). It should also be noted that the same experimental design and during 3-14 weeks of age after which up to 22 weeks quails were maintained at a 16 hours a day program of continuous light.

CONCLUSIONS

In 29-50 weeks of laying period, the quails in the experimental group recorded an average lay of 71.89 ± 1.21%, with 16.16% higher, while the average mortality was lower for quails in the experimental group as quails to the control group (1.11%), the differences between the two groups being very significant. The body weight, egg weight and feed intake were higher for quails in the experimental group (with 19.61% average live body weight, with 18.89% average egg weight and 12.32% average daily consumption of compound fodder) compared with quails in the control group; the differences are very significant. The specific consumption was 6.96% lower at quails in the experimental group compared with the control group; the differences are very significant.

It can be asserted that the use of a lighting divided programme into the period after the laying peak leads to improvement of production performance of quails hens (the case of experimental group), which otherwise would further decline curve pronounced after the laying peak (the case of the control group).

Given the superior performance recorded in case of experimental batch, it is recommended to use the lighting fractionated system with asymmetrical hour intervals at quails in the period after laying peak. Further, it is necessary to carry out studies on production of quails using fractionated lighting programme since the start of laying and even of their youth.

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CREATING RESILIENCE FOR TRANSHUMANT AND SMALL FARM SYSTEMS - TURKISH AND ROMANIAN PARADIGMS

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Abstract

Transhumance is a resource efficient means of livestock production by seasonally moving grazing animals to utilize pastures between varying ecological zones. This article investigated two separate transhumant societies in Romania and Turkey, countries that resemble cultural and environmental likeness. The data for the Turkish component of this study was collected in 2015 by interviewing Turkish transhumant populations during their migration route through the Taurus mountains. Romanian data for the Carpathian Transhumant came from personal communications with Romanian authors and also from Romanian and English written sources. Both nations possess rare natural environments with high nature conservation value open grassland habitats that benefit from traditional, low-impact agriculture of the transhumance. After a millennia of practicing conservation and natural resource management skills it is a lifestyle that is all but lost. Ecosystem services provided by the Romanian and Turkish transhumant family farming systems include preservation of biodiversity, providing substantial carbon sinks and reduction of CO2 emissions assisting to mitigate climate change. The system also maintains erosion control, improves soil quality and deters the likelihood of forest fires, whilst weaving a resilient social web. The survey also indicated a clear link between social and ecological resilience emphasizing that sustainable development relies on the interconnectedness between biological and cultural diversity and as such they merit strong policy recognition and support. The economic, social and environmental costs of losing them would far outweigh the costs of support. To loose this animal production system with its rich cultural heritage would be a tragic deficit for both biological and cultural conservation.

Key words*: Transhumance Social and Ecological resilience, resource management, pastoralism*.

INTRODUCTION

Origins of Transhumance in Romania and Turkey

Transhumance, also referred to as mobile pastoralism has been practiced since the Neolithic, the late stone age period (Nandris 1985; Arnold and Greenfield 2006). With the onset of cultural evolution or the technological development among prehistoric humans, dependence on domesticated animals and settlement in permanent villages saw pastoralism take on a new light. Herders began appreciating the benefits of natural resource management and started moving animals between seasonal pastures. Earliest evidence of herding in the Carpathians comes with the finding of a pair of sheep shears dating back to the Dacian period (c. 500 BC to 106 AD), and there are records of ancient Romans renting land to shepherds in the same area during the same period. Totoianu (2010) considers that long-distance transhumance in what is now Romania could not have begun before the fourteenth century. During 5th century BC, Herodotus also wrote about those who spent their winters in Bodrum (a town in Western Turkey): "Here, I am witnessing another lifestyle of humankind, and the most intelligent of all that we know. There are neither cities nor walls; they carry their homes with them. They do not have farms, but live with their animals''. Cicero in 50 BC also describes nomadic herders moving through the Taurus Mountains in Southern Turkey during winter and summer. The present day transhumant arrived into Anatolia as nomadic Turkic tribes with Oguz and Turkoman lineage, from the Mesopotamian

basin around 5000 years ago (De Blois and Van der Spek, 2008).

MATERIALS AND METHODS

All of the data for the Turkish component of this study was collected in 2015 by interviewing ethnographers, anthropologists, govt. employees, museum curators, historians and most importantly by paying routine visits to the summer locations of various Oghuz lineages of the Turkish Mediterranean and Central Anatolian transhumant populations during their migration route. The Romanian data came from personal communications with Romanian authors but also from Romanian and English written sources. Some of the interviews were walk and talk or carried out during community festive events. The interview topics and the subsequent discussions (Figure 1) were related to the geography of the region, determining factors for the migration routes, constraints and opportunities, animal husbandry skills they practiced; including mating programs and grazing strategy and finally their income sources. All demographic, ethnic, and sociological data presented in the study has been IP approved by the owners of the knowledge.

Study Area

Carpathians are a range of mountains forming an arc roughly 1.500 km long across Central Europe, second-longest mountain range in Europe. The Taurus Mountains also roughly 1.500 km long are a mountain complex in southern Turkey, dividing the Mediterranean coastal region of southern Turkey from the central Anatolian Plateau.

Pasture resources

Romania's current land surface area, unchanged since 1962, is 239.000 km^2. One third of this is mountainous. According to Huband et al. (2010), 'nationally there are an estimated 2.4 million hectares of semi-natural grasslands... and one source estimates 1.2 million hectares of semi-natural pastures and hay meadow habitats in the mountains.' As defined by Huband et al. (2010) semi-natural grasslands are those 'dominated by unsown native plant species that rely on human activities to maintain the condition of the swards and prevent the establishment of shrubs or woodland.' During the communist period 1947-1989, 90% of agricultural areas came under the authority of state farms and collective farms. The remaining 10% of agricultural land, not under state control, was in mountain areas, where the steep terrain and relatively thin and nutrient-poor soils hindered attempts at collectivization. There are 3.9 million farm holdings in Romania, the majority of which are Family Farms of extensive semi-natural grassland pastoral systems and mixed farming systems. These semi-natural small- sale farmed landscapes are of significant economic importance. For example, the 1 million holdings between 1-10 ha (3.1 m ha, 20% of Romania's agricultural area), are classes as semi-subsistence farms producing for home consumption, local sales and for their extended families. Yet these farms are estimated to produce 25-30% of national food consumption. They also provide rural vitality, as compared to the largest farms which are associated with rural poverty (Juler, 2014). Romanian Centre for European Policies, states that agriculture is one of the most important economic sectors in Romania. This sector generates 12% of the country's GDP and around 30% of Romania's active population works in agriculture.

Figure 1. A transhumant goat herd in Turkey

Turkey's current land surface area is roughly 3 times that of Romania at 770.760 km^2 with currently around 14 million hectares as permanent pastureland (TUIK, 2014). During the twentieth century, population pressure resulted in the expansion of farmland. The cultivated area increased from about 8 million hectares in the 1920s to nearly 19 million hectares in 1952 and to almost 28 million hectares by 1991. Using Marshall Plan credits that first became available in 1948, Turkey

began to import large numbers of tractors, which made it feasible to expand cultivation of marginal lands, especially on the Anatolian Plateau. Although total production grew rapidly, average yields did not. By about 1970, nearly all arable land was under cultivation. Cultivation increased primarily at the expense of meadows and grasslands, which diminished from about 46 million hectares in the mid-1920s to the current 14 m. ha (Grant, 2012). There are just over 3 million farm holdings in Turkey with two thirds under 5 ha. in size. Of the total workforce 25% is employed in the agricultural sector producing enough products to make up 9% of the GDP.

Both Turkish and Romanian farmers are extremely hindered by a fragmented ownership system. Semi-subsistence farmers own small pieces of land (average size of 1-5 ha.) trying to maintain productivity with very limited support from government agencies. Governments have opted to give most of the support to the establishment of larger farming units that have a poor natural resource management record. While small farms, as well as micro-sized agro-food businesses, have an important role to play in supporting the local economy and food security in rural areas, they are often placed in contrast with the perceived benefits of large farm structures. The perceived benefits of large farming systems due to economies of scale tends to downplay the efficiency of smallholdings, neglecting the environmental and social aspects of sustainability such as the ability of small farms to better resource manage (both human and natural) their production systems.

Figure 2. Summer migration in the Taurus Mountains

Importance of Pastoralism and SME's vs Industrial Animal Production

Sheep and goat production are very important sources of income for Romania, Turkey and for the world in general. With an estimated 42 million sheep and goat population in Turkey and 11 Million in Romania they are the 1st and 3rd largest flocks in Europe (FAO, 2014). While wool and goat hair in the present day has very little economic significance, sheep and goat's milk dairy products as well as lamb meat hold very high significance for both Romania and Turkey. Post WW2 industrial agriculture was hailed as a technological triumph that would enable the exponentially growing world population to feed itself. However time has shown us otherwise, a growing chorus of agricultural experts, including farmers, scientists and policymakers regard this type of production as a major threat to the healthy survival of our living systems. The impacts of industrial agriculture on the environment, public health, and rural communities deem it as an unsustainable way to grow our crops and raise our animal products (Union of Concerned Scientists).Transhumant societies and the small to medium size enterprise (SME) farms have shown that livestock production does not have to come at the expense of the environment. Transhumance is one of the many customary practices developed by ancient Mediterranean societies to cope with an unpredictable and highly fluctuating climate. It creates a cultural landscape that includes a complex mosaic of habitats, each varying in extent and productivity during the year (Oteros-Rozas et al., 2012). The practice has helped shape a characteristic landscape which has maintained one of Mediterranean's most complex and interesting ecosystems (Ruiz and Ruiz, 1986).

In Romania, small-scale farmers own 70% of the national sheep flock and play a vital role in maintaining large tracts of valuable semi-natural habitats. (PASTORAL 2, 2001). Romania possesses one of Europe's rarest natural environments, one that is to a large extent dependent on traditional, low-impact agriculture, including transhumance, and one that is a 'blueprint' for many more industrialized nations (Akeroyd, 2007). Akeroyd (2007) also notes that: This is a landscape that Europe has mostly lost, where a

wealth of plants and animals thrives alongside traditional agriculture. And there is no reason why its people should not have a happy and secure future, forging new prosperity in this ancient and productive landscape. Nearly a third of this area consists of high nature conservation value open grassland habitats created and maintained by low-intensity livestock farming practices (Webster, 2001). In general, these practices have evolved to exploit natural resources without depleting them, working within the constraints of the carrying capacity of the land. Throughout central and eastern Europe, from the alpine pastures and hay meadows of the Carpathians to the steppes of Hungary traditional (low-input, low-output) livestock farming practices still maintain large tracts of valuable semi-natural habitats.

RESULTS AND DISCUSSIONS

The study showed that the wandering grazing pattern of the mobile transhumant herd has helped spread the seeds of the local endemic varieties and shape the unique ecosystems of the Mediterranean region and maintain the interesting floristic composition of the Anatolian steppes. Resilient social–ecological systems are able to absorb large impacts without change in fundamental ways and, therefore, they can cope, adapt or reorganize without loss on their capacity to generate ecosystem services (Folke et al., 2002). Hence, it is expected that there is a strong link between social–ecological resilience and the ecosystem services associated with transhumant practices. The adaptation of transhumant livestock practice is a means to make optimal use of the resource availability and may possibly be a practice to minimize the impacts on livestock production derived from Climate change (Olea and Mateo-Tomás, 2009). Farming systems more closely connected to nature and small-scale farmed landscapes are more flexible in their farming activities, adapting more quickly to climate change and environmental challenges. They are strongly associated with efficient, low-carbon short food supply chains, through local and direct sales. It can be argued that transhumant and small-scale family farms are in many ways more productive than larger industrial farms, when all products are taken into account, for a variety of reasons including food security; where family farms perform a very significant and underestimated role in providing food to localities and wider families. Empowering families to grow their own food on small plots has been shown to offer solutions to food shortages in many problem regions of the world. The transhumant has created an enduring social fabric which has resulted in sound cultural resilience. Ecosystem services provided by Romanian and Turkish transhumant family farming systems include preservation of biodiversity by maintain the complex mosaic of plant varieties, by protecting natural woodland and permanent semi-natural grassland both act as substantial carbon sinks. Coupled with the low energy use of traditional agriculture, and short food supply chains, these landscapes and systems reduce CO2 emissions and mitigate climate change. Soil erosion is prevented by avoiding disturbance to ground cover. Extensive forest and vegetation cover delays run-off of rainwater, replenishes groundwater supplies and moderating extreme flooding events. Such landscapes also provide water purification services. These farming systems promote healthy populations of insect pollinators, and natural predators of agricultural pests and diseases.

CONCLUSIONS

The transhumant has created an enduring social fabric which has resulted in sound cultural resilience. The honest and sincere interviews held with the transhumant families has clearly shown that they continue to practice this lifestyle because they believe that it is beneficial to their environment, healthy to theirs own and their livestock's' lives. The study found that an important factor which often goes unnoticed in nature conservation and rural development strategies is that of the 'missing' value for low-intensity livestock systems and their products. A scenic landscape produced by High Nature Value farming systems is highly esteemed by visitors as well as by the tourist industry, yet little is invested in their protection. Pastoralism is being increasingly appreciated worldwide as an environmentally friendly practice, which, in the

European Union (EU) is valued as particularly important for the protection and the safeguarding of mountainous areas, defined as "Europe's ecological backbone"'. In 2013, the EU announced important changes to its Common Agricultural Policy (CAP). Among them were the aims of supporting the producers rather than the product, distributing funds more fairly and helping environmental initiatives. The very high estimated value of the ecosystem services provided by Romania and Turkey's transhumant and small-scale family farming systems suggests that they merit strong policy recognition and support. The economic, social and environmental costs of losing them far outweigh the costs of support.

To loose this animal production system with its rich cultural heritage would be a tragic deficit for both biological and cultural conservation.

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HEALTH AND WELFARE OF DAIRY COWS IN SERBIA

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Abstract

Diseases and mortality of dairy cows are significant problems from the aspect of welfare as well as the economy of production. Monitoring and analysis of health and welfare conditions on farms are important prerequisites for their improvement. This paper presents an analysis of health and welfare condition on dairy farms in Republic of Serbia. The study was conducted on 16 commercial farms with total number of 4833 milking cows of Simmental and Holstein Friesian rase. The evaluation of health and welfare indicators was done according to Welfare Quality® Assessment Protocol for Cattle. Results obtained in this study showed that largest share of farms was estimated as enhanced (56.25%) and acceptable (43.75%) in terms of overall health state. The incidences for majority of the diseases below the set alert thresholds indicated no severe risk for dairy cows' welfare on examined farms. The exceptions were determined incidences of laminitis (37.65%), dystocia (4.18%) and mortality rate (6.70%) which nevertheless corresponds to their growing trend in the dairy farming. Although health of skin was evaluated as acceptable almost every fifth cow had at least a portion of the skin without hair while the presence of skin lesions was much less common (6.49%). With high share of dehorned cows in herd (78.9%) another serious welfare risk is the common practice of dehorning without aesthetics and/or analgesics implementation. Analyzing indicators of health and welfare on Serbian dairy farms it could be assumed that the most important risks derived from poor housing conditions and management omissions.

Key words: *dairy cows, welfare, health, diseases, injuries.*

INTRODUCTION

In the last decade, many countries, which have focused their national breeding programs primarily on increase in the milk production, were faced with major problems in the field of health and reproduction of dairy cows. Increased frequency of so-called production diseases (lameness, mastitis, metabolic disorders, technopathy infertility and shorter life expectancy) in modern dairy cattle breeding is reasonably associated with the intensive exploitation of cows in inadequate rearing conditions (Oltenacu and Broom, 2010).

Health and welfare are inseparable concepts, as good health is a prerequisite for the welfare and vice versa. However, if the welfare is viewed as a broader concept, health can be seen as an indicator of its quality. Incidence of respiratory and reproductive disease, as well as locomotive, digestive and metabolic disorders, and the mortality can be used as the so-called, animal-based indicators of the welfare of cows in a herd (Canali et al., 2009). They essentially manifest response of the animal on provided conditions i.e. indicate the level of satisfaction of their needs which is the most important issue in assuring animal welfare. According to Broom and Johnson (1993), the need is the request, part of the biological basis of the animal, to provide adequate resources or responses to specific stimuli from the surrounding environment or its body. Animals in the absence of resources to meet their basic needs are becoming more prone to numerous welfare risks. EFSA (2009) highlighted four key risks to the welfare of dairy cows: housing, feeding, management and genetic selection. The etiology of many diseases is multifactorial and depends largely on the conditions in which animals are grown, which is why the cows must be provided an environment that reduces the occurrence of stress and weakening of

immunity (SCAHAW, 2001). The effective care for the health of cows, therefore, requires the provision of adequate rearing conditions and preventive action.

Monitoring and analysis of welfare conditions on farms are important prerequisites for welfare quality improvement. Regarding this, Welfare Quality Network is a scientific group that enables collection and exchanging of information about farm animals' welfare in order to provide recommendations for its enhancement. Their web-database (2009-2014) contains informations on different aspects of dairy cows' welfare, including a final assessment of the welfare in selected farms of nine EU countries and it is based on the Welfare Quality® Assessment Protocol for Cattle (Welfare Quality Consortium, 2009).

Animal Welfare Law and related regulations for the animal welfare protection on farms, during transport and in the slaughterhouses were adopted in Serbia in 2009. but technical and scientific analysis of their application are still expected. Previous national studies in the field of dairy cows' welfare are mostly fragmented and analyze certain aspects of their welfare while not sufficiently investigated the relation of health and welfare.

Therefore, the main objective of this study was to analyze health and welfare of dairy cows in Serbia, to compare it with established results of Welfare Quality Network, to define the most important welfare risks and propose measures for its improvement.

MATERIALS AND METHODS

The study was conducted in Serbia during 2012 on 16 dairy farms in which the cows of Simmental and Holstein-Friesian breeds were reared (N=4833). Minimum number of cows in the sample was 30 and the average per farm was 64 animals in two repetitions - during winter and summer season. Health condition and welfare of dairy cows were evaluated according to Welfare Quality® Assessment Protocol for Cattle (Welfare Quality Consortium, 2009) where detailed information about the methodology of assessment can be found.

Protocol includes 29 indicators that are used to determine four basic principles of welfare: good nutrition, good housing, good health and appropriate behavior. This paper focused on Principle of good health (PGH) as a part of overall welfare assessment on Serbian farms. Obtained data, indicators, were expressed as the number of animals affected out of the total number of animals assessed on each farm. Total score of PGH was determined by aggregation of corresponding indicators and criteria using the Welfare Quality® scoring system software program. Established values for criteria and principle were then compared to stated welfare categories (not classified, acceptable, enhanced and excellent) in order to provide information about health and welfare condition on Serbian dairy farms.

Data processing and categorization of welfare quality of the investigated dairy farms was conducted using software specially developed under the Protocol, and the respective statistical parameters were analyzed with the program StatSoft.Inc (2004), Statistica for Windows version 7.

RESULTS AND DISCUSSIONS

Distribution of farms according to PGH score is given in Figure 1. Acceptable health condition (21 - 50 point) was estimated on 81.25% of farms, while 18.75% of farms were estimated as enhanced (51 - 60 poena). PGH average value of 41.17 points was similar to those determinated on EU farms of 37 points (Welfare Quality Network, 2012) where, however the 2% of farms had unacceptable and only 13% enhanced evaluation of dairy cows health state. Poorer health of cows on European farms may be linked to breeding under higher selection pressure and higher milk yield as stated by Oltenacu and Broom (2010).

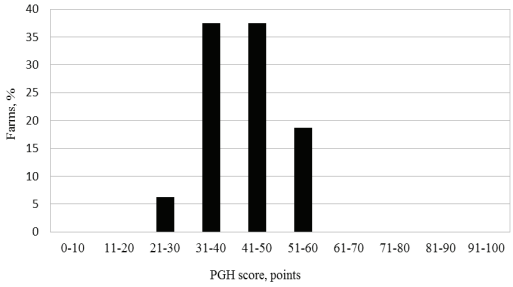

Figure 1. Distribution of farms according to PGH score

Average score for Criterion absence of injuries (CAI) indicate no severe risk from injuries for dairy cows' welfare (Figure 2). The largest share of farms (56.25%) was estimated as enhanced and acceptable (43.75%). Mean value for CAI was 57.57 points, similar to Welfare Quality Network (2012) results of 48.10 points for EU farms.

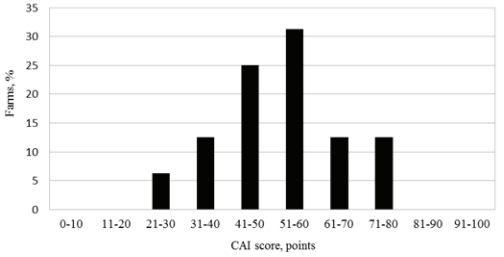

Figure 2. Distribution of farms according to CAI score

Estimated value for Criterion absence of disease (CAD) indicated low welfare risk in terms of frequencies of examined diseases. The largest share of farms was estimated acceptable (43.75%) or enhanced (37.5%) and almost fifth as excellent (Figure 3). Average value for CAD of 59.53 points was somewhat higher than on EU dairy farms (42.5 points) where 8% of farms had unacceptable and only 4% excellent estimation of cows health (Welfare Quality Network, 2012).

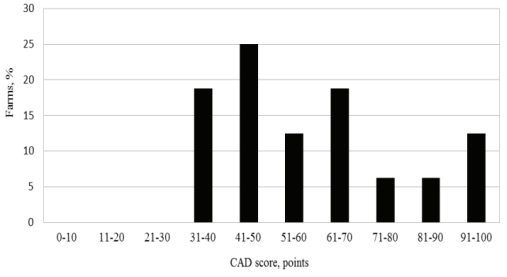

Figure 3. Distribution of farms according to CAD score

Value of the Criterion absence of pain caused by management procedures (CAPIMP) was determined based on the intensity of the implementation of zoo technical procedures, such as tail docking and dehorning. In the defining of the final value of the assessment, the manner of its execution (chemical, thermal, physical) and the application of anesthetics and analgesics were of great importance.

Survey results (Figure 4) show that only 6.25% of farms were rated as unacceptable, 18.75% excellent and most of them -75% were acceptable in relation to this criterion. In the EU, the situation is somewhat better considering that the majority of farms (59%) are rated adequate, as well as higher average value of criterion of 45.9 points in relation to the average of 41 points identified in Serbia. Better assessment of this criterion in the EU countries can be explained by more frequent application of anesthetics and analgesics (Gottardo et al., 2011) than is the case in our country where this practice was absent on studied farms.

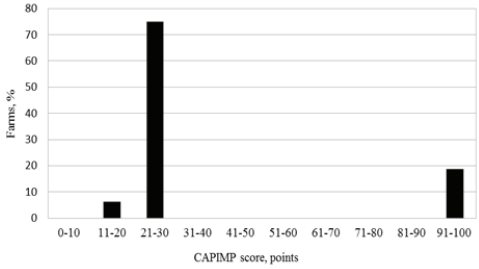

Figure 4. Distribution of farms according to CAPIMP score

Diseases and mortality of dairy cows are significant problems from the aspect of welfare as well as the economy of production. Effective care for the health of cows, therefore, requires the provision of adequate farming conditions and health care and protection. Canali et al. (2009) suggest that the diseases of the highest importance for assessing the welfare of cows can be divided into several groups: respiratory diseases (cough, sneezing, discharge from the nose, rapid breathing), digestive disorders (diarrhea, enteritis), diseases of the eye (discharge from the eye), reproductive diseases (metritis, mastitis, dystocia) and metabolic disorders (downer cows). In addition, the health condition of cows can be estimated on the basis of skin lesions (Schulze et al., 2009) and locomotor disorders (Borderas et al., 2004) and by the intensity of pain arising from the various zoo technical interventions (mutilation), such as shortening the tail or dehorning (Vickers et al., 2005; Anderson and Muir, 2005).

The results of studied indicators included in the PGH (Table 1) show that the average incidence of diseases such as discharge from the nose and

vagina, cough, difficult respiration, tachypnea, mastitis, diarrhea and downer cows is not a risk to the welfare of farmed cows. Those diseases are below the alert threshold (2.25-5.00%) in terms of welfare according to the recommend-dations of Forkman and Keeling (2009). Interestingly, poor cow hygiene that was also determined earlier in this research (Ostojić Andrić et al, 2015) did not increase the incidence of mastitis corresponding to the results of Ellis et al. (2007).

Estimated prevalence of dystocia of 4.18% corresponded to the range from 1.9 to 13.7% global prevalence indicated by Mee (2008), but still exceeds the threshold of high risk for the welfare of 2.75% (Forkman and Keeling, 2009). In study by Webster (2005) it is stated that the incidence of dystocia on farms with best quality of welfare (A, B) was zero while in the other categories (C, D, E) it ranged from 1% to over 40%. With regard to the causes of dystocia and its consequences for health, welfare and production economy (Mee, 2008) possibility for diminishing its occurrence in herds of dairy cows lies in the implementation of adequate breeding - selection programs, providing of good rearing conditions and comfort, balanced diet and the professional and timely veterinary supervision and monitoring of cows.

Mortality of cows was determined based on the number of dead, euthanized and emergency slaughtered cows in one-year period. The value of this indicator of 6.7% determined for the studied farms is alarming in terms of the welfare of farmed animals (Forkman and Keeling, 2009) but also corresponds with the growing trend in the dairy industry (McConnell et al., 2008). Thomsen and Houe (2006) report diseases of legs and reproductive organs, metabolic disease and fractures as the most common causes of mortality of cows.

Lameness is one of the most important welfare problems in cattle production because it causes pain (Whay et al., 1997) and changes in normal behaviour (Singh et al., 1993). Identified high prevalence of lameness of 37.65% on the farms included in the study indicates a significant risk to the welfare, as well as the derived consequences. Studies of lameness prevalence in dairy cattle in European countries show that it ranges from 22% (Whay et al., 2003) to 45% (Winckler and Brill, 2004) in free housing systems, and from 1% to 21% in housing systems where the cattle are periodically kept tied (Sogstad et al., 2005). In the study of Webster (2005), lameness prevalence ranged from 0 - 23% on farms with a satisfactory quality of animal welfare (A - C) as compared to 31 - 50% on farms with endangered welfare (E). In dealing with this disorder great care must be taken to remove the cause which may be genetic (breed, selection) but they are often induced by the influence of an unbalanced diet and poor comfort in housing (Nocek, 1997; Donovan et al., 2004).

Skin alterations may be due to various causes (infectious diseases, technopathy) but it is important to emphasize that in addition to keeping and housing conditions (Groth, 1985), parity (Kielland et al., 2009) also the effects of an unbalanced diet negatively affect the condition of skin and hair creating a predisposition to the formation of lesions (Schulze et al., 2009). The research results for this indicator of welfare showed that almost every fifth cow had at least a portion of the skin without hair while the presence of skin lesions was much less common (6.49%). According to study by Webster (2005), the hair loss was present in 33 - 88% cows on farms with poor assessed welfare (E), while its frequency on the best farms (A and B) amounted to 7%. It can be concluded that this phenomenon on studied farms was within acceptable levels in terms of ensuring the welfare of animals.

Dehorning as management-based indicator shows the intensity of the pain which the animals are exposed to during the performance of this procedure. To sustain the welfare of cows it is important to prevent a chain reaction of pain-stress-distress whose activation endangers the physical condition and behaviour of animals (Anderson and Muir, 2005). A number of authors (Vickers et al., 2005; Anderson and Muir, 2005) have studied the impact of dehorning on the welfare of cattle, and found that its negative impact is reflected in the physiological, neuro-humoral and behavioral changes as a result of pain and distress. Additional risks of implementation of such mutilations are increased possibility of infection by viruses and the development of

diseases such as tetanus and leucosis (Karatzias, 1981; Lassauzet et al., 1990).

According to Table 1, dehorning procedure was applied in the studied farms in 79% of cows without the use of anesthetics and analgesics (pain-killers), with the most commonly used procedure of thermo-cauterization (75%) as a better choice of procedure, while the chemical dehorning was much less used (6.25%). In case of three of the surveyed, sixteen farms that represent the best assessed in terms of this criterion, the procedure of dehorning was not applied. Study of Gottardo and al. (2011) showed that in Italy dehorning is carried out on 80% of dairy farms, where the heat dehorning is implemented in 91% of cases and the remaining is chemical dehorning. Using local anesthetic was part of the protocol in 10% of farms, while only 5% of farms practiced application of analgesics (pain-killers).

Table 1. Indicators of Health and Welfare on Dairy Farms in Serbia

Number of farms, N			N=16		
Principle, criteria and indicators	x	*SD*	S^2	*Min*	*Max*
Principle: Good health (PGH)	41.17	8.11	65.78	23.90	56.60
1. Criterion: Absence of injuries (CAI)	51.57	14.85	220.40	21.00	81.10
Not lame cows, %	64.56	17.59	309.40	20.60	90.00
Lame cows, %	26.50	13.73	188.45	6.98	61.80
Severely lame, %	10.95	15.00	225.06	0.00	86.55
Cows with at least one part of skin without hair, no lesion, %	17.80	16.45	270.48	0.00	73.68
Cows with at least one skin lesion, %	6.49	6.95	48.24	0.00	30.00
Cows without skin lesion, %	93.51	6.95	48.24	70.00	100.00
2. Criterion: Absence of disease (CAD)	59.53	21.67	469.70	30.20	100.00
Cows with nasal discharge, %	0.94	3.06	9.34	0.00	15.18
Cows with hampered respiration, %	0.06	0.25	0.06	0.00	1.00
Cows with ocular discharge, %	3.98	7.06	49.87	0.00	29.17
Cows with diarrhoea, %	2.01	2.44	5.97	0.00	8.16
Cows with vulvar discharge, %	1.45	1.51	2.28	0.00	5.55
Frequency of coughing per cow per 15 min	0.10	0.30	0.09	0.00	1.00
Frequency of mastitis, %	2.29	1.02	1.03	0.70	5.26
Frequency of dystocia, %	4.18	5.38	28.94	0.00	21.30
Frequency of downer cows, %	1.10	1.40	1.95	0.00	5.10
Frequency of mortality, %	6.69	5.91	34.89	0.00	21.30
3. Criterion: Absence of pain induced by management procedure (CAPIMP)	41.00	28.86	833.03	20.00	100.00
Share of dehorned cows, %	78.69	39.13	1531.03	0.00	100.00

CONCLUSIONS

The results presented in this paper are very encouraging when compared with estimates in EU countries for given year (2012). Namely, taking into account poor estimation of housing conditions (from previous researches known as major welfare risk in Serbia) health and welfare of dairy cows in our country may be considered as satisfactory. The cows' health was rated as acceptable to enhanced and on average better than on the farms in the EU, probably due to higher selection pressure and milk yield of cows on EU farms. The frequency of injuries and diseases that threaten the welfare of dairy cows was within the acceptable values. The exception was determined by incidences of laminitis and dystocia which represents a serious risk to the welfare of cows on examined farms. High average mortality rate of 6.7% corresponds to its growing trend in the dairy industry, but also exceeds the alarming level in terms of providing welfare. The common practice of dehorning without the use of anesthetics and analgesics in Serbia, presents a serious risk to the welfare as opposed to European countries where their application is increasingly common. However, ensuring the cows welfare in our country certainly contributes to the fact that the tail docking is almost entirely eradicated in breeding practice. In general, it can be concluded that the most important health and welfare risks on Serbian dairy farms derived from poor housing conditions and management omissions.

Analysis of welfare state in European countries in the period 2009-2014 showed that significant progress has been made in welfare ensuring as a result of monitoring, implementation and compliance with welfare standards. In Serbia recent years also, the growing importance is paid to respect for the principles of welfare, the implementation of legalislations and strengthening the organization for farm animal welfare protection. For promoting and ensuring farm animals' welfare of great importance is development of consumer awareness about the impact of animal welfare on the quality of foods of animal origin. In this way, the welfare becomes an important part of the contemporary concept of food quality.

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ECONOMIC EFFICIENCY - A KEY TO BIODIVERSITY CONSERVATION. ANALYZE PRODUCT COST STRUCTURE AND PROFIT CALCULATION IN THREE TYPES OF BUFFALO FARMS IN FAGARAS AREA

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Abstract

The work aims to study the economic status of some buffalo farms in Fagaras Area. These information are absolutely necessary for developing a program for active conservation of Indigenous Romanian Buffalo. Biological and economic efficiency is an objective of any farm to obtain expected benefits. Lower production costs are a goal of all producers in the field. In accordance with the purpose, they were followed two aspects: study the influence of farm size on parameters that influence production costs and economic efficiency of the unit; to establish to what extent the economic efficiency of the farm is influenced by how the production is harnessed. Whatever the size of the farm, milk and Telemea cheese are produced under conditions of total economic inefficiency. It appears advisable to increase the global production of milk at the farm level. Increasing the volume and quality of milk production and diversification of products offered for sale are efficient ways to minimize the cost per unit of product. Diversification of production at farm level should be a strategic objective of buffaloes exploitation in Romania in order to preserve the genetic resources and biodiversity.

Key words*: buffaloes, biodiversity conservation, economic efficiency, cost structure.*

INTRODUCTION

Current concerns for halting the loss of biodiversity are justified by the enormous rate with which it is lost, being in real danger of extinction entire categories of its components. Biodiversity is under unprecedented threat due to human pressure (Cogălniceanu, 1999).

In animal husbandry, conservation of biodiversity appears nowadays as a necessity because intensification of farming has led to the imposition of certain breeds exploitation and exclusion of others. As a result, some of them have become cosmopolitan and others have disappeared or have entered into an unprecedented numerical decline.

Economic inefficiency is the main factor favoring the decline or disappearance of domestic animal populations. As a result of this, populations either has suffered continuous numeric decreases to a size that determined entered in genetic drifting, or were subject of absorption (Popa, 2009).

Bringing vulnerable species or breeds to the attention of breeders, change selection objective, increasing economic efficiency to increase competitiveness in the natural life, are paths for specific and genetic biodiversity conservation (Grosu, 2003). In order to develop such programs are necessary analyzes of the concrete situation in growth area. In this context, the work aims to study the economic status of some buffalo farms in Fagaras Area. This information is absolutely necessary for developing a program for active conservation of Indigenous Romanian Buffalo. Biological and economic efficiency is an objective of any farm to obtain expected benefits. Lower production costs are a goal of all producers in the field.

In accordance with the purpose, they were followed two aspects: study the influence of farm size on parameters that influence production costs and economic efficiency of the unit; to establish to what extent the economic efficiency of the farm is influenced by how the production is harnessed.

MATERIALS AND METHODS

The research was conducted based on a questionnaire that was distributed via Buffaloes Breeders Association of Romania, headquartered in Şercaia, Brasov County. It aimed to identify at the farmers in Fagaras/Şercaia, the size of conventional farms, herd structure, associated costs and the main categories of incomes by capitalizing production. Based on information from these questionnaires, it was tried to simulate farm modules that correspond as closely as reality in the field.

Evaluation of production unit costs for each of the studied variants (determined by analyzing of questionnaires) was made based on the classification of expenses into two categories: fixed expenses and variable expenses (Oancea, 1999). In this way, the costs per unit of product were quantified by determining the unit cost of production, the latter in his turn is made up of fixed unit cost and unit cost variable.

In the category of fixed costs were not taken into account leaseholds, rents, interest on credits, various types of insurance, depreciation of fixed capital, some of the common and general expenses. In the analyzed farms, these categories of expenses are not included. In the category of fixed costs we consider for our analysis only on those associated with permanent staff.

In the variable expenses, to determine their level, they were established following feed prices (note that all categories of feed are produced under own):

- 0.08 lei per kg green grass forage;
- 0.25 lei per kg silage;
- 0.5 lei per kg hey (hill's hey);
- 0.4 lei per kg coarsely forage (harvest straw, etc.);
- 1 lei per kg concentrated feed mixture.

Also, although we were not given such expenditures, their lack we consider negligence or incapacitated/unable of farmers evaluation. Therefore, we appreciate in the determination of cost structure, an average price of 50 lei/head/year costs associated with veterinary care (including preventive treatment, curative, mandatory review).

From analysis of the questionnaires, the majority of buffalo farms are subsistence farms, with an average of 5 milk females per farm, plus a few heads of youth and one bull. However, during the research, were noted two larger units, one located in the Arpaşu de Sus village (Dan Cristian Naucsi owner) and the other in Grid village (owner Victor Draghici). We analyze the cost of production for each of the two, to which we add a third type, subsistence farm environment respectively, which prevailing in the Şercaia.

RESULTS AND DISCUSSIONS

A. Farm owned by Naucsi Dan Cristian, located in Arpaşu de Sus, is considered to be a big size one (over 20 heads). Table 1 presents the herd structure as it was indicated to us by the owner.

Table 1. Herd structure in farm owned by Naucsi Dan Cristian, located in Arpaşu de Sus

Animal category	Number of heads
Female buffaloes for milk	46
Heifers	28
Bulls	2
Female youth 0-3 months	10
Male youth 0-3 months	25
Female youth 3-6 months	10
Male youth 3-6 months	10
Female youth over 6 months	10
Youth male for fattening (over 6 months)	8

As indicated in the questionnaire, owner of the farm has 4 employees, 2 tractor drivers and two animal caretakers, with 1100 lei net pay each month. Because the employee is paid monthly with such an amount, the employer spends 1876 lei/month (according to legal regulations in force at the time of the research). Table 2 and Figure 1 present the production cost structure of the products produced on the analyzed farm.

Table 2. Production cost structure of the products produced in farm owned by Naucsi Dan Cristian, located in Arpaşu de Sus

Specification	Lei	Structure (% of total)
Fixed expenses	90048	26.29
Staff expenses	90048	26.29
Variable expenses	252441	73.71
Expenses for electricity	3600	1.05
Fuel expenses	6000	1.75
Expenses for feed	235391	68.73
Expenses for water	0	0.00
Veterinary assistance expenses	7450	2.18
Supply and transportation expenses	0	0.00
TOTAL EXPENSES	342489	100.00

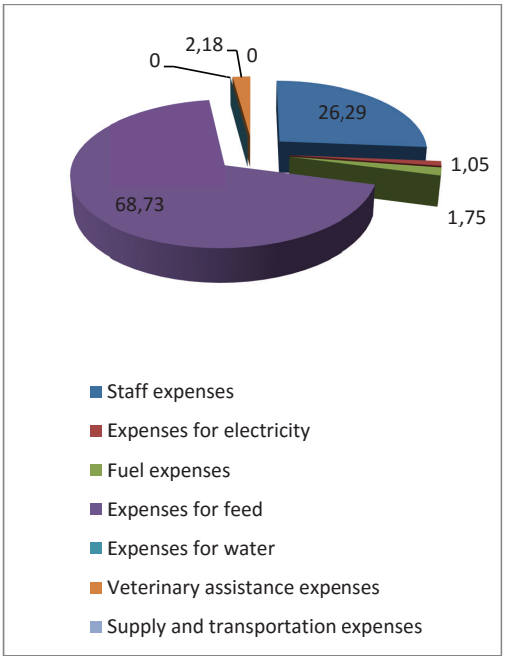

Figure 1. Production cost structure of the products produced in farm owned by Naucsi Dan Cristian, located in Arpaşu de Sus

Estimated production costs on the analyzed farm are:

1. Fixed unit cost:

$$C_{uf} = \frac{Fixed\ expenses}{Production\ volume} = \frac{90048}{36500} = 2.467\ lei\ per\ milk\ liter$$

2. Variable unit cost:

$$C_{uv} = \frac{Variable\ expenses}{Production\ volume} = \frac{252441}{36500} = 6.916\ lei\ per\ milk\ liter$$

3. Total unit cost:

$$C_{ut} = \frac{Production\ expenses}{Production\ volume} = \frac{342489}{36500} = 9.383\ lei\ per\ milk\ liter$$

We present in Table 3 profit calculation for analyzed farm.

It is observed so those, for one liter of milk, are necessary expenses amounting to 9.38 lei. It highlights the very high proportion of feed costs in total expenses for one liter of milk (68.73%) and the large share of staff expenses by 26.29% from total.

As stated in the questionnaire, the owner of analyzed farm deliver daily 50 liters of milk to a processing unit at a price of 3 lei/liter and 50 liters is processing the cheese, within his own farm, which capitalizes 15 lei/kg.

Table 3. Profit calculation in farm owned by Naucsi Dan Cristian, located in Arpaşu de Sus

INCOMES	✓ Milk for sale: 18250 liters x 3 lei/liter = 54750 lei/year ✓ Telemea Cheese: 4562,5 kg x 15 lei/kg = 68437.5 lei/year ✓ Subsidies: - 46 female for milk x 1393 lei/head = 64078 lei/year - 103 heads (others categories) x 575 lei/head = 59225 lei/year
	TOTAL INCOMES: 246490.5 lei/year
EXPENSES	✓ Staff expenses: 90048 lei/year ✓ Expenses for electricity: 3600 lei/year ✓ Fuel expenses: 6000 lei/year ✓ Expenses for feed: 235391 lei/year ✓ Veterinary assistance expenses: 7450 lei/year
	TOTAL EXPENSES: 342489 lei/year
BENEFIT	-

As a result, it may be noted that the capitalization of production in the form of raw milk delivered to processing unit is economically inefficient. The production unit cost was more than 6 times higher than the selling price. Cheese processing within their own farm is also economically inefficient because, if the entire volume of production would be processed into cheese, the production unit cost would be 37.53 lei per kg.

Even taking into account subsidies cannot put into discuss the existence of any benefit.

B. Farm owned by Drăghici Victor, located in Grid village, is considered to be a big size one (over 20 heads). Table 4 present the herd structure as it was indicated to us by the owner.

Table 4. Herd structure in farm owned by Drăghici Victor, located in grid village

Animal category	Number of heads
Female buffaloes for milk	19
Heifers	1
Bulls	1
Female youth 0-3 months	3
Male youth 0-3 months	14
Female youth 3-6 months	-
Male youth 3-6 months	-
Female youth over 6 months	-
Youth male for fattening (over 6 months)	-

As indicated in the questionnaire, owner of the farm has 1 employee (animal caretakers), with 800 lei net pay each month. Because the employee is paid monthly with such an amount, the employer spends 1335 lei/month (according to legal regulations in force at the time of the research).

Table 5 and Figure 2 present the production cost structure of the products produced on the analyzed farm.

Table 5. Production cost structure of the products produced in farm owned by Drăghici Victor, located in Grid village

Specification	Lei	Structure (% of total)
Fixed expenses	16020	19.99
Staff expenses	16020	19.99
Variable expenses	64125	80.01
Expenses for electricity	960	1.20
Fuel expenses	5400	6.74
Expenses for feed	54525	68.03
Expenses for water	240	0.30
Veterinary assistance expenses	1200	1.50
Supply and transportation expenses	1200	1.50
Other expenses	600	0.75
TOTAL EXPENSES	80145	100.00

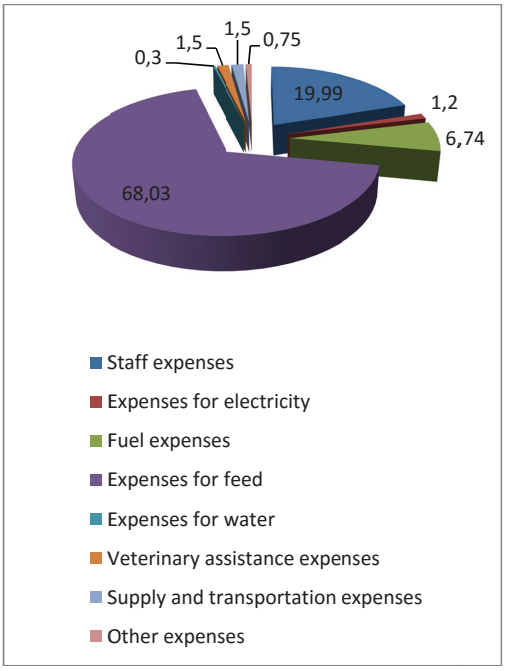

Figure 2. Production cost structure of the products produced in farm owned by Drăghici Victor, located in Grid village

Estimated production costs on the analyzed farm are:

1. Fixed unit cost:

$$C_{uf} = \frac{Fixed\ expenses}{Production\ volume} = \frac{16020}{12775} = 1.254\ lei\ per\ milk\ liter$$

2. Variable unit cost:

$$C_{uv} = \frac{Variable\ expenses}{Production\ volume} = \frac{64125}{12775} = 5.019\ lei\ per\ milk\ liter$$

3. Total unit cost:

$$C_{ut} = \frac{Production\ expenses}{Production\ volume} = \frac{80145}{12775} = 6.273\ lei\ per\ milk\ liter$$

We present in Table 6 profit calculation for analyzed farm.

Table 6. Profit calculation in farm owned by Drăghici Victor, located in Grid village

INCOMES	✓ Telemea cheese: 3193.75 kg x 15 lei/kg = 47906.25 lei/year ✓ Male youth: 14 heads x 800 lei/head = 11200 lei/year ✓ Subsidies: - 19 female for milk x 1393 lei/head = 26467 lei/year - 5 heads (other categories) x 575 lei/head = 2875 lei/year
	TOTAL INCOMES: 88448.25 lei/year
EXPENSES	✓ Staff expenses: 16020 lei/year ✓ Expenses for electricity: 960 lei/year ✓ Fuel expenses: 5400 lei/year ✓ Expenses for feed: 54525 lei/year ✓ Expenses for water: 240 lei/year ✓ Supply and transportation expenses: 1200 lei/year ✓ Veterinary assistance expenses: 1200 lei/year ✓ Other expenses: 600 lei/year
	TOTAL EXPENSES: 80145 lei/year
BENEFIT	Total income - total expenses = 8303.25 lei
PROFIT TAX	1328.52 lei
NET PROFIT	6974.73 lei

It is observed so that, for one liter of milk, are necessary expenses amounting to 6.273 lei. It highlights the very high proportion of feed costs in total expenses for one liter of milk (68.03%) and the large share of staff expenses by 20% from total.

As stated in the questionnaire, the owner of analyzed farm capitalizes the entire production of milk by processing in cheese, which sells for 15 lei/kg. Also it offered for sale 14 heads of youth male for fattening at a price of 8 lei/kg bodyweight.

Cheese processing within their own farm is economically inefficient because the production unit cost would be 25.09 lei per kg.

Regarding the relationship between incomes and expenses, profit can be achieved only under subsidies accessing.

C. Average subsistence farm is considered to be one of the small size (5 heads). Table 7 present the herd structure as it was indicated by in field analysis.

Table 7. Herd structure in a subsistence farm

Categoria de animale	Număr de capete
Female buffaloes for milk	5
Heifers	1
Bulls	
Female youth 0-3 months	2
Male youth 0-3 months	-
Female youth 3-6 months	-
Male youth 3-6 months	-
Female youth over 6 months	-
Youth male for fattening (over 6 months)	-

Within subsistence farms, staff costs are zero since work is non-quantified, unpaid default. Family members are running the daily activities of the farm, including field labor.

Table 8 and Figure 3 present the production cost structure of the products produced in a subsistence farm.

Table 8. Production cost structure of the products produced in a subsistence farm

Specification	Lei	Structure (% of total)
Fixed expenses	0	0.00
Staff expenses	0	0.00
Variable expenses	26780	100.00
Expenses for electricity	1200	4.48
Fuel expenses	1200	4.48
Expenses for feed	21340	79.69
Expenses for water	240	0.90
Veterinary assistance expenses	400	1.49
Supply and transportation expenses	600	2.24
Other expenses	1800	6.72
TOTAL EXPENSES	26780	100.00

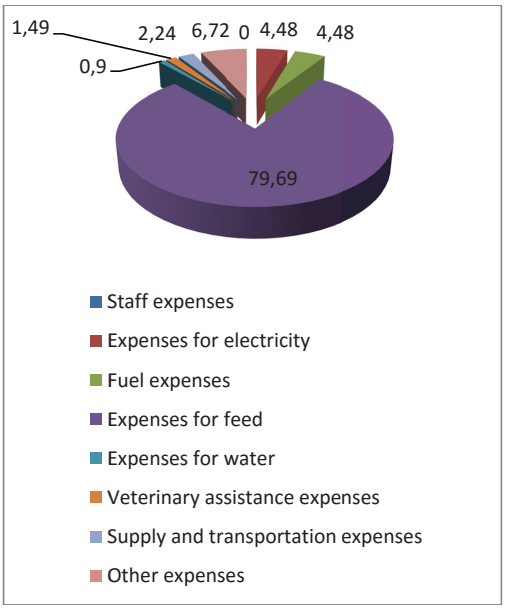

Figure 3. Production cost structure of the products produced in a subsistence farm

Estimated production costs on the analyzed farm are:

1. Fixed unit cost:

$$C_{uf} = \frac{Fixed\ expenses}{Production\ volume} = \frac{0}{5475} = 0\ lei\ per\ milk\ liter$$

2. Variable unit cost:

$$C_{uv} = \frac{Variable\ expenses}{Production\ volume} = \frac{26780}{5475} = 4.891\ lei\ per\ milk\ liter$$

3. Total unit cost:

$$C_{ut} = \frac{Production\ expenses}{Production\ volume} = \frac{26780}{5475} = 4.891\ lei\ per\ milk\ liter$$

We present in Table 9 profit calculation for analyzed farm.

Table 9. Profit calculation in a subsistence farm

INCOMES	✓ Telemea cheese: 1368.75 kg x 15 lei/kg = 20531.25 lei/year ✓ Male youth: 3 capete x 800 lei/cap = 2400 lei/year ✓ Subsidies: - 5 female for milk x 1393 lei/head = 6965 lei/year - 3 heads (other categories) x 575 lei/head = 1725 lei/year
	TOTAL INCOMES: 31621.25 lei/year
EXPENSES	✓ Expenses for electricity: 1200 lei/year ✓ Fuel expenses: 1200 lei/year ✓ Expenses for feed: 21340 lei/year ✓ Expenses for water: 240 lei/year ✓ Supply and transportation expenses: 600 lei/year ✓ Veterinary assistance expenses: 1800 lei/year ✓ Other expenses: 600 lei/year
	TOTAL EXPENSES: 26780 lei/year
BENEFIT	Total income - total expenses = 4841.25 lei
PROFIT TAX	774.6 lei
NET PROFIT	4066.65 lei

It is observed that for one liter of milk are necessary expenses amounting to 4.89 lei. It highlights the very high proportion of feed costs in total expenses for one liter of milk (79.7%) given that staff costs are zero.

As stated in the questionnaire, in subsistence farms the entire production is processed in Telemea cheese, which sells for 15 lei/kg. Also it offered for sale 3 heads of youth male for fattening at a price of 8 lei/kg bodyweight.

Cheese processing within subsistence farms is economically inefficient because the production unit cost would be 19.56 lei per kg.

Regarding the relationship between incomes and expenses, profit can be achieved in a

subsistence farm only under subsidies accessing.

CONCLUSIONS

Whatever the size of the farm, milk and Telemea cheese are produced under conditions of total economic inefficiency. Total unit cost higher than the selling price per unit of product makes profit impossible. Obviously, this statement refers only to the production of milk and cheese without discuss other salable production of the farm (youth for meat, breeding youth).

In all three analyzed cases, there is a high value of variable unit cost. This value is given, for the most part, by the expenses for feeding. It is known that the variable unit cost decreases as production volume increases (to a point). As a result, it appears advisable to increase the global production of milk at the farm level. This action should cover several aspects: a) increasing the number of animals (at a certain level can be an economically non-viable solution, due to the growth of investments); b) increase the production potential by developing animal breeding or active conservation programs; c) improving environmental conditions (maintenance and feeding) in order to fully exploit the genetic potential of animals.

Economic losses in the three analyzed cases are determined by the fact that the sale price is well below the variable unit cost, and can not diminish losses due to staff costs.

Minimize unit cost of production should be permanent objective of farms whereas in relation to the price at which products are sold, determine the level of profit.

Increasing the volume and quality of milk production and diversification of products offered for sale are efficient ways to minimize the cost per unit of product. Diversification of production at farm level should be a strategic objective of buffaloes exploitation in Romania. Meat and meat products (beef jerky, dried raw salami, sausages, liver pate, etc.) and diversification of products from milk (precursor of Mozzarella, milk for coffee, plain and fruit yogurt, buffalo milk desserts, sweet cream for whipped cream, Mediterranean dishes (soak cheese in herbs and olive oil, etc.) may be viable long-term solutions. But these actions require, on the one hand, effective strategies in the medium and long term, developed by local authorities, on the other hand farmers association.

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PRODUCTIVE PERFORMANCES OF HYBRIDS DEPENDING ON GENOTYPES OF MATERNAL AND PATERNAL FORMS OF SWINE

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Abstract

In this paper there are presented the results of hybrids appreciation obtained by combining Large White mixed production, Landrace breeds maternal forms and Pietrain, Hampshire – paternal forms. It was proven that for the formation of qualitative carcasses it would be rational to use tri-racial hybrids LA x L x P in production units, which are characterized by globular hams and an intensive development of main muscles that are qualitative meat providers.

Key words: *meat, hybrids, breed, carcass, muscle.*

INTRODUCTION

The creation of hybrids and swine cross requires the improvement of breeds genetic potential, types and parental lines.
For the selection of advanced hybrid, the appreciation of combinative capacity of genotypes on hybridization is needed.
The combination of different genes provides the modification of heredity, the growth of vitality, prolificacy and increase of swine meat production by using every genetic possibility such as the selection effect, the crossbreeding and heterosis.
The implementation of hybridization on swine depends on the results of swine selection, the amount of breeds and lines controlled by the combinative capacity and scientific provision of this amelioration method.
It is important to do researches concerning the production of qualitative meat which corresponds with the consumers preferences. In such conditions the quality of carcasses had a direct influence on the quantity and quality of products which should be prepared.

MATERIALS AND METHODS

The research was done at the abattoir "FARM MEAT CENTRU" from Bardar, using biologic material obtained in production units FARM MEAT PROCESSING.
In order to make this study there were formed 4 lots of hybrid obtained from the combination of Large White, Landrace, Hampshire and Pietrain breed which were exposed to fattening until 120 kg (Table 1). From each lot there were sacrificed 6 hybrids of swine.

Table 1. Research scheme

Lot	Parental forms		Animal weight at slaughter, kg	Number of animals
	Maternal	Paternal		
I	Large White	Large White	119.5	14
II	Landrace×Hampshire	Pietrain	120.3	14
III	Large White×Landrace	Hampshire	119.8	14
IV	Large White×Landrace	Pietrain	120.6	14

The carcass weight was determined by their weighing after slaughter, using electronic scale. The length of carcasses was measured with a ribbon, starting with the first cervical vertebra until the pectin. The width of carcasses on the exterior was determined on sixth vertebra, using ribbon, and on the interior on the chest part towards the thoracic vertebras.
The thickness of fat layer was measured with a ruler on thoracic vertebra 6-7, back, chest, croup, abdomen and flank.

The flitch was marked off between the last and penult lumbar vertebra and hocks, and then using electronic scale the weigh was determined.
Long dorsal muscle and the sirloin were prepared according to existing requirements. The results obtained were processed statistically (Bucataru, 1993).

RESULTS AND DISCUSSIONS

As a result of using different swine breeds for the production of hybrids there comes the necessity to determine general combinative and specific capacity. This is why, based on the results obtained there could be recommended different combinations of breed and lines for the obtaining of products requested by the consumer.
The quality of carcasses on swine depends particularly on their length and thickness on the interior and exterior. The longer the carcass will be, the more it will contain lean meat.
The width of carcasses indicates the rate of fat formation on superior and inferior line. The carcasses with a bigger width indicate a bigger amount of fat. (Table 2)

Table 2. The genotype influence on swine carcass quality

Lot	N	Genotype	Carcass weight, kg	Carcass length, cm	Carcass width on exterior, cm	Carcass width on interior, cm
I (control)	6	MAxMA	80.50±0.74	95.27±0.49	38.31±0.87	22.61±0.58
II	6	L×H×P	81.24±0.78	95.51±1.06	35.44±0.94	18.32±0.43
III	6	MA×L×H	82.21±0.96	97.52±0.34	37.80±0.63	20.14±0.66
IV	6	MA×L×P	83.22±0.68	97.41±0.54	34.64±0.79	19.55±0.78

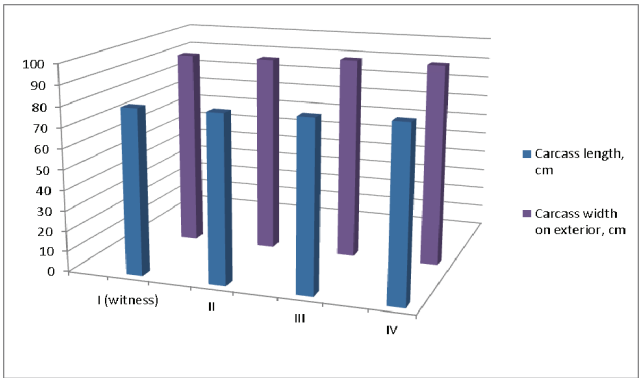

Figure 1. The length and width of carcasses depending on animals genotype

Dates presented in the table prove that carcasses with a length over 97 cm were obtained from III and IV lot, where the maternal form were biracial sows MA x L. The difference between lot III, IV and I was equal with 2.25 cm, 2.14 cm (B≥0.95). In these lots there were obtained 2.72 kg, 1.71 kg heavier carcasses (B≥0.95).
The width of carcasses on the exterior in lots where there were used terminal boar of Pietrain breed was equal with 34.64-35.44 cm, and the difference represents 2.87 -3.67 cm (lots II and IV) (B≥0.95).
Such tendencies were identified by observing the thickness of carcasses on the interior. Comparative differences with control lot were equal with 4.29 and 2.06 cm. These results prove that the layer of fat on swine from these lots was thinner in different parts of carcasses (Table 3).
The thickness of fat layer in towards 6-7 thoracic vertebra was significantly lower in lots II and IV with 10.0 and 11.1 mm (B≥0.999) which confirms the fact that hybridization influences positively the carcass quality.
Such differences were registered in the back part, where there were equal with 13.9 and 8.1 mm (B≥0.999).

Table 3. The thickness of fat layer depending on swine genotype

Lot	N	Genotype	The thickness of fat layer, mm			
			6-7 thoracic vertebras	Back	Chest	Croup
I (control)	6	MAxMA	37.8±0.49	32.6±0.65	26.5±0.70	24.4±0.51
II	6	L×H×P	26.4±0.91	18.7±1.01	15.4±1.4	18.5±0.85
III	6	MA×L×H	30.5±0.67	29.3±1.14	20.8±1.25	22.6±1.3
IV	6	MA×L×P	27.8±0.87	24.5±0.94	19.2±1.09	21.0±0.89

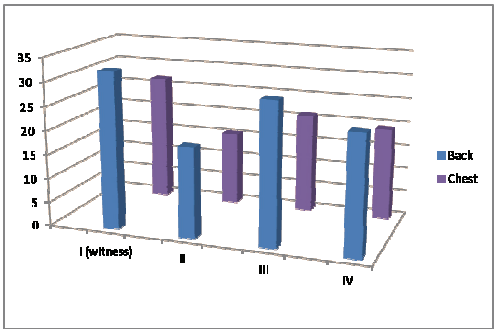

Figure 2. The influence of genotype on the fat thickness on spine and loin

Here, the fat layer was thinner in lot II, equal with 18.7 mm when the obtaining of hybrids was used only in specialized breed for the meat production such as Landrace, Hampshire, Pietrain.

In the chest part, the fat layer varied in experimental lots between 15.4 and 20.8 mm in lot III of young swine. Differences were 11.1 and 5.7 mm (B≥0,999).

The quantity of meat from carcass is influenced by the degree of ham development, long dorsal muscle and sirloin (Table 4)

Table 4. Genotype influence on ham development, long dorsal muscle and sirloin

Lot	Genotype	N	Weight, kg		
			Ham	Long dorsal muscle	Sirloin
I (control)	MAxMA	6	9.20±0.68	1.55±0.06	0.348±0.08
II	L×H×P	6	12.31±0.41	2.49±0.11	0.666±0.06
III	MA×L×H	6	10.52±0.52	1.96±0.16	0.440±0.09
IV	MA×L×P	6	11.24±0.65	2.31±0.22	0.580±0.12

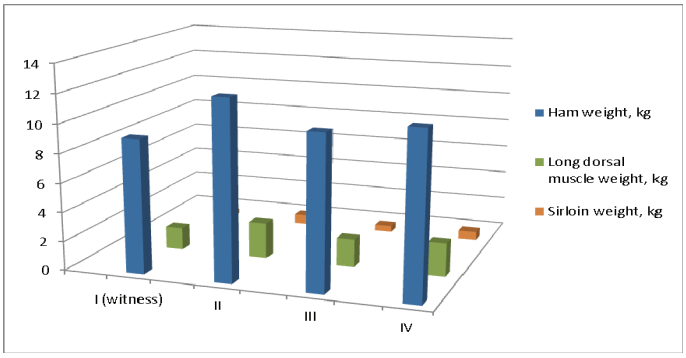

Figure 3. The weight of ham, long dorsal muscle and sirloin

The genotype of animals influences the degree of development of principal muscles from carcass and ham, their weight being equal with lots II, III and IV with 10.52-12.31 kg. The differences between lot being insignificant in comparison with lot I, being 1.32 and 2.04 lot III-IV, 3.11 kg (lot II) (B≥0.95).

The weight of lung dorsal muscle in these lot was of 1.96-2.49 kg, the differences in comparison with control lot (Large White) reached limits between 0.41-0.94 kg. Sirloin reached values that vary in lots II, III and IV from 0.440 to 0.666 kg, increasing compared to witness lot with 0.092 kg-0.318 kg, results which formed these differences from genotypes (B≥0.95).

Qualitative meat quantity formed in carcass depends on the development of ham according to length and perimeter (Table 5).

Table 5. The development of ham and thickness of inferior line carcasses

Lot	Genotype	Ham length, cm	Ham perimeter, cm	Fat thickness on inferior line, mm	
				Abdomen	Flank
I (control)	MAxMA	40.02±0.38	78.16±0.17	27.36±0.36	29.42±1.05
II	L×H×P	45.15±0.77	85.24±0.56	20.11±0.81	24.81±0.39
III	MA×L×H	42.4±0.85	83.18±0.64	23.58±0.95	27.15±0.58
IV	MA×L×P	44.12±0.37	84.42±0.77	21.23±0.40	25.66±0.96

The results obtained according to dimensional measurements, prove that ham with a bigger length, which varies from 42.4-45.15 cm were accomplished by experimental lot hybrids. The best dates were obtained in young swine lot II, where the length of ham was equal with 45.15 or with 5.13 cm (B≥0.999) bigger in control lot (I). The carcasses in lot IV where characterized by a higher length of ham, with 4.10 cm (B≥0.999). We can conclude that in these lots, hams were globular and in the result, their perimeter reached 83-85 cm, the difference in comparison with lot I was equal with 7.08 (lot II) (B≥0.999 and 6.16 (lot IV) (B≥0.999). The quantity of fat from carcasses depend on the fat layer formed on the inferior line, especially in the abdomen are and flank.

The dates presented in Table 5 prove that fat layer in this areas exceeded 20 mm in all lots, but hybrids from lot III formed a fat layer in abdomen area with 7.25 mm (B≥0.999) thinner, in comparison with control lot. On flank, the difference was equal with 4.61 mm (B≥0.999), and in lot IV with 3.76 mm (B≥0.999).

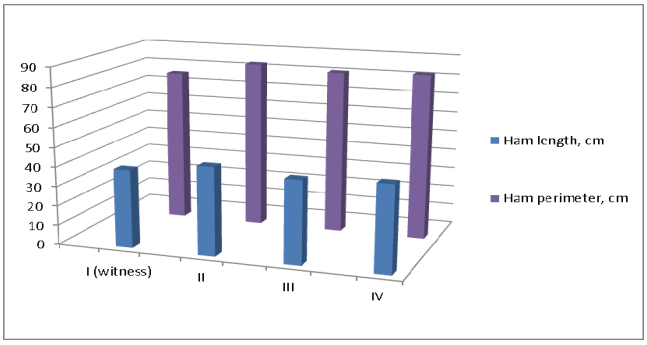

Figure 4. The length and perimeter of ham on swine hybrids

CONCLUSIONS

1. The capacity of combining breeds which influence the degree of development of carcasses, hams and main muscles. The weight of long dorsal muscle in II and IV lots was of 0.94 kg and 0.76 kg heavier (B≥0.95) in comparison with lot I. The weight of sirloin was proved by values, which difer depending on animals genotype, formed by crossing mixt breeds and specialised on meat production.
2. The thickness of fat layer on 6-7 torachic vertebra was less in II and IV lot and 11.4 mm (B≥0.99) than in control lot. On the back part, the differences were equal with 13.9 and 8.7 mm (B≥0.999). This confirms that hybridization influences positively the quality of carcasses and contributes to the increase of superior quality meat amount.

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MANURE FROM LIVESTOCK FARMING IN THE EUPHRATES BASIN AND ITS POTENTIAL ENVIRONMENTAL IMPACT ON WATER RESOURCES

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Abstract

Food of animal origin derived from livestock farming establishments is necessary to meet the needs of human beings. The wastes generated during the production stages of cattle breeding (manure, ground covers, etc.) create a danger to the environment and water resources when they are released to the environment randomly. Therefore, the waste generated by livestock farming establishments should be stored in a controlled manner and should not cause environmental pollution. The research was carried out in the Euphrates basin and covered four provinces (Adiyaman, Sanliurfa, Gaziantep, and Kilis) and 35 districts. In the context of this study, the number of the cattle bred in the establishments in 328 villages which are at least 150 m and at the most 5 km distance to the flow path of the Euphrates was taken into consideration. In the study, Erdas Imagine 9.3 and ArcMAP 10.0 software were used; the number of the cattle and the distance to the rivers were evaluated as a layer. The subject of the study was the investigation of the potential pollution effects of the cattle breeding on the Euphrates River and its tributaries, and it was carried out to determine risky, non-risky and partially risky areas and attract attention to this issue. As a result, it was concluded that 4 districts and villages of Sanliurfa Region would be classified as risky areas, 3 districts and villages of Gaziantep Region would be classified as partially-risky areas, and 3 districts and villages of Adiyaman Region and all districts and villages of Kilis Region would be classified as non-risky areas.

Key words: *Euphrates Basins, Livestock, Manure management, Water resources.*

INTRODUCTION

Animal manure is a natural waste of biological cycle process from feed to product and, in general, it contains soluble and easily degradable organic materials and inorganic components. Nitrogen (N), together with phosphorus (P), is known as the most controversial element in the context of relations between livestock production and the environment. As a result of the chemical transformation, nitrogen contained in the feces can be transformed into various gasses such as nitrate, nitrous oxide (N_2O), nitrogen monoxide (NO) and nitrogen dioxide (NO_2). The characteristics of the manure obtained from livestock shelters largely vary from farm to farm. The age, gender, ration of the animal, production system and other factors can be mentioned as factors affecting the content of manure characteristics (Tamminga and Verstegen, 1996).

Environmental problems are caused by the pollution in soil and groundwater created by the nitrogen compounds in feces and urine. The type of animal, applied farming methods and the level of intensive breeding affect the pressure of the manure on the environment. (Van Horn et al., 1994).

The nitrogen and phosphorus content of the manure is mainly influenced by the style of feeding (Powers and Angel, 2008).

Excessive use of commercial fertilizers and excessive animal manure raise the plant nutrients to an amount which will cause serious pollution to the environment (Atilgan et al., 2006).

Animal manure includes many microorganisms with feces, bedding materials, and feed waste origin (Tanski et al., 2006).

Microorganisms can cause health risks if they each plants, water, animals and people that are more sensitive. Livestock enterprises that are built in locations not suitable for water resources and settlements and have no adequate infrastructure in terms of waste disposal, reclamation and treatment are known as a source for many health problems (Pell, 1997; Gilchrist et al., 2007).

The most negative impact of animal production on the environment is that it creates a source of infectious disease agents. These factors may spread to the environment in the form of direct or indirect disposal. The pits in which the wastes disposed of the sheds are stored create great threat as a source of infection for humans and animals. That some disease agents arising from the animals can live in nature for a period ranging from one week to 3 years is important, for it shows that the pollution to be created may be effective for a very long time (Ergul, 1989).

The internal environment of the shelter consists of temperature, humidity, airflow and various gas, odors and dust while the external environment consists of slaughterhouse products generated by slaughtering and procedures applied to dead animals. Since other auxiliary buildings located in the courtyard outside the shelter create the agricultural enterprise together with the shelter, environmental pollution emerges as a result of the procedures applied in these areas. Therefore, the hazardous waste adversely affecting animal and human health both within and outside the agricultural building must be eliminated, if this is not achieved, it must be kept below the tolerance values not to harm the environment (Alagoz et al., 1996).

Modernization and intensive operations in the rapidly developing livestock enterprises brought a number of problems. Waste which is also an important economic potential is a significant problem for the environment in the direct proportion to the number of animals. If the necessary precautions are not taken, waste generated by the livestock enterprises can contaminate surface and underground water resources as potential pollutants. For this purpose, necessary storage and project designing criteria must be investigated in order the waste generated from livestock enterprises not to create adverse environmental conditions (Karaman, 2005).

The use of manure ignorantly and with inappropriate techniques in the agricultural production also reduces the effectiveness of manure. As a result of keeping manure under unsuitable conditions or implementing it randomly in the land, it loses a significant amount of nutrient composition due to washing and evaporation, and the expected benefits from agricultural manure in terms of agricultural production and soil fertility cannot be achieved (Boyaci, et al., 2011).

Livestock sector in our country still has the largest share after agriculture. Due to the significant increase in the capacities of farms and hence the amount of manure in recent years, environmental problems caused by the manure came to the agenda. Due to the development of agriculture and the increase in the number and capacity of integrated animal farms, environmental problems caused by animal waste are increasing (Inan, 2012).

This study covers Adiyaman, Gaziantep, Kilis and Sanliurfa provinces in the Euphrates Basin, and its aim is to determine the effect of the potential pollution caused by cattle breeding enterprises on water resources using the Geographic Information System (GIS) methods. In this context, the study was carried out to determine risky, non-risky and partially risky areas and attract attention to this issue.

MATERIALS AND METHODS

The research was carried out in the area covering cattle breeding enterprises located at least 150 m and at most 5 km distance to the river coast in Adiyaman, Gaziantep, Sanliurfa and Kilis provinces and their districts which are within the flow path of the Euphrates.

The Euphrates River is the most productive river in Turkey with the highest water potential. After determining province borders of Erzincan, Elazig, Malatya, Diyarbakir, Adiyaman, Gaziantep, Sanliurfa provinces, the Euphrates River enters first into Syria and then

Iraq. The most important tributaries of the Euphrates are the Murat River, the Karasu River, the Streams of Tohma Creek, Peri, Calti, and Munzur.

The total length of the river is 2.800 km, and the length of the section within the borders of Turkey is 1.263 km. It has 720.000 km^2 catchment area (Anonymous, 2015).

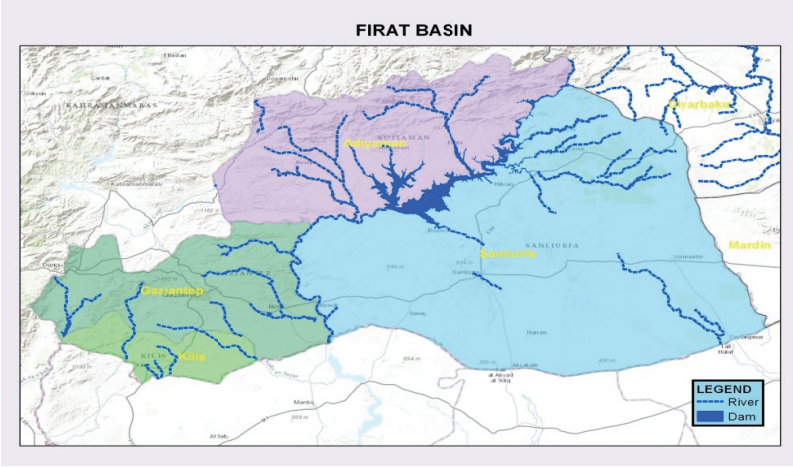

Figure 1. Provinces in the study area

Livestock activities around four provinces and districts covering the research area are being carried out intensively. The number of cattle fed in the agricultural enterprises along the Euphrates River was determined as 17.078 for Adiyaman province, 53.955 for Sanliurfa province, 109.519 for Gaziantep province and 2.978 for Kilis province, and 183.530 in total (Anonymous, 2014).

In the study, in above-mentioned provinces on the basis of districts, it was aimed to evaluate the presence of cattle and the effect of its presence on the environment in the setting of Geographic Information System (GIS). For this purpose, these places were digitized in the setting of GIS as polygons based on first province borders and then district borders. ArcMap 10.0 software was used for this purpose. The topographic base map within the said software was used. Since the program works based on layers, any qualification obtained was considered as a layer. First, the borders of the study area were digitalized by making geographical corrections. The whole boundary was divided into a separate layer on the basis of provinces in order the inquiries for each province to be carried out independently from other provinces. Similarly, the districts of each province were divided as separate layers based on the borders of the districts. Evaluating the present bedding materials and identifying the water resources of the region, they were digitalized as a separate layer. The database (attribute data) was created in these layers prepared using the statistical information obtained from governmental bodies. All water resources (rivers, lakes, etc.) along the Euphrates River were digitalized, and the cattle breeding enterprises were chosen. This process was prepared using Analysis Tools/Buffer command within the ArcMAP 10.0 software. The classification of the number of animals was performed using the attribute table in order to evaluate the effects of the number of animals.

The number of cattle in 2014 was evaluated within 5 layers and shown on the map of the basin by different coloring (Table 1).

Table 1. Representation of the number of Livestock on The Study Area Map

Cattle Number in Enterprises	Display Color on the Map
1- 9	
10- 22	
23- 41	
42- 82	
83- 83+	

RESULTS AND DISCUSSIONS

The most important water resource of the region is the Euphrates River that has a wide range area together with the dams between Adiyaman and Sanliurfa provinces and its tributaries. Animal numbers are quite variable between provinces and regions. While the number of animals in certain regions is over 1500, it remains even below 50 in some regions. When particularly the villages and towns near water resources were considered, it was observed that water resources in the villages and towns having more than 1500 cattle were under a higher pollution risk. 77 villages/towns in Gaziantep, 104 villages/towns in Adiyaman, 26 villages/towns in Kilis and 121 villages/towns in Sanliurfa, 328 villages/towns in total were evaluated by provinces in the basin with particular attention to choosing the villages/towns close to the water resources; 26742 cattle in this region are grown for commercial purposes (Table 2).

Table 2. Livestock and Cattle Population

Study Area	Village	The number of villages close to water resources	Number of cattle in the villages nearby on water resources
Adiyaman	478	104	2.803
Sanliurfa	881	121	11.849
Gaziantep	473	77	10.742
Kilis	113	26	1.348
Total	1945	328	26.742

Gaziantep and Kilis: The number of cattle in the enterprises in these regions is high; therefore, the potential pollution generated by these enterprises may be high.

However, these regions are considered to be partially-risky areas, for there is fertilizer use possibility in the industry in these regions (Figure 2).

In Adiyaman region, the potential pollution effects caused by the livestock enterprises were predicted as low, for the enterprises are not close to water resources, and the number of cattle in the enterprises lose to water resources is low compared to other provinces, and it was concluded that this region may be classified as non-risky area (Figure 3).

In Sanliurfa Region, the number of cattle in villages/towns located in the areas close to water resources was determined as high (Figure 4). Considering that cattle produces 1 ton/month manure (Bengtsson and Whitaker, 1986), 11849 tons/month manure that will have an effect on water resources will be produced in Sanliurfa region.

Therefore, the potential pollution effects caused by livestock enterprises in the province were predicted to be high compared to other provinces, and this region was classified as the risky area (Figure 4).

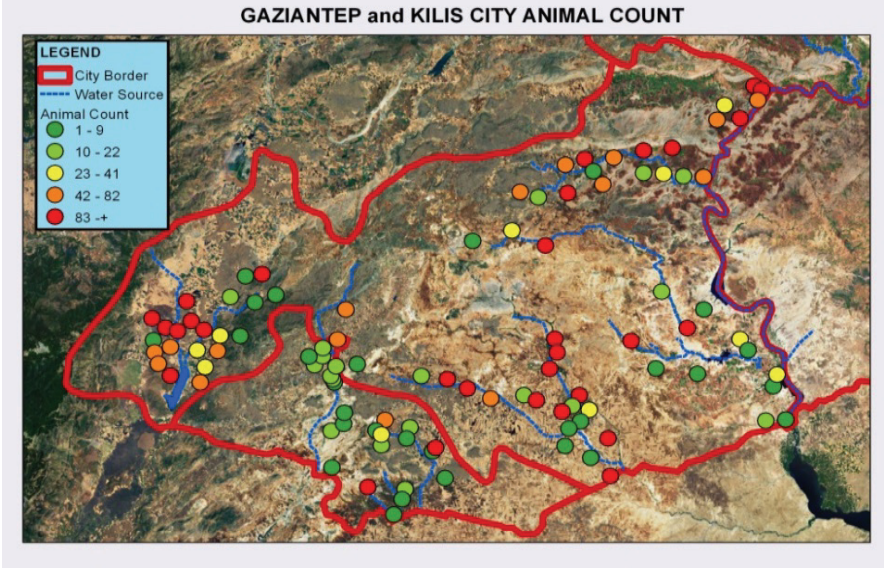

Figure 2. Gaziantep and Kilis City Cattle Population

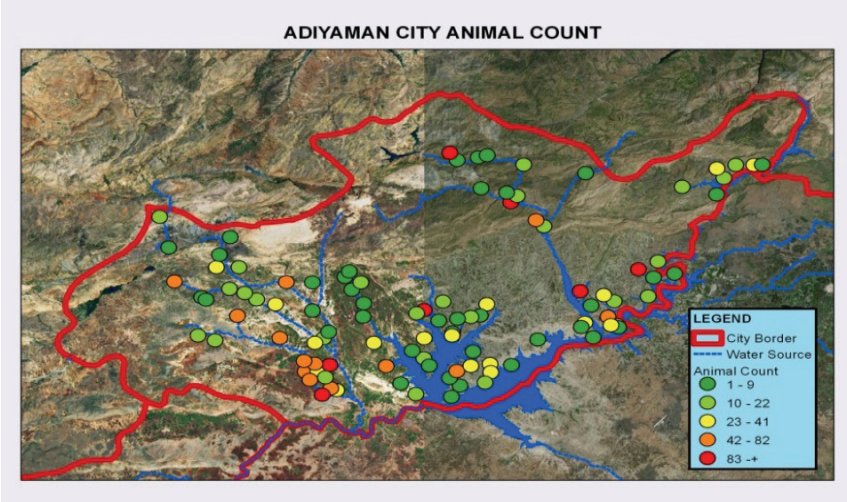

Figure 3. Adiyaman City Cattle Population

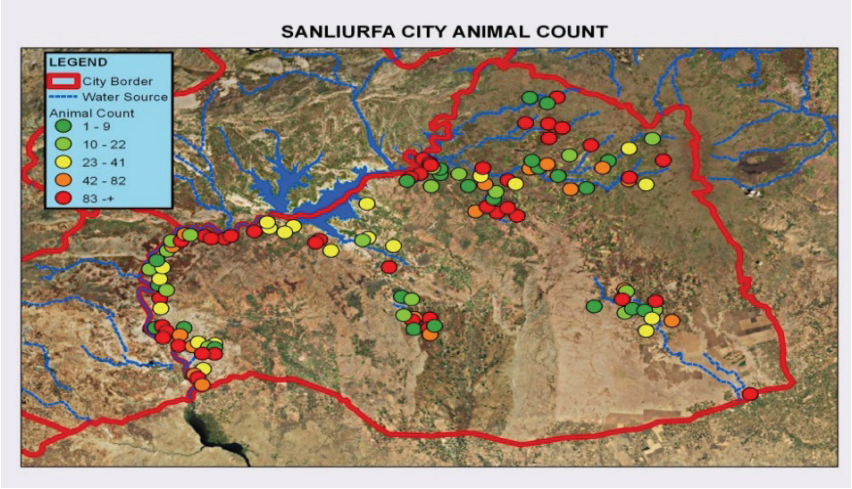

Figure 4. Sanliurfa City Cattle Population

When the general structure of livestock enterprises in the Euphrates Basin was examined, it was observed that intensive cattle breeding activities were carried out in the areas close to water resources, but operators were not sensitive to the issue of waste management. It was observed that the waste storage buildings are within the boundaries of Gaziantep province, and many establishments were insufficient in this regard (Figure 5).

It is understood that manure and waste generated from the production activities of enterprises must be stored appropriately not to pollute water resources and the environment. If the manure generated from these enterprises is stored outside randomly in piles, they can be a potential source of pollution for water resources by causing environmental pollution, visual pollution, and odor pollution. Due diligence must be taken when storing manure generated from livestock enterprises and burying it for crop production.

The interference of animal waste with water resources during manure management reduces the aquatic quality and causes the death of aquatic life. Ammonia, pathogens and organic substances with biological oxygen values contained in the waste lead to water pollution.

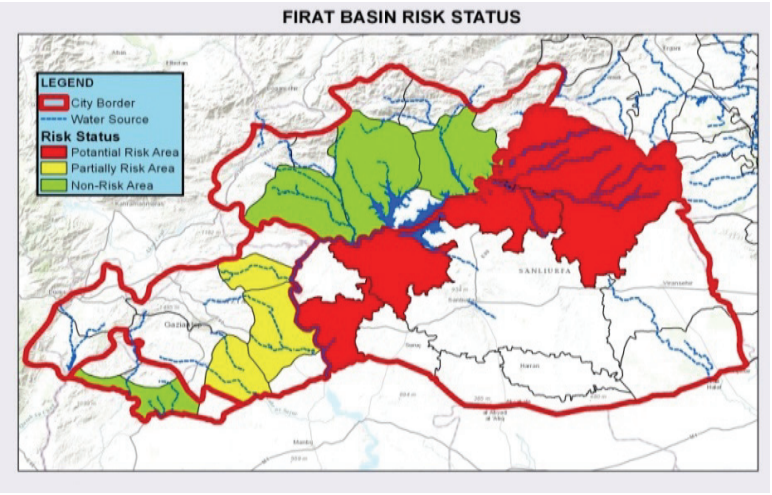

Figure 5 Euphrates Basins Potential Risk Area

CONCLUSIONS

In this study, it was tried to determine the risky areas for the environment and water resources that would be created by the manure generated by cattle breeding enterprises operating along the bank of the Euphrates River. According to this, it was concluded that Bozova, Siverek, Hilvan and Birecik districts and their villages located close to the part of the Euphrates River within the boundaries of Sanliurfa province could be classified as risky areas, Nizip, Yavuzeli and Oguzeli districts and their villages in Gaziantep province could be classified as partially risky areas, City center, Besni and Kahta districts and their villages of Adiyaman province and all districts and villages of Kilis province could be classified as non-risky areas. The results of this study are expected to contribute to the improvement of manure management implementations of the establishments in the study area and attract attention to the pollution potential of the manure for water resources.

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USE OF SYNCHRONIZATION IN HATCHING OF POULTRY EGGS

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Abstract

The essence of the synchronization is the reduction of hatching window to a minimum, so that the main mass of the chicks hatched in the same time just before picking them up from the cabin. For this at 19 days of incubation it is necessary to open the flap of the machine and the fresh air is forced to enter, and CO_2 level at the same time lowers to the level of 0.2%. Thus there are created favourable conditions for a longer stay in the egg for the embryo. In this mode, the egg is incubated for 24 hours. Then, at 20 day of incubation, the damper is closed, CO_2 level raises to 0.55% and the chicks begin to hatch intensively. The hatcher is set up so that the flap is entirely dependent on CO_2 level, so than higher is CO_2 level, the more opens the valve and more air goes into the cabinet. As a result of hatching synchronization process it was possible to mention that the time of first appearance of chicks was 19 days and 12 hours in the experimental group and 19 days and 22 hours in the control group from the beginning of incubation period.

Key words*: eggs, hens, hatching period, synchronisation.*

INTRODUCTION

The increase in poultry meat consumption is associated with a number of key factors, first and foremost, it is a growing population and the need for protein of animal origin, as well as lower prices for chicken products as compared with the prices of competitive meats.

One of the important technological aspects of poultry production is eggs incubation on proper carrying out of which is largely dependent on both economic efficiency of breeding farms themselves, and maintenance performances, growth and development of poultry during its subsequent growth.

Modern incubation is a high-tech process, implementing the most advanced technical solutions to achieve the biological sciences, information technology and provides highly accurate and flexible regulation of micro-climatic conditions, allowing to receive high levels of egg hatchability. In such circumstances, further progress in the field of incubation is seen on the one hand in the automation and robotics of all links of the process chain and control of the process of eggs incubation, on the other hand - in-depth study and taking into account the biological characteristics of the eggs caused by genetic, age, morphological and climatic factors (Тагиров et al., 2009).

The industrial incubation of eggs is of a great importance in poultry reproduction. During the time of intensification of poultry brunch had increased the capacity of Incubation Park and the volume of eggs incubation.

New specialised incubators provide incubation of eggs of large lots, higher rates of hatching index and reduced labour costs (Кочиш И. И., et al., 2004).

The main problem of hatching management is to receive a large number of homogeneous strong day - old chicks. The viability is the main criterion of health that originates in embryo and directly relates to performances and endurance of individual batches of chickens.

Results of eggs incubation, depend on the compliance of technologic processes and requirements. The main goal of every farm is to get healthy and strong chicks. Modern technologies involve the introduction of a variety of ways to improve the quality of day old chick.

The scientific novelty of the study was that the synchronization of chicks hatching was performed in developing the programs for the first time that is practiced in a country incubation station.

MATERIALS AND METHODS

The experiment was carried out in industrial conditions of hatching station Servalim SRL, of

Floreni Holding. As a biological object of study were eggs of Cobb 500 cross brought from Wimex firm, Germany.

There were studied the incubation quality index of eggs laid by hens of breeding flock of 50-55 weeks old. There were carried out six experimental series.

The eggs incubation was done in the hatching machine Smart Set Pro 77 and the chicks hatching was done in the machines Smart Hatch Pro 4.

Each group consisted of 82.944 eggs. The conditions of eggs incubation were similar for all the series. The eggs were incubated accordingly to the hatching programme (Table 1).

Table 1. Eggs hatching programme

Time of setting change (d/h)	Temperature, °F	Relative humidity, %	CO_2	AMF	Rotation
-0,05/0,08	77.0-81.0	60	0.40	Off	0
0.00	100.4	60	0.40	Off	2
1.00	100.2	60	0.40	Off	2
2.00	100.0	60	0.40	Off	2
3.00	99.9	55	0.40	On	2
4.00	99.9	55	0.40	On	2
5.00	99.9	55	0.40	On	2
6.00	99.9	55	0.40	On	2
7.00	99.8	55	0.40	On	2
8.00	99.8	50	0.40	On	2
9.00	99.7	50	0.40	On	2
10.00	99.5	50	0.40	On	2
11.00	99.2	50	0.40	On	2
12.00	98.8	50	0.40	On	2
13.00	98.5	45	0.40	On	2
14.00	98.3	45	0.40	On	3
15.00	98.0	45	0.40	On	3
16.00	98.0	45	0.40	On	3
17.00	98.0	45	0.40	On	3
18.00	98.0	45	0.40	On	3

Before loading the incubator we determined the quality parameters of hatching eggs, such as weight, diameter of air chamber and index format.

Before loading all the eggs were disinfected in special fumigation chamber at an air temperature of + 21°C using Vapcophore drug based on iodine for 40 minutes.

The eggs were transferred into the setter for preheating for 7 hours at + 25°C, and then the eggs were introduced in the incubation mode.

After the transfer of the eggs at 18 days of incubation for the experimental group the synchronization mode was used, and the eggs from the control group continued to be incubated at normal mode.

The essence of the synchronization is the reduction of hatching window to a minimum, so that the main mass of the chicks hatched in the same time just before picking. For this at 19 days of incubation it is necessary to open the flap of the machine and the fresh air is forced to enter, and CO_2 levels at the same time lowers to the level of 0.2%. Thus there are created favourable conditions for a longer stay in the egg for the embryo. In this mode, the egg is incubated for 24 hours.

Then, on 20th day of incubation, the damper is closed, CO_2 level raises to 0.55% and the chicks begin intensively to hatch.

The hatcher is set up so that the flap is entirely dependent on CO_2 level, so than higher is CO_2 level, the more opens the valve and more air goes into the the cabinet. All hatched chicks were evaluated on a scale Pasgar Score.

RESULTS AND DISCUSSIONS

The eggs weight in the control and experimental groups is almost the same and ranges from 69.9 to 71.9 g in the control group and from 69.5 to 71.9 g, in the experimental group.

The other studied index was diameter of air chamber. Accordingly to the standard the diameter of air chamber must be 18-20 mm, but in our experiences it was larger and it was connected to the eggs age that was 4-7 days.

The eggs of control groups were incubated at normal mode, and for the eggs of the experimental groups was used the synchronization mode. The synchronization programme is shown in the table 2.

Table 2. Synchronization programme

Hatching days	Control group			Experimental group		
	t°F	Humidity, %	CO_2, %	t°F	Humidity, %	CO_2, %
18 d. 5 h.	98	55	0.4	98	55	0.4
19	98	60	0.4	98	60	0.2
20	98	75	0.4	98	75	0.55
21	97	60	0.4	97	60	0.3

Table 3. Hatching results in control group

Series of experiments	Total eggs	Chicks		Waste		Chicks with reed knees	
		heads	%	heads	%	heads	%
1	41472	34660	83.57	992	2.39	464	1.12
2	41472	33695	81.25	1310	3.16	465	1.12
3	41472	33970	81.91	962	2.32	497	1.20
4	41472	33700	81.26	1215	2.93	597	1.44
5	41472	34565	83.35	930	2.24	530	1.28
6	41472	34050	82.10	915	2.21	564	1.36
$\overline{X} \pm S\overline{x}$ $\overline{X} \pm S\overline{x}$	41472 ±0.00	34106 ±170.6	82.24	1054 ±67.9	2.54	519 ±22.1	1.25

Table 4. Hatching results in experimental groups

Series of experiments	Total eggs	Chicks		Waste		Chicks with reed knees	
		heads	%	heads	%	heads	%
1	41472	35320	85.17	600	1.45	132	0.32
2	41472	34185	82.43	747	1.80	232	0.56
3	41472	34660	83.57	962	2.32	165	0.40
4	41472	34200	82.47	808	1.95	265	0.64
5	41472	34715	83.71	795	1.92	66	0.16
6	41472	35050	84.51	810	1.95	132	0.32
$\overline{X} \pm S\overline{x}$ $\overline{X} \pm S\overline{x}$	41472 ±0.00	34688 ±184.7	83.64	787 ±47.7	1.90	165 ±29.7	0.40

Incubation parameters as temperature and humidity remain unchanged in both the control and the experimental groups. There is changing only CO_2 level in the experimental group, the figure changed at 19 days of incubation from 0.4% to 0.2%. Thus, a decrease of CO_2 levels at 19th days of hatching from 0.4% to 0.2% makes hatcher to open the valve to maintain the level of CO_2 at a given level. Then the air conditioning system manually changes the level of positive pressure from 6 Pa to 9 Pa thus through the open valve gets plenty of fresh air into the cabinet. Then, on day 20 of incubation, the program changes the level of CO_2 from 0.2% to 0.55% and the shutter begins to close creating critical conditions for the embryo breathing and causing to hatch from the eggs.

With this program the machine is operating for several months, and it may be noted that the quality of the chicks was improved - namely, disappeared red knees and all chicks have become more active. The hatching window was reduced from 36 hours to 26 hours so the period of chicks stay in cabinet decreased with 10 hours in the experimental group compare to the control group.

The results of eggs hatching are presented in tables 3 and 4.

Because of reducing the number of defective chicks we got 1.68% more quality day old chicks in the experimental group.

In the control group, the average number of defective chicks was 2.54% the difference was 0.86%. The number of chicks with red knees was 1.25% in the control group and 0.4% in the experimental group.

About the quality of the chickens can be sorted after evaluation on a scale Pasgar Score.

The final results of chickens' quality are shown in Table 5.

Table 5. Quality of chickens

Series of experiments	Control groups	Experimental groups
1	8.0	9.2
2	7.9	8.6
3	7.7	8.8
4	7.6	8.7
5	7.9	8.9
6	7.8	8.5
$\overline{X} \pm S\overline{x}$ $\overline{X} \pm S\overline{x}$	7.8±0.07	8.8±0.10

The table shows that the results of the control and experimental groups differ. Estimates of the experimental group did not correspond to an ideal chicken of 9 points but they significantly differ from control. In the control group, the average score was 7.8 points, but in the experimental group it was 8.8 points.

After eggs hatching the incubation wastes were studied. We have studied the shell. The remaining shell is a valuable source by which is easy to identify deviations from the normal development of the embryo.

The eggs shell in the control group was dirty with meconium, which tells that the hatched chicks were out and stood longer in the cabinet before taking them out (Figure 1 and 2).

Figure 1. Egg shell in control group

Figure 2. Egg shell in experimental group

After crumbling the shell into the hand it was very dry and crumbly. Dry shell is one of the signs that the chicks were hatched very early and waited for a long time their sorting in a cabinet that usually has a big impact on their quality and further growth.

The shell of egg of the experimental group was clean, and was not stained with meconium. Trying to crush the shell and shell membranes they did not crumble and had broken into large pieces and shell membranes remained intact.

CONCLUSIONS

As a result of held research in the conditions of incubation station SRL «Servalim», it is possible to mention that the time of first appearance of chicks was 19 days and 12 hours in the experimental group and 19 days and 22 hours in the control group from the beginning of incubation and it was possible as a result of synchronization of hatching mode.

For achieving the minimum hatch window of incubation it was developed the program that allowed synchronizing the hatching of chicks and thereby reducing the time spent in the hatcher from 38 hours in the control group and to 28 hours in the experimental group that greatly affected the quality of the chicks.

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SEASONAL CHANGES IN DAILY BEHAVIORAL RHYTHMS OF GÖKÇEADA SHEEP GRAZED INTO RANGELAND WITH INTENSE PRICKLY BURNET (*SARCOPOTERIUM SPINOSUM*) COVER

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Abstract

In this study, seasonal changes in daily behavioral rhythms of Gökçeada sheep into the pastures were investigated. The research was conducted at Gökçeada Island located at the far-west end of Turkey in Aegean Sea. This study is a part of 3-year rangeland reclamation project. Experiments were conducted into 8 plots surrounded with fences including 3-4 years old 40 head sheep (0.15 ha/sheep) grazed into the rangeland for a year. Prickly burnet cover ratio was found between 71.4-88.2% in reclaimed plots 95.7-94.9% in control plots. The sheep were observed through directs observations with sampling method in day-time. Significant differences were observed in grazing behaviors of Gökçeada sheep in different seasons ($P<0.0001$). Grazing frequency was high in winter and spring seasons and low in summer season ($P\leq0.05$). The sheep grazed in winter and spring seasons 2 times higher than autumn season. Grazing frequency was quite low in summer season. The sheep grazed in summer season rather in cool hours of the morning and evening. They spent rest of the day with resting and rumination. They spent majority of the day with grazing in winter and spring seasons. It has been concluded in this study that Gökçeada sheep, raised almost under wild conditions, were able to self-sustain into the rangeland with dominant prickle burnet cover through efficiently benefiting from prickly burnet in all season.

Key words: *Gökçeada Island, pasture reclamation, seeding, day time, climate.*

INTRODUCTION

Prickly burnet (*Sarcopoterium spinosum*) is quite common over the garrigue vegetation of Mediterranean island rangeland (Lanteri et al., 2012). Several methods including burning, herbicide treatments, mechanical control (cutting or grubbing) and fertilization were practiced into the rangeland to control this invasive species (Papanastasis, 1980; Henkin et al., 1998; Perevolotsky et al., 2001; Henkin et al., 2007). It was indicated that grazing could also be a control practice over these sites (Bartolome et al., 2000). Undoubtedly, goats benefit from such sites in a best way (Papanastasis et al., 2008). However, sheep were reported more efficient in control of shrubbery lands with shrubs shorter than 0.5 m (Papachristou, 1997; Ferreira et al., 2013).

As it was in other sites with dominant Mediterranean climate, prickly burnet is widespread over the island vegetation of Gökçeada. Commonly found Gökçeada (Imbros) sheep and Gökçeada goat well adapted to plant cover and land topography are breeding in the island. Goat inventory of Gökçeada Island has been declining since 1982 because of the damages exerted on agricultural fields and pastures.

Prickly burnet is probably getting more common just because of decreased goat inventory of the island. Today, almost all of the dwarf shrubbery rangeland and 36.2% of total island surface area are covered with prickly burnet (Cengiz et al., 2009).

There is a long standing pastoral ruminant production system in Gökçeada Island (Aktürk et al., 2005; Tölü and Savaş, 2011). The island surface area is 286 km^2 and there are 46.414 head sheep and 16.191 head goats in the island (Anonymous, 2013a). While about 70% of ovine is raised freely under almost wild conditions, the rest is raised in sheep barns under human control but still mostly depend on

rangeland. Fully-free ones captured once in each year for lamb and fleece by humans and specially trained dogs.

Previous grazing studies mainly focused on domestic animals and there are several factors affecting in behavioral characteristics of the animals into rangeland (Ouedraogo-Kone et al., 2006; Sanon et al., 2007). Besides, there is limited number of studies carried out with wild or feral animals (Haris and O'Connor, 1980; Arnold, 1982). However, there aren't any studies about the grazing behaviors of free-range intensively raised Gökçeada sheep and their daily behavioral rhythms. Thus, the present study was conducted aimed to investigate the daily behavioral rhythms of Gökçeada sheep into the rangeland in different seasons.

MATERIALS AND METHODS

Study area

The present research was conducted in Gökçeada Island covering 286 km^2 land area and located at the far-west of Turkey in Aegean Sea (40°14'10.82" N latitude, 25°54'30.45" E longitude). Total precipitation in observation year was 869.1 mm (Anonymous, 2013b). Pasture soils are shallow, slightly alkaline, unsaline with sandy-loam texture, low lime content, medium organic matter content, high N, Ca and Mg contents, and very low P content and sufficient levels of K (Gökkuş et al., 2013).

This study is a part of pasture reclamation project implemented for 3 years to remove prickly burnet from the pasture applying different methods and to reclaim the rangeland through seeding with herbaceous plants. In October 2010, cutting, grubbing and burning have been practiced over the rangeland to control prickly burnet shrubs and natural pasture was separated as control plot. The plots with shrub control measures and the natural plots were divided into two parts and one of these parts was seeded with forage crop seeds. For direct seeding, mixture of 20% perennial ryegrass (*L. perenne)*, common crested wheatgrass (*A. cristatum)*, orchard grass *(D. glomerata)*, 15% alfalfa (*M. sativa)*, sainfoin (*O. viciifolia)* and 10% burnet (*P. sanguisorba)* seeds were sown at a rate of 100.75 kg/ha. Two years after rangeland reclamation practices, in December 2012, sheep were placed into the plots and their behavior activities have been observed for a year. In this study, daily behavioral rhythms of the sheep in different seasons were considered free of rangeland reclamation methods and seeding treatments. Each grazing plot was surrounded with fences having a size of 0.15 ha/sheep.

Animals

A herd containing 150 sheep, freely ranged over the rangeland and accustomed to feeding was used to select the experimental sheep. Among them, 40 sheep were selected based on age, live weight and body condition, and then they were randomly distributed to experimental plots having 5 sheep in each plot. Selected sheep with the age of 3-4 years and with the average live weight of 31.18±1.70 kg (Figure 1).

Sheep have been grazed freely into 8 rangeland plots. Paddocks of 12 m^2 were placed in each plot and they were looked like a shelter with close sided windward. Paddocks protected animals to the sun, precipitation and harsh winds. Supplementary roughage has also provided in paddocks. Water was supplied *ad libitum* in 30-liter plastic containers (Figure 1).

The sheep care practices were implemented by herdsman in morning as well as evening hours. For adaptation of sheep to rangeland, maize corn, to which the sheep were accustomed, was supplied from the first day of pasture. Concentrate feed was directly spread over the pasture and roughage was supplied in feeders in paddocks. Feed supply was provided as group feeding within each plot. Based on gestation, birth and lactation of the sheep, they were supplied with 100-300 g/sheep/day concentrate feed and 500 g/sheep/day alfalfa hay. Supplementary feeding did not show any performance between mid-April and the beginning of June.

Births intensified at the end of February and the starting of March. At the beginning of September, 8 Gökçeada sheep breed rams with 25-27 kg live weight and with the age of 2.5 years were placed into each plot. Rams were stayed with the sheep until the removal of the sheep from the pasture. Rams were selected from the sheep herd raised freely over Gökçeada Island pastures.

Behavior observations

The first behavioral observations were performed 15 days after placing the sheep into the rangeland.Then, monthly behavioral observations were performed.

Figure 1. Gökçeada sheep into rangeland plots (By Tölü)

All of the observations have been carried out by 4 observers through direct observations from dawn till dusk. Observers did not always observe the behaviors in the same plots. In behavioral observations, grazing (picking, chewing, searching, walking; etc. for consumption of plants), rumination (rumination in standing or lying position), locomotion (being in action without grazing), resting (lying or standing) like behaviors have been observed for a 10 min of intervals through time-sampling method. The sheep in each group were painted in different colors (Figure 1).

Plant measurements

To determine plant nutrient contents of rangeland vegetation, five sections (1 m^2) were cutting from the bottom in each season. Mowed samples were weighted to determine fresh weights, initially air dried and then dried at 60^oC for 48 hours to determine dry weights (Cook and Stubbendieck, 1986).

Rangeland plant cover was dominantly composed of prickly burnet (70-95%), while prickle burnet ratio was found between 71.4-88.2% in plots where control measures were implemented, the ratio was between 95.7-94.9% in case of natural rangeland plots.

Dry matter and ash analyses were performed in accordance with AOAC (2000), crude proteins determined with Kjeldahl method through wet ashing in salicylic-sulphuric acid mixture (Bremner, 1960).

Structural carbohydrate analyses (NDF, ADF) were carried out in accordance with Van Soest et al. (1991) and tannin analyses in accordance with Makkar et al. (1995).

Statistical Analyses

GEE (Generalized Estimating Equation)-based intermittent model for repetitive binomial distributions has been used to assess the animal behavioral characteristics.

Seasons (winter, spring, summer, autumn) were placed into the model as fixed factor.

Odds ratio, regression coefficients and standard error values of regression coefficients were used in the evaluation of the effects.

Odds ratio was defined as the ratio of observation to non-observation of behavior. Odds ratio was calculated from the equation of $\Psi = e^b$, where ψ is the odds ratio, b is the regression coefficient and e is the exponential constant. The *post-hoc* analyses were made according to the Wald chi-square test.

Then, the plant nutrient contents were subjected to variance analysis for repetitive measurements with a model containing seasons.

Pair wise comparisons were performed through Tukey test. SAS (1999) was used in for statistical analyses.

RESULTS AND DISCUSSIONS

Crude protein content and mineral matter contents of pasture plots increased in spring (Table 1). Natural pasture plots with low protein and ash contents and high NDF and ADF ratios were significantly different from the other groups ($P \leq 0.05$). Grazing behaviors of Gökçeada sheep were significantly different in all seasons (Table 2; $P < 0.0001$).

The largest grazing frequency was observed in winter and spring and the least grazing frequency was observed in summer ($P \leq 0.05$).

The sheep grazed in winter and spring for 2 times more than autumn and 3 times more than summer. Similar to the present study, grazing behaviors of the sheep grazed over natural rangeland of Northern Greece in winter and spring seasons were 2 times more than summer

and autumn (Evangelou et al., 2014). However, it is known that sheep stopped grazing activity throughout the time periods with increased ambient temperatures (Ferreira et al., 2013).

Table 1. Seasonal mean, standard error (SE) and significance levels for nutrient contents of rangeland vegetation

Nutrients	Winter		Spring		Summer		Autumn		P
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	
DM	70.05^{b}	1.14	59.85^{c}	0.78	58.58^{c}	0.89	88.15^{a}	0.39	<0.0001
CP	4.90^{b}	0.09	6.75^{a}	0.22	4.77^{b}	0.15	3.41^{c}	0.09	<0.0001
NDF	50.97^{c}	0.50	49.96^{c}	0.68	53.69^{b}	0.69	59.28^{a}	0.99	<0.0001
ADF	35.50^{b}	0.46	36.66^{b}	0.72	41.29^{a}	0.50	43.17^{a}	0.81	<0.0001
Ash	5.77^{b}	0.18	7.07^{a}	0.18	4.15^{c}	0.24	4.41^{c}	0.13	<0.0001
Tannin	1.48^{ab}	0.05	1.60^{a}	0.03	1.61^{a}	0.06	1.35^{b}	0.04	0.0024

DM: Dry matter, %; CP: Crude protein, % DM; NDF: Neutral detergent fiber, % DM; ADF: Acid detergent fiber, % DM; Ash: Crude ash % DM; Tannin: Tannin, %. The differences indicated with different letter in the same line within each nutrient are significant (P≤0.05).

The sheep rather grazed in morning and evening hours and probably kept grazing at night hours in this period.

Rumination behaviors of Gökçeada sheep were also significantly different throughout the all seasons (Table 2; P<0.0001). The most frequent rumination behavior was observed in summer and the least rumination was observed in spring (P≤0.05). Rumination behavior was reverse of grazing behavior. Consumptive behaviors of sheep may reduce their rumination behavior (Kronberg et al., 1997). Similarly, low rumination behaviors were reported in rainy seasons and high rumination was reported in dry seasons for cattle, sheep and goats (Ouedraogo-Kone et al., 2006; Sanon et al., 2007). However in present study, seasonal changes were found probably because of transition of behavioral frequencies to night and day hours.

Table 2. Estimation (b), standard error (SE), odds ratio (ψ) and significance levels for behavioral characteristics of the seasons

Season	Winter			Spring			Summer			Autumn[1]	P
Behavior	b	SE	ψ	b	SE	ψ	b	SE	ψ	ψ	
Grazing	0.77	0.07	2.15^{a}	0.83	0.09	2.29^{a}	-1.13	0.05	0.32^{c}	1.00^{b}	<0.0001
Rumination	-0.29	0.07	0.74^{b}	-0.69	0.10	0.50^{c}	0.09	0.06	1.09^{a}	1.00^{a}	<0.0001
Resting	-1.02	0.12	0.36^{d}	-0.66	0.14	0.51^{c}	0.78	0.05	2.18^{a}	1.00^{b}	<0.0001
Locomotion	0.23	0.11	1.25^{a}	-0.20	0.18	0.81^{b}	0.08	0.14	1.08^{b}	1.00^{b}	0.0211

[1]In autumn b=0.00 and SE=0.00. The differences indicated with different letter in the same line for seasonal behaviors are significant (P≤0.05).

As it was in grazing and rumination behaviors, locomotion behaviors of Gökçeada sheep were also significantly different in all seasons (Table 2). The sheep were mostly observed in resting position during the day hours of in the months of summer and rested the least in winter months (P≤0.05). Locomotion behavior significantly differentiated in winter along with high frequencies (P≤0.05). Evangelou et al. (2014) reported that the standing behavior of the sheep in winter, spring and autumn recorded as 7.4%, 16.9% and 43.8%, respectively. The resting behaviors of the sheep in rainy, post-rainy and dry seasons were reported as 9.8%, 16.4% and 15.3%, respectively (Sanon et al., 2007).

Grazing behavior exhibited a fluctuating trend with day hours in winter and spring months (Figure 2). The grazing behavior with increased frequencies during the morning and evening hours in the months of summer and autumn decreased to the lowest levels between the hours 10:00-16:00. Such a trend was more distinctive in summer as compared to autumn. Similar findings on grazing rhythms through the day hours were also reported by Dudzinski and Arnold (1979) for different sheep races. It was also reported that the grazing and resting behaviors of the sheep have been found with changes in day light (Haris and O'Connor, 1980) and sheep usually grazed at night, early in the morning

and evening hours during the summer months in long as well as hot day hours (Ferreira et al., 2013).

It was observed in this study that sheep demonstrated rumination behavior in the months of summer and autumn during noon hours in which they didn't graze (Figure 2). In summer and autumn with low grazing and high resting frequencies, the changes in resting behavior throughout the day were reverse of grazing and parallel to rumination behavior as expected.

The sheep started to rest after 08:00 in summer and after 09:00-10:00 in autumn. Sheep demonstrated different resting behavior in spring and winter seasons from the other seasons. It was reported in a study including different herbivores that all animals spent mid-day with resting (Ferreira et al., 2013).

The locomotion behavior with relatively lower frequencies in this study demonstrated quite fluctuating trend throughout the day.

CONCLUSIONS

Behavioral rhythms of Gökçeada sheep were significantly found different in each season. Therefore, the seasonal changes in behavior frequencies and the changes in nutrient contents of rangeland vegetation throughout the year should critically be assessed. Gökçeada sheep minimized their grazing frequencies during the day hours in the months of summer. During this season, an increase was rather observed in grazing frequencies during morning and evening hours. They spent the rest of the day with resting and rumination. On the other hand, sheep spent majority of the day with grazing in winter and spring.

It was also observed in this study that Gökçeada sheep, raised freely throughout the year and they, were able to self-sustain over unfertile pastures with dominant prickly burnet cover with worthless nutrient contents.

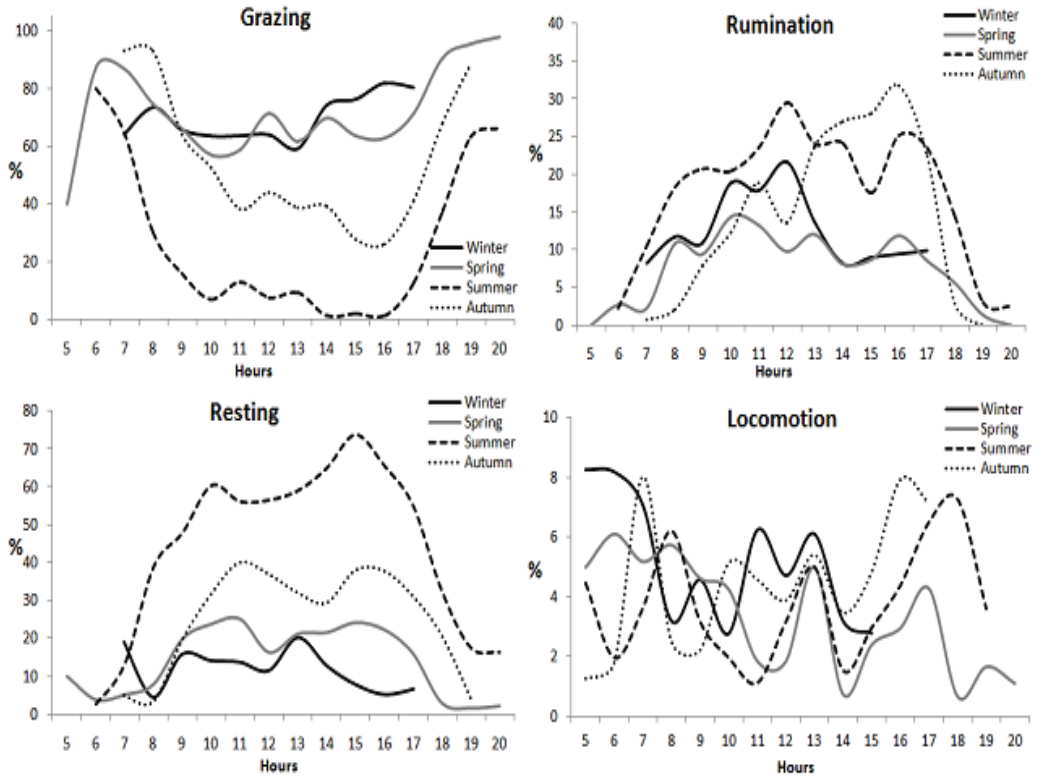

Figure 2. Day-hour behavior rhythms of Gökçeada sheep, %

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PRELIMINARY DATA ON EDUCATION LEVEL OF DAIRY FARMERS PERFORMANCES IN KOSOVO

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Abstract

The comparison of education level and its effect on the performance of the dairy farms in two regions of Kosovo was the objective of this exploratory study, as it is not done up to now from any institution, in the country. The data are collected from 150 respondents randomly selected from farmers' municipality database, in two regions of Kosovo (Gjilan and Ferizaj). The data were collected through face to face interviews and personal visits based on a structured questionnaire, during the period of May-July 2014. The results show significant differences between farmers (higher level of education compare with primary education) in regard with food safety standards, animal diseases, milk yield, animal identification, farm register, and feeding of the animals according to the production level. 64.4% of the farmers of secondary+ education group knew who is the institution in charge with food safety, 72.4% knew about the institution in charge of issuing the animal health certificate, 40.2% new the symptoms of Brucellosis, 43.7% knew the symptoms of TBC compare with 27%, 33.3%, 12.7% and 14.3%, respectively for the primary education group. This research may help agriculture advisory service decision makers improving teaching and training programs for dairy farmers. Better trained farmers can improve animals feeding, cows' milk yield, diseases control of animals, and reduce the public health risk of milk-borne zoonosis. In addition, public and donors support schemes for farmers should be strongly linked to food safety standard implementation.

Key words: *dairy farming, education, dairy farming knowledge, performance, Kosovo.*

INTRODUCTION

Kosovo continues to be predominantly a rural economy with 12 percent of GDP generated by agriculture, and it's the largest employer in Kosovo today, estimated to employ around the agriculture sector nearly 25 percent of the total employment, mainly in the informal sector, while according to the official results from the Population, Households and Housing Census in Kosovo (2011) shows the employment rate in agriculture by 4.4 percent (MAFRD, 2014; IPA, 2013; Qeveria, 2013). The majority of the Kosovo's population (61 percent) lives in rural areas (FAO, 2014).

Agriculture still remains subsistence-oriented due to a very small average size of farms, on average, the farm size is 3.2 ha agriculture land (including common land/pasture) and about 3.9 cattle (about 2 milking cows). Most of the farms are mixed ones and only 1-2 percent of agricultural land is used by commercial farms (more than 10 ha/farm) (MAFRD, 2014). According to Agriculture Census in Kosovo, there are 130.775 agricultural farms, which are breeding 261.689 cattle (51 percent of which milking cows). It is estimated that today there are about 91.200 livestock farms in Kosovo (ASK, 2015).

Due to the favorable natural resources dairy activities have a long tradition in Kosovo. Cow's milk production is concentrated in the private sector since the social sector collapsed during the transition in the 1990s and especially during the war in 1999 (Nushi and Selimi, 2009). In recent years efforts have been made to turn the production of milk from own consumption farms to market-oriented ones.

According to Food and Veterinary Agency of Kosovo (FVA) the number of cattle farms is 70.215 farms and about 26.100 of them are delivering milk to the dairy processors (ASK, 2013).

Farms with more than 5 milking cows which are consider commercial farms, during 2014 were 5.472 (7.8 percent of the dairy farms), These farms are the main suppliers to the dairy processing industry with a total of about 62 million liters of milk per year, or about 18 percent of the milk produced in the country (AAD, 2015; MAFRD, 2015; and author calculations). The rest is used for feeding calves, for own consumption, sold as raw milk or white cheese on the various local unregulated (green) markets. Commercial and semi-commercial farms sell milk to one of the 19 dairy processors (Nushi, 2010). Traditional dairy products are yogurt, butter, curd and different kinds of cheese.

Low productivity characterized the dairy farming as the result of poor farming technologies and lack of capital by smallholder farmers. In addition, research and training programs needs to be planed, which would be beneficial to the farming environment. Due to the increase in purchasing power, demands for local dairy products are expected to mark an increase (MAFRD, 2014).

The average age of the holders of agricultural farms is 52 years. As far as education/training in agriculture, more than 95% of managers have only practical experience in agriculture (ASK, 2015).

Farmers' Education. The question addressed in this paper is: What impact does farmers' education have on farm business practice?

According to several authors (Hicks, 1987; Hanushek and Wößmann, 2010) the education and training are: (i) widely acknowledged as contributors to national economic wellbeing and growth; (ii) explaining differences in productivity and income between countries is the level of education and human capital, which includes both formal education and informal on-the-job training; (iii) can facilitate the diffusion and transmission of knowledge and information for implementing the new technologies.

Lockheed et al., (1980) have synthesized the conclusions of a number of studies of the positive effect of a farmer's educational level and exposure to extension services on the farm productivity.

In our research, is investigated the relation between the educational levels of dairy farmers with practices implemented on the farm, such as: farm size, farmer age, farmers experience in livestock milk production, number of animals raised, farmers' awareness about food safety standards and animal diseases.

MATERIALS AND METHODS

The present study was conducted in the regions of Gjilan and Ferizaj, Kosovo. The data was collected during the period of May-July 2014.

A structured questionnaire was used for collection of all information related to dairy farming. To avoid confounding questions and for clarity, the questionnaire was pre-tested on a pilot group of 10 farmers. In the case of inconsistent questions, it was modified accordingly. Face-to-face interviews were conducted. The questionnaire contained both open-end and closed questions. Observation is the most direct way of collecting data. According to Gillham (2003) a risk of choosing observation is that people observed may play some kind of a role when observed. For this reason both observation and interviews were used and also the animals and their environment were observed carefully. Farms with more than 4 dairy cows were interviewed.

The obtained data was stored in Excel-2000 and imported to software SPSS 22.0 for analysis. Stored data were tabulated and arranged as percent value. Descriptive statistics (i.e. means, frequencies etc) was done to estimate the different variables.

RESULTS AND DISCUSSIONS

This section analysis the current situation and the sector's developments with focus the relationship between level of formal education and farmers practices, in two regions of Kosovo. A six point Likert scale was used for farmers' education: 1-no education, 2- primary education (up to 4 years of school), 3- obligatory education (up to 9 years of school), 4- agricultural middle school (12 years of school), 5- other middle school(12 years of school), and 6-university degree.

The results of group 1-3 (primary education) are compared with those of better educated farmers group 4-6 (secondary + education).

Sample socio-demographic and farm indicators

Table 1. Main sample socio-demographic and farm indicators

Education level	Sample farm household indicators			
	Age		Working experience	
	Mean	Std. Dev.	Mean	Std. Dev.
Primary education	48.98	12.432	23.29	15.731
Secondary+ education	44.02	8.357	17.5	10.823

Education level	Sample farm household indicators			
	Farm size		No. of cows	
	Mean	Std. Dev.	Mean	Std. Dev.
Primary education	6.29	6.433	9.0	4.708
Secondary+ education	9.02	9.699	12.07	7.835

Since the typical sample farm had more than 4 cows and the average farm size was 9.0 to 12.07 cows, it may be concluded that they were market oriented (Table 1). We targeted market oriented farms, as usually they are more aware for standards; invest more in the future toward improving standards; and compare with small subsistence farms (1-2 cows) are more likely to "survive" the growing competition in the future.

Effect of education on milk yield and milk sold to the processors. The difference in milk production performance between primary education group of farmers and secondary+ group (Table 2) is an established fact: hence the effect of education level on milk yield was assessed in both groups of farmers. Milk yield, increased with the level of farmer's education.

Table 2. Effect of education on milk yield

Education level	**Milk/cow/day (kg)**	
	Mean	Std. Dev.
Primary education	9.97	4.490
Secondary+ education	14.06	4.921

Table 3. Answer the question "What part of your milk production is sold to the processors"?

Percentage of the production	Selling milk to the processors			
	Primary education		Secondary+ Education	
	Freq.	Percent	Freq.	Percent
0	46	73.0	40	46.0
1-50	2	3.2	8	9.2
51-100	15	23.8	39	44.8
Total	63	100	87	100

From table 3, we figure out that 73 percent of the primary education farmers are not selling milk to the processors. This group mainly is selling the milk directly to the consumer houses, in the green market (open market), and only 27 percent are selling to the processors. While for the secondary+ group of farmers the figures are 46 and 54 percent, respectively.

Food Safety Institution. According to the Food Law[1] the official control of food shall be carried out from the inspectorate under the Food and Veterinary Agency. The farmers were asked to choose the institution in charge of food safety in Kosovo providing them several options from which to choose. 64.4 percent of the secondary+ education group stated they know that FVA is in charge with food safety compare with 27 percent of the primary education group. 69.8 percent of farmers of the primary education group stated that they do not know–indicating the law awareness level among farmers about food safety (Table 4).

Table 4. Answer to the questions: "Which is the institution in charge of food safety? and Which is the institution that issues animal health certificate?

Education level	Institution in charge of food safety			
	Primary education		Secondary+ education	
	Freq.	Percent	Freq.	Percent
FVA	17	27.0	56	64.4
MAFRD	1	1.6	4	4.6
Municipality	0	0.0	2	2.3
I don't know	44	69.8	23	26.4
No answer	1	1.6	2	2.3
Total	63	100	87	100

Animal Health Certificate. The Law on "Veterinary"[2] (article 6, 14, 16, and 19), emphasis that veterinary service is responsible for issuing veterinary certificate for: (i) the movement of the animal or the products, (ii) trade of animals, (iii) slaughtering of animals. Farmers were asked to choose the institution in charge of issuing animal health certificate providing several options from which to

[1] Republic of Kosovo (RoK): Law on Food No. 03/L-016. Assembly of Kosovo, 12 February 2009. Official Gazette of the Republic of Kosovo No. 49, 25 March 2009

[2] Republic of Kosovo (RoK): **Law on Veterinary No 2004/2**. Assembly of Kosovo, 16 June 2004, Official Gazette of the Provisional Institutions of Self-Government in Kosovo No. 18, 01 November 2007

choose. 72.4 percent of the farmers from the secondary+ education group provided the right answer while almost 62 percent of the farmers from primary education group stated that they do not know (Table 5), indicating the lack of information about this important aspect.

Table 5. Answer to the questions: "Which is the institution that issues animal health certificate?

Education level	Institution that issues animal health certificate			
	Primary education		Secondary+ Education	
	Freq.	Percent	Freq.	Percent
FVA	21	33.3	63	72.4
MAFRD	1	1.6	2	2.3
Municipality	1	1.6	1	1.1
I don't know	39	61.9	20	23.0
No answer	1	1.6	1	1.1
Total	63	100	87	100

Farm Register. According to the article 27 of law on "Veterinary" the farmer must keep the animal register. Keeping a farm register is very important, not only for farmers but also for animal and public health experts**,** because through records could be identified the movement of animals from one farm to another, from one farm to the market or to a processing facility (slaughterhouses and/or meat processors). The record keeping of animals' movement helps to find the sick ones, and also to find the farm and the area where other animal may have come into contact with and potentially exposed to a disease. Using the farm records, the experts can determine if those animals need to be tested, treated, or even quarantined to prevent further spread of disease. These are very important step in securing the safety of our food supply.

Only 14.3 percent of the farmers of primary education group and 35.6 percent of the farmers of the secondary +education group stated that they have farm register book (Table 6).

Table 6. Answer to the questions: "Do you have a farm book/register on livestock"?

Education level	Farm register			
	Primary education		Secondary+education	
	Freq	Percent	Freq	Percent
Yes	9	14.3	31	35.6
No	54	85.7	56	64.4
Total	63	100	87	100

Animal diseases. Most of the farmers of primary education group stated that they don't know the symptoms of Brucellosis and TBC, 87.3 percent and 85.7 percent, respectively (Table 7). While the answer from the secondary + education group is much better: 40.2 percent of them knew the symptoms of Brucellosis and 43.7 percent knew the symptoms of TBC.

Feed ration. Most of the farmers of the both groups are not consulting the feed expert and they continue to feed the animals without making a distinction of the ration according to the milk production. This is one of the reasons why the milk yield in Kosovo is below potential breed averages.

Table 7. **"**Do you know the symptoms of the following diseases?

Education level	Brucellosis			
	Primary education		Secondary+education	
	Freq.	Percent	Freq.	Percent
Yes	8	12.7	35	40.2
No	55	87.3	52	59.8
Total	63	100	87	100
Education level	TBC			
	Primary education		Secondary+Education	
	Freq.	Percent	Freq.	Percent
Yes	9	14.3	38	43.7
No	54	85.7	49	56.3
Total	63	100	87	100

However there is a considerable difference between the two groups, where 1/3 of the secondary+ education farmers are feeding the cows according to the milk production (Table 8).

Table 8. "Do you use the feed ration of cows according to the milk production?

Education level	Feed ration			
	Primary education		Secondary+education	
	Freq.	Percent	Freq.	Percent
Yes	8	12.7	28	32.2
No	55	87.3	59	67.8
Total	63	100	87	100

CONCLUSIONS

The aim of this exploratory study was to assess the impact of farmers' education on dairy farming knowledge, national food safety standards, animal diseases, and farm production. According to our findings, the milk yield of the cows managed by the farmers of the secondary+ education group is 41 percent higher than those of the primary education group. 54% of the farmers of the secondary+ education group sell milk to the processors compare with 27% from the primary education

group. In addition 64.4% of the farmers of secondary+ education group knew who is the institution in charge with food safety, 72.4% knew about the institution in charge of issuing the animal health certificate, 40.2% new the symptoms of Brucellosis, 43.7% knew the symptoms of TBC compare with 27%, 33.3%, 12.7% and 14.3%, respectively for the primary education group.

In addition significant differences between two groups of farmers (in favor of secondary +education group), are also for the animal identification, farm register, feeding the animals according to the production level, etc.

These findings are indicating the low awareness level among farmers about farm management, animal diseases and food safety.

Public and donors support schemes for farmers should be strongly linked to farm management and food safety and animal health standard implementation.

Well established extensionist/ veterinarians - farmer relationship will lead to economic gains for farmers.

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RHEOLOGICAL PROPERTIES DESCRIPTION OF MYOFIBRILLAR PROTEIN HOMOGENATES AND CONCENTRATES OBTAINED BY DIFFERENT METHODS AND FROM DIFFERENT SPECIES

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Abstract

In our study we aimed the rheological behavior of fish myofibrillar proteins and of the homogenates of which these were obtained. For protein extraction and concentrates purification different methods were used. We tracked the variation of elastic modulus and phase angle according to temperature.
Myofibrillar proteins determine the water retention and meat hydration capacity, fat emulsifying and gelling capacity.
The source and the method of extraction influence the gelling properties of muscle protein. The protein concentration plays a key role in determining the heat induction properties of gels.
Gelling properties of the muscle proteins are also influenced by heating temperature, temperature rise speed and of various adjuvants.
Solubility and gelling properties are also important for obtaining edible films based on these proteins and also to obtain microcapsules.

Key words*: elastic modulus, fish protein concentrate, gelling properties, myofibrillar proteins, phase angle.*

INTRODUCTION

The manufacture of meat processed products involves the use of different types of meat, organs, edible subproducts and fat (as raw materials), derived mainly from cattle, sheep and swine, along with a large number of non-meat ingredients, with a very important role in the formulation of various products (Banu, 2009). These ingredients stabilize mixtures and add specific features and flavors.
The chemistry and functional behavior of meat, as a raw material for processing, are derived from the characteristics of muscle tissue. The muscle is a biological tissue, highly organized, with a complex intrinsic structure, a unique and very active composition from biochemical point of view.
The composition of the meat is very important for the final product. From a nutritional standpoint, muscle tissues are rich in protein, containing all essential amino acids and is also a good source of zinc, selenium, phosphorus, iron, vitamins B6, B12, niacin, choline (Lawrie, 2006), some meats being rich in vitamin K (Schürger et al., 2000).
The proteins functional properties are the physico-chemical properties that affect their behavior during processing, storage or consumption of food systems and contribute to the quality and sensory attributes of the food (Kinsella, 1976).
Gelling properties of the muscle proteins are influenced by:

- the source and type of muscle;
- the protein concentration plays a key role in determining the properties of thermal induction for gels;
- gelling properties of the proteins are dependent on pH;
- gelling properties of the muscle proteins are influenced by heating temperature, by the rate of temperature increase and by various adjuvants.

Gelling properties of the myofibrillar proteins have been studied in different species of animals.

Currently, the meat can be used in its natural state, or muscle proteins can be separated and used as functional ingredients, such as structural protein concentrates (surimi, surimi-like) protein isolates and hydrolysates (Lee, 1986; Ionescu et al., 2006).

MATERIALS AND METHODS

Raw materials

Hypophthalmichthys nobilis and Abramisbrama specimens were procured fresh from the local fish store. The fish was transported to the laboratory in a cool bag and then stored at 4°C until processing. After weighing the fish was descaled, gutted, beheaded and filleted. Fillets were boned and skinned by hand. Red muscles were detached manually and separate from the white muscles. White muscle, resulting after weighing, was minced using an electric mincing machine, fitted with a sieve with mesh size of 3 mm.

Minced meats were divided into equal parts, in order to obtain muscle myofibrillar protein concentrates by various methods: repeated washing of the meat with cooled water (3 washes), followed by centrifugation to remove the washing water; repeated extraction of minced meat with cooled solution of KCl 0.15M and 1 mM EDTA; acid solubilization of proteins and their precipitation from the solution at the pH of the isoelectric point of the muscle protein; alkaline solubilization of proteins and their precipitation from the solution at the isoelectric pH point of muscle protein.

Determining the approximate chemical composition

The contents of water, protein, fat and ash were determined using standard method of analysis (AOAC, 1990; Ionescu et al., 1992). Also, moisture was determined by fast drying to constant weight using the thermobalance "Precisa XM 60". Total nitrogen was determined by Kjeldahlsemi micro method, mineralization being performed in the "Trade Raypa" facility. Total proteins were calculated by multiplying the total nitrogen content by a factor of 6.25. All chemical analyzes were carried out in duplicate.

The pH was measured potentiometrically using the pH meter type "Hanna" using protein dispersions with a concentration of 10% (G/V), at a temperature of 22 ± 1^{0}C.

Protein solubility

The solubility of proteins in wet protein concentrates was studied in the pH range from 3.0 to 11.0.

Gelling properties

The gelation properties were determined by dynamic rheological measurements at oscillations of small amplitude, performed by a voltage-controlled rheometer (AR 2000, TA Instruments, New Castle, DE), attached to a control software computer (Rheology Advantage Data Analysis Program, TA , New Castle, DE). The temperature was monitored using a Peltier temperature control system. All rheological measurements were made using a cone plate geometry of 40 mm with an angle of 2° and a gap of 2000 μM. Samples were run in duplicate.

RESULTS AND DISCUSSIONS

Determining the approximate chemical composition

Table 1 describes the type of concentrates/isolates of myofibrillar proteins, separation methods used and their concentrations in the dry matter and protein.

Table 1. Chemical composition of fish meat (Abramis brama, Hypophthalmichthys Nobilis) and a protein concentrate obtained

Indicators	ABRAMIS BRAMA				HYPOPHTHALMICHTHYS NOBILIS			
	MHAB	CPMAH 1	CPMAH 2	CPMAH 3	MHAB	CPMAB 1	CPMAB 2	CPMAB 3
Water, %	76.52	82.05	84.38	85.82	80.86	83.82	85.23	83.55
Proteins, %	17.21	16.42	13.84	12.75	17.83	14.66	13.36	13.75
Fat , %	4.28	0.52	0.59	0.56	2.38	0.12	0.17	0.46
Ash, %	1.26	0.06	0.05	0.09	1.06	0.16	0.05	0.12
Other, %	0.71	0.93	1.12	0.75	0.53	1.24	1.19	0.75

MHAB - Muscle homogenate of Abramisbrama
CPMAB 1 -Protein concentrate of Abramisbrama - Alkaline extraction
CPMAB 2 - Protein concentrate of Abramisbrama – Acid extraction
CPMAB 3 - Protein concentrate of Abramisbrama - KCl and EDTA extraction
MHAH - Muscle homogenate of Hypophthalmichthys nobilis
CPMAH 1 -Protein concentrate of Hypophthalmichthys nobilis - Alkaline extraction
CPMAH 2 - Protein concentrate of Hypophthalmichthys nobilis- Acid extraction
CPMAH 3 - Protein concentrate of Hypophthalmichthys nobilis - KCl and EDTA extraction.

Myofibrillar proteins, derived from different sources, were functionally characterized by determining the proteins solubility and gelling properties.

The quality and stability of the final protein product are affected by the functional properties of the proteins (Xiong, 2000). The most important functional properties, when muscle protein concentrates or isolates are obtained, for use in food products are the following: solubility, viscosity, water retaining capacity, emulsifying and gelling capacity (Hultin et al., 1999).

Solubility characteristics of myofibrillar proteins are interesting due to their relationship with other functional properties, especially the gelling and water retention properties (Hultin et al., 1995;Dagher et al., 2000).

Protein solubility

Myofibrillar proteins are generally soluble in solutions with ionic strength >0.3. Modification of muscle protein solubility can be obtained in different ways, by changing the ionic strength, types of ions, pH and/or temperature, this way being affected the hydrophobic and/or ionic nature of proteins. For a long time, there was a general conviction that in order to form good protein gels, the solubilization of mofibrilare proteins in high saline concentration (0.3 - 0.6 M) is required, characteristic for meat products with added salt and polyphosphates. However, Stefansson and Hultin (1994) showed that code myofibrillar proteins are soluble in solutions with less than 0.3M ionic strength, at both neutral pH and acidic pH, because the repulsion forces due to negative charges of the side chains of the proteins are sufficient to maintain the protein molecules separated, when there is enough water available (Stefansson et al., 1994).

The solubility of proteins depends on the species of animal, the type of muscle, postmortem changes, the exposure to pH values lower than 6.6 (Hultin et al., 2002; Ionescu et al., 2002, 2003, 2006), the treatment applied (freeze-thaw).

Protein solubility curves are shown in Figures 1 and 2. The solubility profiles were similar for all protein pastes which were analyzed.

The fish concentrates showed minimum solubility in isoelectric domain, with pH range between 5.5 - 7.0, characteristic for most muscle proteins (Xiong, 1997), the lowest protein solubility values were observed at pH 5.5. For protein concentrates/isolates obtained by alkaline and acid solubilizing, higher solubility values were observed at pH 5.5, than for protein concentrates obtained from washing with water or various solutions of minced meat.

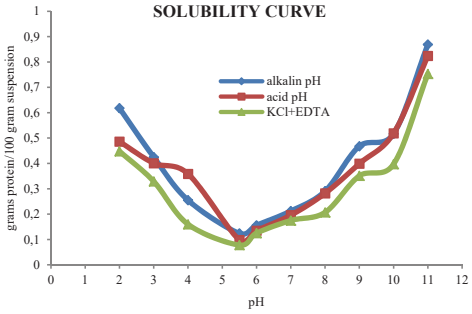

Figure 1. Protein solubility curves - Abramis brama

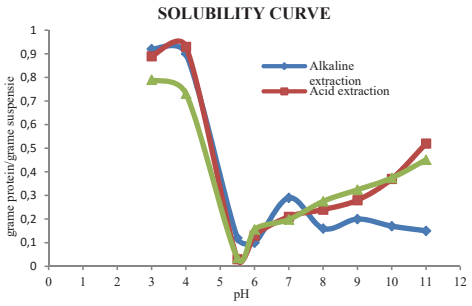

Figure 2. Protein solubility curves – Hypophthalmichthys nobilis

This can be explained by the presence in the constitution of this protein concentrates of sarcoplasmatic proteins soluble in water and low ionic strength solution and which represents 20-30% of the muscle proteins (Haard et al., 1994; Ionescu et al., 2009). The proteins soluble in isoelectric point domain were mostly sarcoplasmatic proteins and possibly dissociated actin. As can be seen from Figures 1 and 2, over 20% of the proteins were soluble in the physiological pH range (6.5-7.0), pH found in some of the meat products salted with added polyphosphates.

The decrease of solubility at very low pH values (1.5) could be due to aggregation induced by anions, as more hydrochloric acid into the environment will increase the ionic strength of the solution and can reduce the electrostatic repulsion between proteins (Goto et al., 1994; McClements, 1999; Damodaran, 1989).

Gelling properties

Myofibrillar proteins are responsible for the textural properties of the processed meat products (Yasui et al., 1980; Asghar et al., 1984). In general, the proteins extracted in saline solutions with high ionic strength (0.3-0.6M), also known as salt-soluble proteins (SSP) represent 55 to 60% of the total muscle protein or 10% of the skeletal muscle weight (Asghar et al., 1985). Among the myofibrillar proteins, myosin and actomyosin contribute most to the development of gel characteristics of the processed products obtained from salted meat (Ionescu et al., 2008, 2010).

In our study, we followed the rheological behavior of protein suspensions by scanning a wide temperature range (4.3-74.8^{0}C or 31-80^{0}C) and monitoring parameters: elastic modulus and phase angle (delta). Rheological measurements were determined by dynamic rheological method at small deformation, non-destructive, conducted in the linear region of viscoelasticity, which enables the determination of the elasticity and viscous nature of the tested sample.

Elastic shearing modulus (storage or storage facilities, G') is a measure of the released energy per cycle of deformation per unit volume and the property which makes the correlation with the elastic nature of the material. Phase or deformation angle (δ) is a measure of the prevalence of viscous properties (characteristic to the liquids) and elastic properties (characteristic to the solids) in the viscoelastic behavior of a material. The phase angle is related to the formation of bonds in the gel during the heating/deformation, mainly in temperature increase/oscillation frequency decrease.

As can be seen from Figures 3 and 4, values of the elastic modulus and phase angle (δ), in case of the homogenate and protein derivates from Abramis brama and Hypophthalmichthys nobilis muscle, have evolved differently depending on the temperature domain and on the nature of the sample.

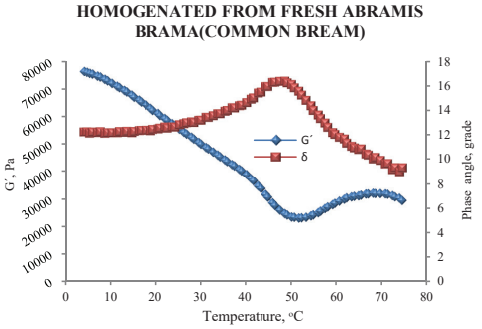

Figure 3. Shows the rheological behavior of Abramis brama (Common Bream)

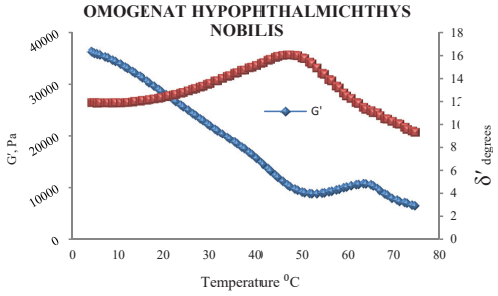

Figure 4. Shows the rheological behavior of Hypophthalmichthys nobilis

As can be seen, the values of the elastic modulus and phase angle (δ) of the homogenate and the Abramis brama (Common Bream) muscle protein derivatives have evolved differently depending on the temperature domain and the nature of the sample.

In the case of homogenated Abramis brama (Common Bream) muscle (pH 6.3), elastic modulus had a moderate downward trend in the

temperature domain between 4.3-35.9°C, characterized by high values of G', 76140 Pa at 4.3°C and 43700 Pa at 35.8°C. For the homogenated of Hypophthalmichthys nobilis muscle (pH 6.3), elastic modulus had a moderate downward trend in the temperature domain between 4.3-35.9°C, characterized by high values of G', 36240 Pa at 4.3°C and 17620 la 36.8°C. This interval is followed by another temperature domain (35.9-51.7°C) characterized by a more significant reduction of this parameter to a minimum of 23150 Pa (51.7°C) for homogenated Abramis brama (Common Bream) muscle and 8972 Pa(51.8ºC) for Hypophthalmichthys nobilis muscle. In these temperature ranges, the reduction of storage module can be attributed to the complex structure of fish muscle proteins due to denaturation of certain protein fractions.

The thermo-rheogram, shows below, a portion close to a plateau in the 50.7-59.7°C domain, possible characteristic to the denaturation and simultaneous aggregation of some protein fractions, given the complex nature of the system investigated.

Our findings are in agreement with those reported by Westphalen etc. (2005, 2006), who found the existence of the plateau in the range of 50-57°C, for myofibrillar protein samples with a 6.0 pH and lower concentration.

Starting with the inflection point of the curve (51.7°C), elastic modulus values increased very slowly at first, then the increase was accelerated when the temperature was raised above 59.7°C to the finalization of the heating process at 74.6°C.

The thermo-rheogram of the phase angle indicates a reverse trend relative to the elastic modulus. Low values of the phase angle, between 8.998-16.34 grade, across all the temperature domain of 4.3-74.6°C is specific to the visco-elastic bodies at which elastic component was permanently predominant relatively to the viscose component. The base zone of the elastic modulus in the thermo-rheogram corresponds to the highest value of phase angle > 12.0°.

Figures 5 and 6 are presented thermo-rheogram elastic modulus and phase angle for wet protein concentrates extracted from moss Abramis brama and Hypophthalmichthys nobilis, by the process of alkaline acid and wash with KCl.

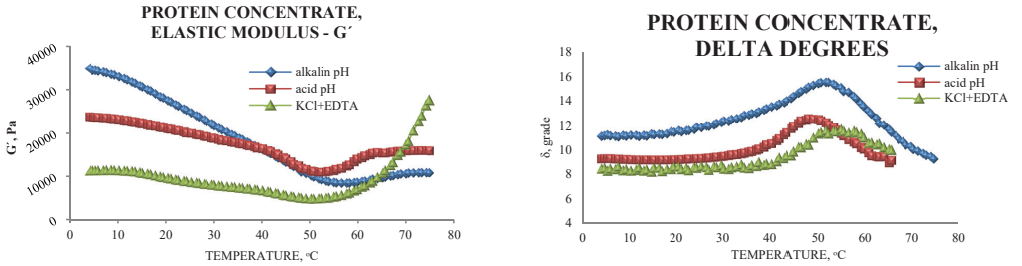

Figure 5. The elastic modulus change depending on temperature and changing of the phase angle depending temperature for Abramis brama

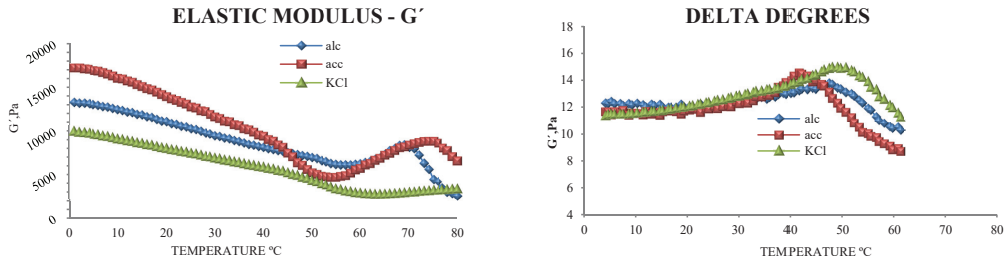

Figure 6. The elastic modulus change depending on temperature and changing of the phase angle depending temperature for Hypophthalmichthys nobilis

Protein concentrates thermograms profile was similar to that of muscle homogenate except that the elastic modulus values were different, being much higher in the muscle homogenate. If we compare the three types of protein concentrates (acid, alkalin and wash with KCl) it can be seen that the values of G' were higher for alkaline protein concentrate relative to the acid and not to KCl. For the two types of protein concentrate transition temperature of the ground to the gel was the same (50.8^{o}C), slightly lower than that recorded in the muscle homogenate (51.9ºC).

The modifications of the rheological properties on heating of the Abramisbrama (Common Bream) protein concentrates compared to the Abramisbrama (Common Bream) muscle homogenate we ascribe on the greater complexity of the homogenate, differences in protein content and characteristic pH values and potential denaturing changes in the protein system during extraction treatments (Yongsawatdigul and Park, 2004). Protein concentration and pH are very important parameters in thermal gelation of meat protein (Lesiów et al., 2003). In addition, it is well known fact that during the extraction of muscle proteins by the acid procedure, due to the high concentration of hydrochloric acid suffers modifications which influence the functional and rheological properties.

Reduced capacity to form gels of acid treated protein, when compared to those treated under alkaline conditions may be attributed to conformational changes (partial loss of myosin heavy chain) or due to the unfavorable conformation of the protein during the acid treatment (several hydrophobic groups leading to larger aggregates and to a less ordered gel). Another explanation could be that related to the presence of denatured sarcoplasmatic protein that are retained in the acid process, but not in the alkaline one (Ingadottir, 2004).

CONCLUSIONS

The functional properties of protein derivates were dependent on the protein source and separation techniques

The solubility profile of the muscle protein concentrates/isolates varied depending on the nature of the product and the pH of the protein solution. All protein derivates showed a minimum protein solubility at pH 5.5 and two areas of maximum solubility in strongly alkaline pH (11) and strongly acid pH (3).

The protein concentrates/isolates obtained by alkaline or acid solubilization and precipitation at pI are characterized by better solubility in the isoelectric range 5.5-6.5, pH values commonly encountered in various sausages formulations or in restructured products, where they may be used as functional ingredients.

Studied protein concentrates behaved, from rheological point of view, as viscoelastic systems with high elastic component, but variable depending on the temperature, protein source and extraction method.

For the protein concentrate, lower values of the elastic modulus were found at the beginning of the heating treatment, than for the homogenate at the same temperature, and higher values at end of the heating process for the concentrate compared to the homogenate.

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ECONOMICAL PROSPECTS ON MEDIUM TERM IN THE CONTEXT OF MILK QUOTA ABOLITION

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Abstract

The dairy market has a major contribution to the agricultural turnover in the EU as a whole as well as in most of the Member States (MS) of the EU. The milk quota system was introduced in 1984, in order to limit public spending on the sector, to control milk production, and to stabilize milk prices and the agricultural income of milk producers. The abolition of milk quotas was aiming to improve the competitiveness of European dairies, making production more sensible to market variations. The removal of milk quotas at the same time intensifies the economic outlook of certain areas/categories of dairy production with comparative disadvantages. Medium and long term prospects are favorable for the dairy sector in line with population growth and appetite for Western-style diet in emerging economies. This does not prevent, however, short-term market fluctuations. Finally, the current difficulties faced by the milk producers milk in certain areas worst affected by feed price increases cannot hide the overall positive image of the sector.

Key words: *dairy, milk quota, price, milk producers, EU.*

INTRODUCTION

The Common Agricultural Policy (CAP) is confronted with a set of challenges, some unique in nature, some unforeseen, that invite the EU to make a strategic choice for the long-term future of its agriculture and rural areas. To be effective in addressing these challenges, the CAP needs to operate within the context of sound economic policies and sustainable public finances contributing to the achievement of the EU objectives.

Common Agricultural Policy (CAP) has changed several times throughout its history, as a reaction to the enlargement process and under the pressure of globalization.

EU dairy market is governed by the Common Market Organization (CMO, where the milk quota system was one of the most notable elements. The milk quota system was originally introduced in 1984 to limit public expenditure in the sector, to stabilize milk price and also milk producer's income as well as to limit public sector spendings (Hemme and Otte, 2010)

The Common Market Organization (CMO) for the milk sector suffered successive reforms in the last decade. Based on Agenda 2000 Reform guidelines, the 2003 Reform started with a relaxation of the milk quotas system. However, it was abolished in 2015. This date was confirmed under the 2008 CAP Reform known as the Health Check of the CAP. The reform established a progressive increase of quotas for each Member State separately. It also led to the suspension of seasonal storage aid for certain cheeses and for butter used in pastries and ice cream.

A key element of this document was the proposal to abolish milk quotas starting with 2015, as this instrument has been known as obsolete in the current market developments and the new CAP philosophy. The reform proposed the abolition of this instrument to take place when the sector would show signs for such a radical measure. Therefore, a gradual increasing of quotas by 1% each year, until 2015, was proposed.

The main concerns were related to the possibility of milk production concentration in areas where this activity involved lower costs and the disappearance of production from certain areas (e.g. mountain areas, vulnerable areas).

For a sector that had been using production limitations for more than 25 years, the end of quotas created new challenges for economic operators and also for the sheep and goat sector. In order to prepare them to the new context, the Commission proposed measures to strengthen the organization of milk producers and their bargaining power, as well as measures to improve cooperation and relations between the various links of the supply chain (Ernst & Young, 2013).

MATERIALS AND METHODS

The study intends to analyze the evolution of the milk sector on medium term (2016-2025) after the milk quota abolition with special reference to milk production, number of producers, livestock, products evolution and trade balance.
To achieve this goals we analyzed the data of official statistics provided by different institutions (Eurostat, FAOSTAT, Ministry of Agriculture and Rural Development from Romania), after that the forcast were studied in order to interpret the availble data and finally to issue several conclusions arising from this study.

RESULTS AND DISCUSSIONS

The milk sector represents 15% of total EU agricultural production, with a production value of nearly 55 billion euros. EU milk production represents approximately 20% of world production and in 2014 about 159 million tonnes were produced at EU level. In the first eleven months of 2015, EU cow's milk production increased by 1.5% (aprox. 1.7 million tons), compared to 2014.
Germany and France are the main producers covering around 40% of EU production, followed by UK, Poland, Netherlands and Italy.
The main milk product is represented by cheese products and it uses approximately 50% of EU milk production.Only 8% of total EU cheese production is exported (Agriculture, forestry and fishery statistics, 2014).
Approximately 11% (in milk equivalent) of EU milk production is exported while the rest is consumed domestically.
Main dairy exported are milk powder, cheese, butter and fresh dairy products. Netherlands, France, Germany, Belgium, Poland and Denmark export individually more than one million tonnes of milk equivalent, which cumulated represents 70% of EU exports.
As mentioned in the document "Evaluation of CAP - measures applied in the dairy sector", the EU has been over the year's one of the major players on the global market, with considerable influence on the price of dairy products. EU share of global exports should increase slightly due to the considerable potential of the sector (unlike New Zealand, EU's main competitor which is more constrained by the availability of natural resources).
The potential for an increased production is mainly a consequence of the milk quota abolition, livestock, production capacity, favorable climatic conditions and processing capacity.
A production increase on medium term is expected due to lifestock increase. These changes have occured after decades of continuous decline. Although, lower than in the last decade, an annual 1% increase is expected for the milk production, reaching in 2025 a production 172 million tonnes. This will take place in the context of a 2% annual increases of global imports and an increase in demand for dairy products on the EU internal market (Figure 1).

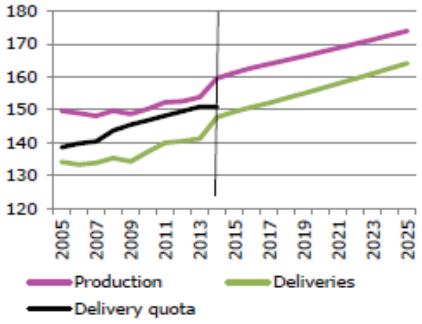


Figure 1. Milk supply and deliveries in the EU (million tones)

This represents an increase of about 15 million tonnes over 10 years and from this

quantity less than 3 million tonnes will be produced by the EU-13 (New Member States). Significant increase in deliveries is expected in Ireland, Poland, Denmark, Estonia and Latvia. In Germany, France, Britain and the Netherlands, deliveries should follow the EU average. The aforementioned Members States will generate 74% of EU production in 2025, compared with 72% in 2015. Therefore, the concentration of milk production will be quite limited.

Meanwhile, drop of supplies are expected in countries such as Finland, Sweden, Greece and Romania. In these countries, milk production increased in 2014, based on favourable market context, but it's expected to return to a downward trend if the milk price is going to decrease.

Furthermore, investments in processing capacities and farms could help to reverse, from negative to positive, the trend of production in Member States such as: U.K., Czech Republic, Slovakia and Hungary.

In the new Member States (EU-N13), it is likely that a higher proportion of milk will be delivered factories (80% in 2025 compared to 73% in 2015). In addition, a substantial growth of productivity is expected: milk yield will increase by 2.5% per year and will reach an average production of 6460 kg/cow in 2025. Consequently, a fall by 1.9% per year, in the number of dairy cows is expected, slightly lower than in the last 10 years.

In the old Member States (EU-15), the yield is expected to grow slightly faster compared to the past decade, approx. 8400 kg/cow in 2025, which represents an increase of 1.4% per year.

Among the factors that will play a role in the improvement of efficiency, we shall include: genetics, wider use of robots, better pasture management and a higher proportion of concentrates in diets. Taking into account that in the post-quota period herds cannot be taken over if farmers leave the sector, the number of dairy cows could decrease by 0.5% per year (European Commission, 2015).

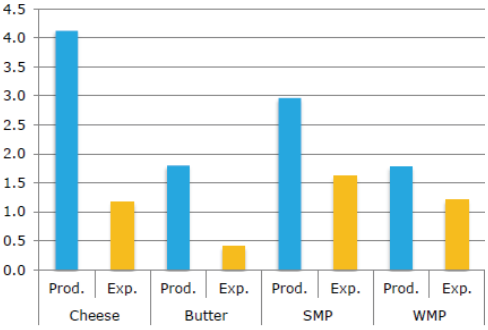
EU dairy market is currently characterized by an imbalance both in terms of raw material, and milk products.

Since 2007, EU prices for raw milk and milk products have fluctuated significantly. Such variations will continue in the following years as a response to: the impact of weather conditions on production, fluctuating energy prices, exchange rates and animal health issues.

On medium-term, the dairy sector will continue to expand as a response to a steady growth of domestic and world demand, while prices are expected to reach moderate levels in the following years. Import demand from other regions of the world increased significantly and is expected to grow steadily (approx. 2.4% annually), driven mainly by population growth and a change in diet in favour of dairy products. On the demand side, the most remarcable change in 2015 was the increase of imports in countries such as: Mexico, Japan, USA, Philippines and Malaysia (FAO - Global dairy sector. 2013).

In the next decade, about half of the `EU milk production will be further converted into milk powder (skimmed milk powder mainly) and 30% will be used for cheese. (Figure 2)

While the bigger share of the milk powder surplus will be exported, the main driver for cheese consumption remains the domestic market.



Note: Milk equivalent total solids coefficients used: 3.6 for cheese, 6.57 for butter, 7.6 for SMP, 7.56 for WMP.

Figure 2. Increase in production and exports of dairy commodities in the next 10 years (2025 vs. 2015, million t of milk equivalent)

Almost half of EU production of skimmed milk powder (SMP) is exported and the rest is used for domestic consumption (e.g. for processing of fresh milk, chocolate, biscuits, etc.) or stored. After several years of decline the use of SMP for household purposes began to rise again in 2009 and it is expected to increase by more than 200 thousand tons in

the next 10 years. The EU SMP production will stabilize around 1 million tons.
Skimmed milk powder is also the basis for the whole milk powder production, which is exported mainly to countries with low-income from Africa.
The low SMP price and a competitive euro have allowed other countries from the Middle East and Southeast Asia to purchase larger quantities. EU skimmed milk powder exports continued to rise so far, despite of a drop in sales in the case of Algeria, China and Indonesia. Currently, there are some concerns regarding Algeria, which has purchased smaller quantities in the context of reduced oil prices, but this is not expected to be maintained throughout the period analyzed. Demand is expected to increase in Asia (including China) and Africa. With 900 thousand tons exported in 2025, the EU could maintain a market share of 32% on the world market (Agricultural Outlook 2015-2024, 2015).
As regards whole milk powder (WMP), from 2013 the production began to rise after a fairly long period of decline, mainly based on the increase of domestic consumption.
Regarding export, the main EU clients are represented by Oman, Algeria, Nigeria, China and Hong Kong. Medium-term outlook, estimates an increase of exports with 150 thousand tons generated by a strong increase in imports to African countries and Asia.
China will remain the main buyer and will absorb almost 30% of world trade. However, its imports of WMP will increase much less between 2015 and 2025 than in the previous decade (with less than 200 thousand tons). (Milk and Milk Products Price and Trade Update, 2015)
Cheese production is expected to increase by 1.15 million tons in the next decade, reaching a total of 11.2 million tonnes in 2025. If the proportion intended for exports increases, in the following period, it will still be less than 10% of the production obtained in 2025, emphasizing the importance of the domestic market. Consumption per capita is rising, determined by a favorable economic situation and also by consumer preferences. The increase rate will be higher in the new Member States (EU-N13) than in the old Member States (EU-15). (Figure 3)

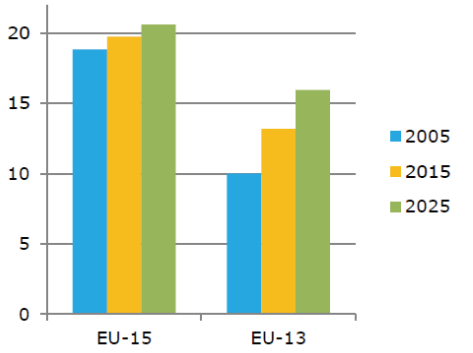


Figure 3. Consumption of Cheese (kg per capita)

On short term, cheese exports were affected clearly by the introduction of the Russian embargo. Russia was EU's main customer for this category, accounting for over 30% of its exports. However, EU traders have been successful in directing a significant proportion of their exports to other destinations, such as US, Japan and South Korea (USDA Outlook, 2015).
By 2025, it is expected that the EU will export around 1 million tonnes of cheese that is with 230 thousand tons more than 2013 exports (before the Russian embargo). The average export price for EU cheeses, in 2014, was 5 euro/kg compared to US and New Zeeland export cheese price 3.5 euro/kg (the EU's main competitors). The EU cheese high prices reflect the diversity of varieties that are exported. The EU is the largest exporter of cheese in the world and could attain by 2025, a market share of 37% (Medium-term prospects 2015-2025, 2015).
Butter production will be higher as a result of the increased (2.6 million tonnes in 2025, with 12% higher than 2015). While in the past, it burdensome to extract milk fat, market trends have reversed at EU level and worldwide, reaching high prices in 2014 and 2015. Retail sales of butter rose by almost 20% over the past 10 years, while the margarine and vegetable oil seeds fell steadily. (European Milk Market Observatory, 2015)
Industrial use of butter (approx. 40% of the butter consumption) is also increasing. Butter is used extensively for manufacturing biscuits

and pastry, representing 50% of the butter used industrially and is facing a growing demand. Butter is also used in the cheese and chocolate industry.
The EU butter market relies more on domestic consumption. EU exports accounted for 15% of production in 2005 (when export subsidies were granted), and only 6% in 2015 - a proportion which should remain stable in the next decade.
Over the analized period 2015 -2025, the US is expected to resume exports, while New Zealand will maintain its position as number one exporter, covering 50% of world trade. However, a growth to 210 thousand tons is estimated for EU exports.
In light of the above mentioned, the EU butter consumption is expected to grow by 9% in the medium term reaching 4.6 kg/capita. The growth will be higher in the new Member States, reaching only 3.9 kg/capita by 2025. Nevertheless, a gap of 1 kg/capita remains between new Member States and old Member States (CLAL, 2015).
Fresh products category includes milk (including UHT), fermented milk products and fresh cream. Retail sales for these products increased significantly: 90% for yogurts, almost 80% for drinking milk and 60% for cream. In volume terms, expresed in milk equivalent, drinking milk holds the biggest share of fresh dairy products. For this reason, in the following 10 years, a reduction 2 kg/capita is expected. Taking into account the population growth, this will translate into a stabilization of total volume consumed.
Exports of fresh dairy products (especially UHT milk) increased by nearly 15% every year for the past 10 years: from very small volumes of approx. 200 thousand tons in 2005 to 800 thousand tons in 2015. (European Milk Market Observatory, 2015)
Export of milk does not seem very profitable because of the high water content and low added value, but the market has grown due to the low transport cost towards China.
EU exports will further develop in the next 10 years reaching 1.3 million tonnes. In spite of that, fresh products will remain a minor market (representing less than 3% of EU production).

CONCLUSIONS

In the following period global dairy consumption will grow annually by 1.9%, according to FAO-OECD estimations.
This is slightly lower than in the last decade (2.1%), but in terms of volume represents an additional 16.1 million tonnes of milk produced annually, as compared with 14.5 million t between 2005 and 2014. However, the global market should trade cautiously. By 2025, only 7.5% of world milk production should be marketed in order to maintain under control possible imbalances that may arise on the market.
On medium term, consumption of dairy products will increase, especially for butter (+ 9%, 4.6 kg / capita), cheese (+ 3%, 18.6 kg / capita), cream (+ 9%), but will decrease for milk (-6.2%) and fermented products (-0.8%).
Milk price will recover on short term and it's expected to grow on medium term reaching 360 eur/t in 2025 (the European average). By 2020, the average EU milk price is expected to fluctuate between 320 eur/t and 330 euro/t in the context of lower energy and feeding costs. After 2021, the milk price will increase simultaneuosly with dairy commodity prices, oil prices and feed costs.
In the following 10 years milk fats will be used more intensively, butter and cheese price are expected to rise to 3.800 eur/t in 2025. Skimmed milk powder prices may increase from the level of intervention price, recorded in 2015 (1698 eur/t), to an average price of 2.500 eur/t by the end of the projection period.
Agricultural income per annual working unit (AWU) in the EU-28 is expected to increase substantially by around 16 % in real terms over the 2015-2025 outlook period, as a combined effect of a strong increase in income in the EU-N13 by close to 40 % and a much smaller one in the EU-15 by 2 %. Consequentely, EU-15/EU-N13 income gap will continue to narrow, but still remain substantial.
Given the large number of small farms and the age of farmers throughout the EU, structural change should continue over the outlook period, but at a slightly slower pace than in the pre-crisis period. The total EU

agricultural labour force is expected to fall from 9.9 million AWU in 2014 to 7.3 million in 2025.

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DAIRY GOAT PRODUCTS OF EAST MEDITERRANEAN REGION OF TURKEY: KÜNEFE AND SÜNME CHEESES

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Abstract

Goat products have historically been used for multitude of purposes; forming an integral component of the livestock industry, plays a vital role in the socio economic structure of the rural community. Turkey has the highest goat population in Europe with 8 million head however; the population has a decreasing trend due to poor performance of native breeds, lack of state support policies and migration from the rural to urban areas by 40% from 13.3 million down to 8 million between the years of 1985 and 2014. Dairy goat products and cheeses constitute of a long historical back ground in Turkey, as well as many toher Mediterranean countries. Künefe and Sünme cheeses are traditional dairy goat products that are produced in the Eastern Mediterranean region of Turkey with local artisan cheese-making procedures. They are mostly made from goats', sometimes cows' milk or a mixture of both. With the high industrialisation like in many fields, artisanal cheese types are diminishing. While Künefe cheese is sold fresh as soft cheese for a special dessert (Künefe), Sünme cheese is usually consumed in the breakfast. The objectives of the present paper were to characterize the processing stages of Künefe and Sünme cheeses produced from goats' milk and to describe their compositional characteristics. A better knowledge of their characteristics would support the improvement of the production technology and help to obtain a constant quality product capable of being successfully introduced into national and international markets. Apart from the present situation, production methods and steps affecting consumption of above mentioned cheese types are elaborated in this paper. Information gathered from this study may provide a better understanding of Künefe and Sünme cheese and therefore could be useful to disseminate artisanal cheese types in the industry.

Key words: *Goat cheese, Künefe, Sünme, traditional dairy products, production technology.*

INTRODUCTION

Goat milk production is a dynamic and growing industry that is fundamental to the well being of millions of people worldwide and is an important part of the economy in many countries (Thum et al., 2015). It has been used throughout the world since ancient times for the manufacture of different types of cheeses (Park, 2001; Mehaia, 2002). Goat cheese demand is also rising quite considerably, especially among gourmets and consumers of health and diet products due to its high nutritional value in terms of proteins and fat (Fresno et al, 1997; Caponio et al, 2000; 2001). Mediterranean countries are important producers of goat milk and most of it is used for cheese production (Juan et al., 2016). Goat milk differs from cow milk from its higher digestibility, alkalinity, buffering capacity, and certain nutritional and therapeutic properties (Park, 1994). These nutritional features have contributed to the growth of the market for goat dairy products, and consequently have attracted the interest (Golinelli et al., 2014). Goat milk presents some specificities related to its chemical properties, specially due to the characteristics of its proteins, which display reduced levels, or even a lack of, α_{s1}-casein, as well as structural differences in α-lactalbumin and beta (β) lactalbumin. These characteristics make it less allergenic when compared to bovine milk. Therefore, cheeses prepared using goat milk present a number of desirable properties to many consumers, especially those who are allergic to the type of protein present in bovine milk (Bezerra et al., 2016). Goat milk exceeds cow milk in monounsaturated (MUFA), polyunsaturated fatty acids (PUFA), and medium chain triglycerides (MCT), which all are known to be beneficial for human health, especially for cardiovascular conditions. This

biomedical superiority has not been promoted much in marketing goat milk, goat yoghurt and goat cheeses, but has great potential in justifying the uniqueness of goat milk in human nutrition and medicine for treating the various gastro-intestinal disorders and diseases, besides its value in alleviating cow milk allergies (Haenlein, 2004).

Goat breeding is a common activity in the Mediterranean region of Turkey as a result of their good adaptability to the harsh environmental conditions and their converting ability of low input to high valuable products. Most of the milk is processed by household to cheese and sold in the nearby region since many years. Traditional dairy goat products are usually manufactured by the small scale producers and consumed locally. With the increased industrialization in the recent years some of the traditionally produced cheese types have begun to disappear. A large number of goat cheeses are produced at the industrial scale but only a few are still remains as traditional and supports the rural area's income. It is important to keep traditional types both as a cultural heritage and for supporting the rural income.

One of the important cheese type in the Eastern Mediterranean Region of Turkey is Sünme and Künefe cheese. These types are not produced under industrial scale and are region specific. We aim to introduce these special types of cheeses with a wide description of their processing steps as well as the properties of goat milk and its benefits for human health.

COMPOSITION/NUTRITIONAL VALUE OF GOAT MILK

The popularity of dairy products made from milk of small ruminants is increasing among researchers and the dairy industry, due to their peculiar taste and nutritional proprieties (Niro et al., 2014). Compositions of goat, sheep, cow and human milks are different (Table 1), but vary with diet, breed, individuals, parity, season, feeding, management, environmental conditions, locality, stage of lactation, and health status of the udder (Park et al., 2007).

Cow milk and goat milk both have similar protein content, but goat milk proteins are assimilated more easily (Witczak et al., 2016). The principal caseins in goat milk are about the same as in the milk of sheep or cows, α_{s1}-casein, α_{s2}-casein, β-casein and κ-caseins (Park et al., 2007). It is poor in casein; casein micelles contain more calcium, in organic phosphorus, and non-centrifugal caseins, they are less solvated, less heat stable, and lose β-CN more quickly than cow milk casein micelles (Niro et al., 2014). Sheep and goat milk proteins are also important sources of bioactive angiotensin converting enzyme (ACE) inhibitory peptides and antihypertensive peptides. They can provide a non-immune disease defence and control of microbial infections. Important minor milk proteins include immunoglobulins, lactoferrin, transferrin, ferritin, proteose peptone, calmodulin (calcium binding protein), prolactin, and folate-binding protein. Non-protein nitrogen (NPN) contents of goat and human milks are higher than in cow milk. Taurine in goat and sheep milk derived from sulphur-containing amino acids has important metabolic functions as does carnitine, which is a valuable nutrient for the human neonate. Goat milk contains higher concentrations of nucleotides, polyamines and some of the essential amino acids (Thum et al., 2015). Goat milk with the genetic trait of low or no α_{s1}-casein, but instead with α_{s2}-casein, has less curd yield, longer rennet coagulation time, more heat lability, and weaker curd firmness, which also may explain the benefits in digestibility in the human digestive tract (Haenlein, 2004).

Triacylglycerols (TAG) constitute the biggest part of milk lipids (nearly 98%), including a large number of esterified fatty acids (Park et al., 2007). Nevertheless, the main characteristic of small ruminant milk fat is the high content in short- and medium-chain fatty acids (MCFA), especially in goat milk fat, which has at least twice as many C6–C10 fatty acids as cow milk fat: 8%, 12% and 16% total fatty acid for cow, ewe and goat milk fat, respectively (Raynal-Ljutovac et al., 2008). Sheep and goat milk also have simple lipids (diacylglycerols, monoacylglycerols, cholesterol esters), complex lipids (phospholipids), and liposoluble compounds (sterols, cholesterol esters, hydrocarbons). The average fat globule size is smallest (<3.5μm) in sheep milk followed by goat and cow milk. Five fatty acids (C10:0,

C14:0, C16:0, C18:0, and C18:1) account for >75% of total fatty acids in goat and sheep milk (Park et al., 2007). Average goat milk fat differs in contents of its fatty acids significantly from average cow milk fat (Jenness, 1980), being much higher in butyric (C4:0), caproic (C6:0), caprylic (C8:0), capric (C10:0), lauric (C12:0), myristic (C14:0), palmitic (C16:0), linoleic (C18:2), but lower in stearic (C18:0), and oleic acid (C18:1) (Haenlein, 2004).

Milk sugar, lactose, is the major carbohydrate in goat, sheep and cow milk. Lactose is a valuable nutrient, because it favors intestinal absorption of calcium, magnesium and phosphorus, and the utilization of Vitamin D (Park et al., 2007). Goat milk has higher digestibility and contains less lactose. Therefore, it can be recommended for those who suffer from lactose intolerance. In addition, it has a lower casein content (and is hence considered a hypoallergenic milk), and a similar oligosaccharide content to human breast milk (Vieitez et al., 2016).

Overall, goat milk has more Ca, P, K, Mg and Cl, and less Na and S contents than cow milk. Trace mineral contents of goat milk are also affected by diet, breed, individual animal, and stages of lactation, mean levels of Mn, Cu, Fe, and Zn in goat milk were 0.032, 0.05, 0.07, 0.56 mg/100 g respectively. Levels of folate and Vitamin B12 in cow milk are five times higher than those of goat milk, and folate is necessary for the synthesis of hemoglobin. Vitamin B12 deficiency can cause a megaloblastic anemia in infants, but the anemia has been attributed mainly to folate deficiency in goat milk (Park et al., 2007).

Table 1. Average Composition of Basic Nutrients in Goat, Sheep, Cow and Human Milk (Park et al., 2007)

Composition	Goat	Sheep	Cow	Human
Fat (%)	3.8	7.9	3.6	4.0
Solids-not-fat (%)	8.9	12.0	9.0	8.9
Lactose (%)	4.1	4.9	4.7	6.9
Protein (%)	3.4	6.2	3.2	1.2
Casein (%)	2.4	4.2	2.6	0.4
Albumin, globulin (%)	0.6	1.0	0.6	0.7
Non-protein N (%)	0.4	0.8	0.2	0.5
Ash (%)	0.8	0.9	0.7	0.3
Calories/100 ml	70	105	69	68

GOAT MILK PRODUCTION IN THE WORLD AND TURKEY

Goat milk and products of goat milk are important in human nutrition and have become a part of the current trend of healthy eating around the world. Goat milk consumption is increasing and consequently, the goat population has considerably increased during recent years (Sosnowski et al., 2016). At present, the global production of goat milk is estimated at 2025 million L. In the world the largest producers of goat milk are the USA (100 million L), Canada (60 million L), and Mexico (50 million L). Goat milk production in European countries accounts for 26% of world production (Witczak et al., 2016).

Turkey is among the 10 largest milk producers in the world (FAO 2014). The production of raw milk is mainly from cows and accounts for an average of 91-92 % of the total production dairy products have an important role in the Turkish diet.

Consumption level of liquid milk is very low; the most common form of milk consumption is yoghurt, followed by white cheese (feta type) and ayran, a liquid salted milk drink (Bor, 2014).

As it is seen from Table 2, the number of cattle is important for the milk production. There is a stable increase in the number of dairy goats, cattle and sheep since 2011. Animals that are milking shows an increasing trend with the years according to the TUİK (2014) and number of dairy goats are 3.9 million in 2014. Milk yield from the dairy goats are estimated as 415.000 tons.

Table 2. Total Numbers of Milking Animals (Millions) in Turkey (TUİK, 2014; USK, 2014)

Yıl	Goat	Cow	Sheep
2011	3.033.111	4.761.142	11.561.144
2012	3.502.272	5.431.400	13.068.428
2013	3.943.318	5.607.272	14.287.237

Table 3 shows milk production indices of sheep, goat and cattle in Turkey and in the World between 2006-2013.

The highest increase is has been observed in goat milk. Total global milk production is comprised of 2.4% goat and 1.3% sheep milk. World goat milk production is dispersed between Asia, Africa and Europa at 60%, 22% and 15%, respectively.

While sheep milk is utilised in Asia and Europa at 46% and 32%, respectively. Annual per head production of milk in 1930 was 80L, whilst in 2013 this figure grew to 105 L (USK, 2014).

Table 3. Total Milk Productions in The World and Turkey (tonnes) (FAOSTAT, 2016)

Milk Production	2006		2013		Change (%)	
	World	Turkey	World	Turkey	World	Turkey
Goat	15259583.00	253759.00	17957372.00	415743.00	+18	+64
Cow	563402301.00	10867302.00	635575895.00	16655009.00	+13	+53
Sheep	9338076.00	794681.00	10137749.00	1101013.00	+9	+39

Total cheese production in Turkey in 2013 was 600.266 tons, which consisted of 95.6% cattle milk and the rest was from sheep, goat and buffalo milk (USK, 2014).

GOAT CHEESES

The chemical characteristics of goat milk can be used to manufacture a wide variety of products, including fluid beverage products (low fat, fortified, or flavored) and UHT (ultrahigh temperature) milk, fermented products such as cheese, buttermilk or yogurt, frozen products such as ice cream or frozen yogurt, butter, condensed/dried products, sweets and candies (Ribeiro and Ribeiro, 2010). Goat cheese was originated in Mesopotamia and the milk was probably made into soft cheese, hard and ripened goat cheeses were later developed in the Mediterranean basin countries (Park, 2001). A considerable number of goat milk products have a strong regional and artisanal character (Sosnowski et al., 2016). Cheeses made from goat's milk are greatly appreciated because of their particular organoleptic characteristics. The composition of the lipid fraction plays an essential role in the sensory attributes of these products. The fatty acids hexanoic (caproic), octanoic (caprilic) and decanoic (capric), together with certain branched-chain free fatty acids, are responsible for the characteristic 'goaty flavour' of goat cheeses (Galiou et al., 2015).

The main characteristic of the Mediterranean area is the importance of sheep and goat milk production, mainly processed as cheeses. The small-scale and farm-made cheeses, mostly with sheep or goat milk, are generally considered a possible vehicle of economic and social development for the less favored rural areas; mountainous areas are seen as holders of local know-how (Dubeuf et al., 2010). Cheeses (Erzincan Tulum cheese, Izmir Brined Tulum cheese, Cimi Tulum cheese, Carra cheese, Kopanisti whey cheese, Sepet cheese), fermented dairy foods (Kefir, Salted yoghurt, Ayran), butter, ice cream (Kahramanmaraş ice cream) and milk based desserts made by using goat milk have very desirable sensory characteristics for the consumers in Turkey (Hayaloglu and Karagul-Yuceer, 2011). In the Mediterranean region, the other traditional goat cheeses (Antep Cheese, Çepni Cheese, Çimi Cheese, EğridirKelle Cheese, GönenYörük Cheese, Kartal Cheese, Kelle Cheese Koponesti Cheese, Sütlü Cheese (Toros), Teleme Cheese, Urfa White Cheese, Yayladağ Goat's Cheese and Denizli White Goat's Cheese) are also common produced (Kamber, 2015). In this study, we aim to describe Künefe and Sünme cheeses from the Eastern Mediterranean Region of Turkey.

KÜNEFE CHEESE

Künefe cheese is consumed as a fresh and unsalted dessert cheese and has melting properties (Table 4). At the same time, Künefe is a special dessert name in this region, making by Tel Kadayıf (a sweet pastry with a texture similar to that of shredded wheat. Shredded dough baked in syrup topped with crushed nuts, a kind of sweet pastry). It is used for production of some special desserts especially Semolina halva and Künefe. This cheese is also the main component in many cheese varieties produced in the same region such as Yuvalama, Dil and Sünme cheeses and Künefe cheese is manufactured before Sünme cheese production. Above mentioned cheese types are produced by making some modifications on Künefe cheese (kneading, shaping, stretching etc.).

Figure 1. Künefe cheese

No starter culture in used in Künefe cheese. The raw milk of goat or cow is coagulated with commercial rennet at 35±2 °C for 60 min or longer. The curd is cut into 2-3 cm^3 pieces and sometime after cutting (15-20 min), the curd need to be put into traditional cheese cloth (muslin) in steal or wood box for separating the whey by itself and tied four-sided of cloths and then covered box should be removed. After keeping for about 30 min to drain off the whey, the cheese cloth is tied very tightly for 30 min again. The pressing process need to be applied for 1-2 h by piled up one cloth on the top of the other. After whey separation, this raw cheese is cut into 4-6 big pieces. Cheeses are left fermentation either at room temperature 6 h in summer about, 12 h in winter or 24 h at +4 °C in refrigerator until the cheese curd reach to around 4.9-5.3 pH. The end of the fermentation is determined by the producers with stretching test in hot water.

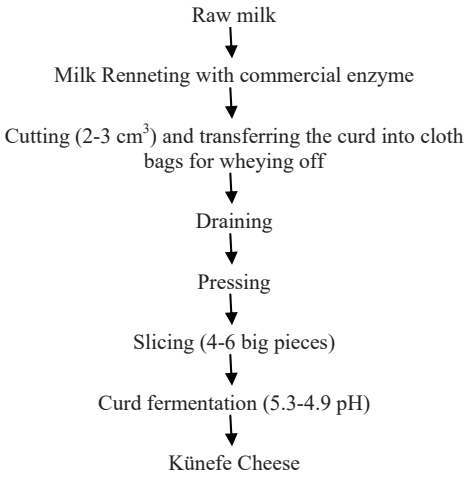

Figure 2. Production of Künefe cheese

The Künefe curd is cut into slabs that are kneaded and stretched in hot water (80-85 °C 1-2 min). Squeezing is applied the curd within two hands, excessive water is removed and given a ring-shaped for making longer or stretching stage. The hot curd is kneaded and then stretched (sometimes 2 meter long) by hand until it becomes shiny, smooth and elastic. The stretching process is repeated until the curd has a fibrous character. The stretched curd is given into string shapes by folding up and then tied a knot to top of the cheese. The shaped curd is then chilled in cold water to harden, and salted for several days in cool brine. Frequently, after brining, the cheese is packaged with a vacuum plastic or can be stored in freezer. Its yield is about 10%.

Table 4. Composition of Künefe Cheese (Karaca et al., 2008)

Properties	Künefe Cheese
Acidity (% la)	0.63
pH	5.36
Dry Matter (%)	46.43
Protein (%)	19.47
Protein in Dry Matter (%)	42.15
Fat (%)	24.19
Fat in Dry Matter (%)	52.07
Salt (%)	0.24
Salt in Dry Matter (%)	0.53
Ash (%)	1.88

SÜNME CHEESE

Sünme cheese is a semi-hard cheese variety, has a fibered structure and it is consumed for breakfast. Originally, Sünme cheeses are made from goat's milk; it is now also derived from cow's milk. After production of Künefe cheese, Sünme cheese can be obtained by making some application stages, stretching, shaping into traditional forms and salting in brine. These stages are similar to Pasta filata cheeses, having a unique plasticizing and kneading treatment of the fresh curd in hot water, which gives the product a fibrous structure and melting and stretching properties. It is being made totally by the hand of man.

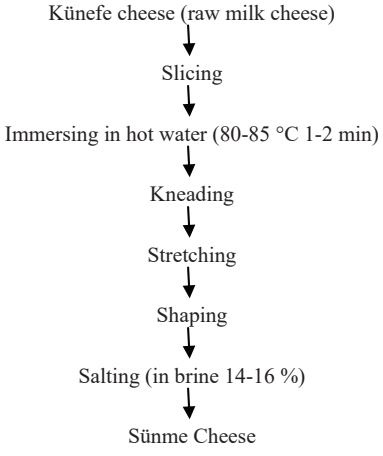

Figure 3. Traditional Sünme cheese production method (Mutlu, 2000)

Table 5. Composition of Sünme Cheese

Properties	Biçer et al., 1995	Mutlu, 2000 (n:20)	Karaca and Güven, 2004 (n:30)
Acidity (% la)	2.0	0.27	1.41
pH	-	5.05	5.60
Dry Matter (%)	63.1	54.09	53.74
Protein (%)	21.4	24.38	26.12
Protein in Dry Matter (%)	-	-	48.51
Fat (%)	31.7	21.45	20.07
Fat in Dry Matter (%)	-	39.66	37.20
Salt (%)	9.3	6.59	2.65
Salt in Dry Matter (%)	-	12.30	4.98
Ash (%)	1.9	-	8.54

Composition of Sünme cheese, determined by some researchers is given in Table 5. In addition to Table 2, the average nitrogen fraction results were obtained as the follows; total nitrogen 4.09%, water soluble nitrogen 0.66%, casein nitrogen 3.44% and ripening index 16.60% by Karaca&Güven (2004).

Physical, chemical and organoleptic properties of Sünme cheeses made from raw and pasteurized cows' milk, the two different brine including 14 %, 16 % ratio salt were investigated by Mutluer (2007) and ripened during 90 day.

From the results, it was found that using of brine including different levels of salt significantly had effect on pH value, titratable acidity, total solids, fat, fat in the dry matter, protein, protein in the dry matter, salt, salt in the dry matter, melting, total nitrogen, water soluble nitrogen, 12% trichloroacetic acid-soluble nitrogen, 5% phosphotungstic acid-soluble nitrogen, casein nitrogen, protease-peptone nitrogen values and water-soluble nitrogen, 12% trichloroacetic acid-soluble nitrogen, 5% phosphotungstic acid-soluble nitrogen, casein nitrogen, protease-peptone nitrogen in the total nitrogen ($p<0.05$) and organoleptic properties significantly had effected ($p<0.05$). It was determined that produced from raw milk and matured cheese in brine 16% was most preferred by panelists and Sünme cheese could be produced from heat-treated milk but these cheeses should be ripened up to 45 days to eliminate the negative impacts arising from the rapid maturation during storage or raising the percentage of salt in brine or packaging under vacuum.

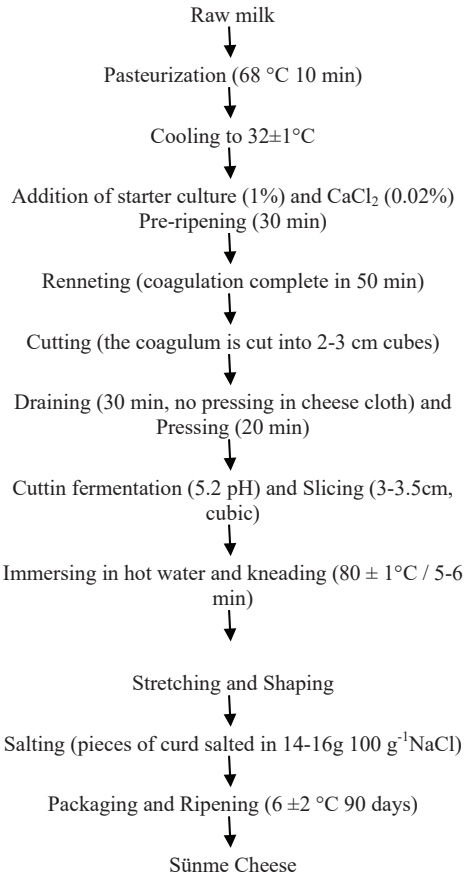

Figure 4. Standardized Sünme cheese production method (Mutluer, 2007; Mutluer et al., 2014)

Figure 5. Sünme cheese

CONCLUSIONS

There is quite limited information available about Sünme and Künefe cheeses in the literature. Since Künefe and Sünme cheeses production mainly take place on a non-industrial scale, quality is not consistent and the cheeses are not appropriately promoted or appreciated. However this traditional product is recently started to be produced in modern plants, however, it is not enough well known in Turkey as well as in the other countries. In addition, it can be concluded that modern technologies and facilities could be used instead of traditional processing methods for production of Künefe and Sünme cheeses in order to disseminate it worldwide.

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DETERMINATION OF MICROBIOLOGICAL QUALITY OF KOKOREÇ SOLD IN ISPARTA

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Abstract

In this study, a total of 30 kokoreç samples (10 raw, 10 cooked and 10 cooked-seasoned) collected from 10 different restaurants in Isparta district of Turkey were investigated for microbiological quality. The raw, cooked and seasoned kokoreç samples were subjected to total aerobic bacteria count, total coliforms, yeast and mould. The results of this study revealed that raw kokoreç samples had 10^5-10^8cfu/g aerobic mesophilic viable bacteria, 10^4-10^5cfu/g total coliforms, 10^3-10^6cfu/g yeast and molds. On the other hand, cooked kokoreç samples had 10^2-10^5cfu/g aerobic mesophilic viable bacteria, $<10^1$cfu/g coliforms, $<10^1$cfu/g yeast and molds. Furthermore, cooked-seasoned kokoreç samples had 10^5-10^6 cfu/g aerobic mesophilic viable bacteria, 10^4-10^5cfu/g coliforms, 10^3-10^4cfu/g yeast and molds. This study results showed that even though cooking process decreased the microbial load significantly, the use of seasonings resulted in a significant increase in the microbial load of cooked kokoreç samples. This study indicated that kokoreç sold in Isparta market is not at the desired level as far as microbiological quality is concerned and the necessary measures should be taken for protection of consumer health and satisfaction of consumer expectations.

Key words: *Kokoreç, microbiological quality, food market.*

INTRODUCTION

Hygienic quality problems of foods cause food borne diseases. Worldwide, many people die each year due to food-borne diseases. Food-borne diseases remain one of the major public health problems in our country as well as all over the world. Besides, deficiencies in the quality of food causing malnutrition negatively affect the health of society as well as adversely affect the sale of products leading to economic losses. Thus food safety and quality are an important factor. Therefore, scientific studies about foods sold in market have an important functionality for protection of consumer health and satisfaction of consumer expectations.

Kokoreç is a dish of the Balkans, Greece, Azerbaijan, Iranian Azerbaijan and Turkey consisting of lamb or goat intestines wrapped around seasoned offal, and typically cooked on a griddle. The intestines of suckling lambs are preferred. The ingredients are sliced and seasoned with lemon, olive oil, oregano, salt, and pepper. The intestine is cleaned especially thoroughly. The filling meats are threaded onto a long skewer and wrapped with the intestine to hold them together. Kokoreç is usually roasted on a horizontal skewer over a coal, gas, or electrical burner. A quite different preparation mixes the chopped innards with chopped tomatoes and green peppers, and then cooks them on a large griddle with hot red pepper and oregano added. When done, kokoreç can be served in half a baguette or in a sandwich bun, plain or garnished, almost always with oregano and red pepper.

There is no doubt that foodborne pathogenic bacteria are the cause of illness and death for many people each year, at great economic cost and human suffering. Any outbreak of food borne illnesses triggers recalls and can cause consumers to decrease their meat purchases. That domino effect can have exhausting consequences on the meat production industry. As a result, meat processors constantly research for inventions to eliminate potential bio-hazards. As the consumers are wary of chemical solutions being used on products, natural inventions are especially appealing for consumers. Two major sources of bacteria causing foodborne disease in meat and meat products may be identified. The living animal

carries pathogenic bacteria while the processing environment harbours them. In addition, the human being is also an important source of pathogenic bacteria, most frequently indirectly by cross contamination. Limiting the contamination and subsequent inactivation of occurring pathogenic bacteria is decisive to the safety of meat and meat products.

Food market studies have the essential function of providing insight into consumer purchasing patterns and provide directions to researchers and regulatory agencies for controlling safe and nutritious food production. Several studies have been conducted to determine microbiological quality of kokoreç sold in Turkish markets. These studies revealed that there are variations in microbiological parameters of kokoreç samples sold in the market.

In the present study, the objective was to determine microbiological quality of raw, cooked and seasoned kokoreç samples collected from 10 different restaurants in Isparta district of Turkey.

MATERIALS AND METHODS

Samples

Ten samples (about 100 g) of raw kokoreç samples, ten samples (about 100 g) of cooked kokoreç samples and ten samples (about 100 g) of seasoned kokoreç samples were collected from ten different restaurants in Isparta district of Turkey. Each kokoreç sample was placed in an individual sterile plastic bag and transported on ice to the laboratory immediately after collection. Kokoreç samples were tested upon arrival for microbiological quality.

Microbiological analysis

To analyze raw, cooked and cooked-seasoned kokoreç samples, kokoreç samples (10 g) were aseptically weighed, added to sterile buffered peptone water (90 ml) and homogenized in a stomacher at room temperature. Decimal dilutions in buffered peptone water were prepared. Aerobic plate counts (TVAC) were measured using the spread plate method on aerobic plate count agar (Merck, Darmstadt, Germany). The Petri dishes were incubated at 30°C for 24–48 h. Mould and yeast counts were measured using the spread plate method on Potato Dextrose Agar (Merck, Darmstadt, Germany). Petri dishes were incubated at 25°C for 2–5 days. Total coliform bacteria counts were carried out using the spread plate method on Eosin Methylene Blue Agar (Merck, Darmstadt, Germany). Petri dishes were incubated at 37°C for 24–48 h (Karahan et. al., 2002; Maturin & Peeler, 2001).

RESULTS AND DISCUSSIONS

The microbiological count in raw, cooked and cooked-seasoned kokoreç samples are shown in Table 1. The results indicated that there were a significant differences for total aerobic bacteria count, total coliforms, yeast and mould among in raw, cooked and cooked-seasoned kokoreç samples.

The results of this study revealed that raw kokoreç samples had 10^5-10^8cfu/g aerobic mesophilic viable bacteria, 10^4-10^5cfu/g total coliforms, 10^3-10^6cfu/g yeast and molds. On the other hand, cooked kokoreç samples had 10^2-10^5cfu/g aerobic mesophilic viable bacteria, $<10^1$cfu/g coliforms, $<10^1$cfu/g yeast and molds. Furthermore, cooked-seasoned kokoreç samples had 10^5-10^6cfu/g aerobic mesophilic viable bacteria, 10^4-10^5cfu/g coliforms, 10^3-10^4cfu/g yeast and molds.

Table 1.Microbiological count (cfu/g) in raw, cooked and cooked-seasoned kokoreç samples

		Raw	Cooked	Cooked-seasoned
Total aerobic bacteria	Minimum	$7.3x10^5$	$2.4x10^2$	$2.1x10^5$
	Average	$2.5x10^7$	$5.3x10^3$	$1.1x10^6$
	Maximum	$1.5x10^8$	$1.2x10^5$	$3.5x10^6$
Total coliform bacteria	Minimum	$3.0x10^4$	$<1.0x10^1$	$6.7x10^4$
	Average	$1.3x10^5$	$<1.0x10^1$	$5.7x10^5$
	Maximum	$4.0x10^5$	$<1.0x10^1$	$7.1x10^5$
Mould and yeast	Minimum	$7.0x10^3$	$<1.0x10^1$	$1.0x10^3$
	Average	$1.5x10^5$	$<1.0x10^1$	$5.5x10^3$
	Maximum	$1.6x10^6$	$<1.0x10^1$	$1.6x10^4$

The study results revealed that the microbiological quality of raw materials used for kokoreç manufacture is not appropriate and hygienic conditions for kokoreç production was not achieved as shown on Table 1. Similar observations on microbiological quality of kokoreç samples manufactured in Ankara were reported by Yentür et. al. (1989). This results showed that the intestines used in production during the preparation of kokoreç did not meet the required the microbiological criteria and might contain potential pathogenic bacteria.

As far as cooked kokoreç samples are concerned, results indicated that thermal process resulted in a decrease in the number of microbial load in kokoreç samples. However, the results of the study showed that reduction in a number of microorganisms due to cooking process was not sufficient. This may be the indication of insufficient thermal process.

The addition of seasonings to kokoreç resulted in a significant increase in microbial load. This results showed that seasonings used kokoreç manufacture have a significant contribution for a contamination. In this study, microbial load of seasonings were also determined and the results indicated that seasoning was one of the main reasons for kokoreç samples with high microbial load. Therefore it is important to take an action to control microbial load in seasonings used for kokoreç manufacture (Aksu et.al., 1997; McKeen, 1995; Geeta and Kulkarni, 1987).

CONCLUSIONS

The results suggest that changes such as improving the hygienic properties of the intestines and seasonings used in the production of kokoreç and more effective cooking process in kokoreç manufacture should be applied to improve safety traits. Even though there is no official report, possible food borne illnesses are major concerns surrounding kokoreç. Therefore, further research and a broad control system are needed to improve safety of kokoreç.

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QUALITY PRODUCT – HISTORY OR REALITY

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Abstract
The paper was performed to improve the competitiveness of primary processors through a better integration of those into agri-food chain by quality schemes, increasing added value of agricultural products, promotion on local markets and short supplying network, inter-branch and producer organizations. The restoration and development of villages and especially resources preservation as an essential requirement to improve life quality occurs as an important element into utilization of local resources and environment protection. The paper aimed to present the evolution of traditional food production during the period 1990 -2011 in the North West Region, Center, North East, Muntenia Regions, South West, West and South East Regions.

Key words*: quality product, physical person, authorized person, legal entity, individual and family enterprises, family associations, commercial societies, joint-stock companies.*

INTRODUCTION

The paper was performed to improve the competitiveness of primary processors, through a better integration of those into agri-food chain by quality schemes, increasing added value of agricultural products, promotion on local markets and short supplying network, inter-branch and producer organizations. The restoration and development of villages and especially resources preservation as an essential requirement to improve life quality occurs as an important element into utilization of local resources and environment protection (Banu, 1998; Barariu, 1992).

MATERIALS AND METHODS

Research was performed by collecting operative data from County Agricultural Directorates regarding the claims of producers with various legal statutes to certify them as producers of **quality products**, on different activities: food products – meat, milk, fish, bakery/pastry, vegetal fats, alcohol, vegetables/fruits and so on. The data collected were centralized at national level, on development regions, on activity domains mentioned above. The paper presents the evolution of number of quality product producers, number of quality products on economical development regions, during eight years of study, 2005 – 2012, at both national and regional level.

RESULTS AND DISCUSSIONS

Following the dynamics of **quality product** producers at national level, depending on legal status during 2005 – 2012, one can ascertain that there is a permanent annual fluctuation of number of new certified producers, with a maximum in years 2006 **(228)** and 2007 **(248)** and a minimum in years 2010 **(73)** and 2012 **(80)** (Figure 1).

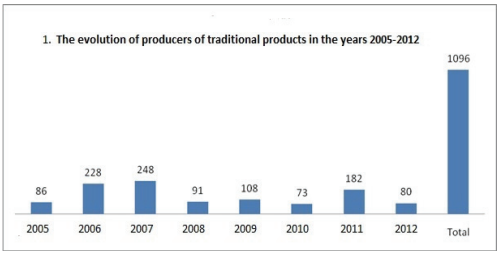

Figure 1. The evolution of producers of traditional products in the years 2005-2012

From the viewpoint of quality product producer legal status (Table 1, Figure 2), the many certifications are those achieved by the Commercial societies (591), followed by Physical persons (247) and Family associations (120), the fewest being achieved by the farming societies (3), Joint-stock companies (7) and Associations (17).

Table 1. Dynamics of quality product producers depending on legal status during 2005 – 2012 (Ministry of Agriculture and Rural Development)

Specification	Period under study 2005 - 2012								
	2005	2006	2007	2008	2009	2010	2011	2012	Total
Physical person	16	25	72	17	19	9	66	23	247
Authorized person (PFA)	0	0	20	2	13	7	13	5	60
Individual enterprises (I.I.)	0	0	0	0	4	4	15	6	29
Family enterprises (.I.F.)	0	0	0	0	3	6	8	4	21
Family association (AF)	3	28	74	11	2	1	0	1	120
Commercial society (S.C.)	64	173	72	56	65	44	78	39	591
Joint-stock company (S.A.)	1	1	0	1	1	0	2	1	7
Farming society (S.C.A.)	1	1	0	0	1	0	0	0	3
Agricultural universities	1	0	0	0	0	0	0	0	1
Association/Ordinance no.26 din 30 January 2000 with regard to associations and foundations	0	0	10	4	0	2	0	1	17
TOTAL	86	228	248	91	108	73	182	80	1096

Figure 2. The legal forms of traditional producers in the years 2005-2012

The Table 2 presents the dynamics of quality product categories on economical development regions, to establish their repartition on Romanian territory and as follows of covering degree of local market demands versus them.
Thus, one can ascertain that regarding the quality milky products, they are very well represented and diversified in Economical Development Regions Center and Muntenia, relatively well represented in North West and North East Regions and lower represented in South West, West and South East Regions. The meat quality products have a territorial repartition similar with milky ones, being very well represented in Center, North East, North West and Muntenia Regions and a lowest presence in South West, West and South East Regions. The bakery/pastry quality products have a well representation in Center and North West Regions, while in the rest of development regions, the market demand is less covered. For alcoholic beverages one can clearly ascertained a very well representation in North West and Muntenia Regions. The quality vegetables/ fruits are relatively uniform represented on development regions, excepting West one.

Table 2. Dynamics of quality product categories on economical development regions (Ministry of Agriculture and Rural Development)

Development Region	Milk products	Meat products	Bakery/Pastry products	Alcoholic beverages	Vegetables/ Fruits	Vegetal fats	Fish products	Other agri-food products	Total
West	38	68	60	2	5	0	0	0	173
North West	222	308	223	173	48	4	0	12	990
South West	9	24	30	7	19	1	0	0	90
North East	126	292	24	6	22	0	4	6	480
South East	49	69	34	5	19	0	12	5	193
Muntenia	459	209	22	69	34	0	1	0	794
Center	553	475	339	17	27	1	12	0	1424
Ilfov Bucharest	55	51	0	0	5	0	11	4	126
Total country	1511	1496	732	279	179	6	40	27	4270

At the level of whole country (Fig. 3), from the total of 4270 certified quality products, many of them are from Central Region (1424) and North-West Region (990), while the areas least covered are South West Region (90), West Region (173) and South East one (193).

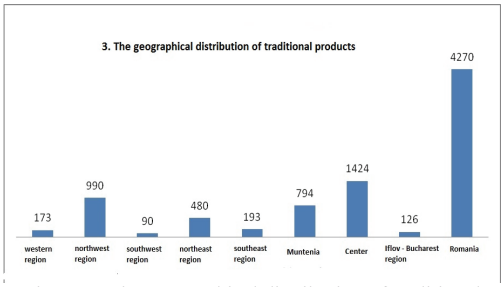

Figure 3. The geographical distribution of traditional products

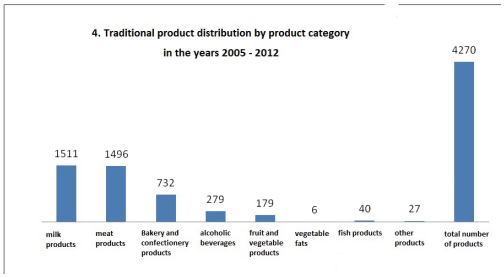

Figure 4. Traditional products distributions by product category in the years 2005 - 2012

CONCLUSIONS

1. Certification of a higher number of quality products means major advantages, such as:
   - Promotion of food chain organization, including processing and commercial-lization of agricultural products;
   - Support of patrimony preservation and local traditions;
   - Increasing life quality into rural areas;
   - Stimulation of rural tourism as activity;
   - Development of local brand and releasing of jobs;
   - Stimulation and on-going progress into rural area.
2. Repartition of certified quality products on development regions is non uniform one, which means yet large possibilities for future certification of new quality products, which ensure consumer demands for this product category.

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A REVIEW OF THE QUALITY STANDARDS FOR FROZEN BEEF MEAT AND FISH

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Abstract

One of the fastest growing sectors in food service industries is that of frozen foods. Beside the volume, the effective management of interdependent operations regarding the production, storage, distribution and retailing of frozen foods ("the cold chain") is a key factor of success and a must for preserving the safety and quality of frozen foods.
Therefore, the main concern is to regulate the aspects regarding safety, identification, quality, labelling and advertising of foods, in order to inform and protect the consumer, establish the traceability of the product and also to sustain a fair-trading. The final goal of this research is to presents the relevant standards for two categories of foods, related to hygiene, food additives, pesticide residues, contaminants, labelling and presentation, along with the proper methods for sampling and analysis. The paper will present key recommendations for processing, handling, distribution and storage of frozen beef meat and fish.

Key words*: frozen fish, frozen beef meat, quality standards.*

INTRODUCTION

Based on legislation, food represents any processed, partially processed or unprocessed product, designed for human consumption. As referring to quality, food laws deal with:
- *safety issues*: hygiene standards are implemented; undesirable substances, additives and contaminants are restricted and controlled;
- *composition issues*: producers must declare the nutritional and energetic value of food, as well as the presence of allergenic factors;
- *fair trade and consumer protection:* the consumer is protected against fraud by preventing the sale of altered, short-weighted, impure or low quality foods, along with preventing any false claims being made on labels and advertisements.

Food quality is a concept associated to the requirements that products must meet in order to be in compliance with their specifications, standards and consumer's expectations.
Also, the concept of *fair trading* statutes that labeling, advertising, packaging and food presentation, including the way food is arranged and displayed as well as promoted in any media, shall not mislead consumers (EuroIMM).

Beef meat and fish are products with a high nutritional value, containing good quality proteins and lipids (fish, especially), vitamins and minerals. The issue associated with these foods is the preservation of their qualities along the distribution chain. Refrigeration and freezing are the main preservation techniques used in meat technology, but these methods also determine the decreasing of the nutritional value of products (ISO/TC 34/SC 6).

MATERIALS AND METHODS

This research is part of a project that investigates the influence of freezing on beef meat and fish sensorial and physicochemical properties.
The present paper presents an overview of the literature and legal resources related to food quality and legislation (printed and/or available online) that apply to frozen beef meat and fish.

RESULTS AND DISCUSSIONS

Human health and wellbeing protection is mainly based on risk analysis in three different but interconnected areas:
- *risk assessment*- a scientifically based process consisting of:

- hazards identification;
- hazards types assessment;
- assessment of hazards exposure;
- risk assessment.

- *risk management*- the process of selecting appropriate prevention and control options.
- *risk communication*- the interactive exchange of information and opinions through the risk analysis process.

European Union and its Members are working tightly with the main international organizations for standardization and legislation involved in the area of food and feed, such as:

- Codex Alimentarius Commission (CODEX);
- World Organisation for Animal Health (OIE);
- IPPC International Plant Protection Convention (WOAH, Codex Alimentarius Commission).

The Romanian national stakeholder responsible for standardization is the Standards Association of Romania (ASRO). ASRO is a private, non-profit, non-governmental and apolitical entity of public concern, recognized as the only national standardization body by GD 985 of 7 July 2004, organized and existing under GO no. 39/1998 on the national standardization activity in Romania, approved with amendments by Law no. 355/2002(GD 985/2004, ASRO).

National standardization in Romania, similarly to standardization in most of the European countries, defines its duties as follows:

– Adoption of the European and international standards as national standards;
– Development, approval, revision and revocation of national standards;
– Providing public information by editing, publishing and disseminating of the new adopted standards;
– Romania's representation and involvement in the activities of international standardization bodies, regional and European;
– Ensuring the functioning of inquiry point for technical regulations and standards;
– Provision of consultancy services and training in standardization.

Thus, in Romania there are several technical committees aimed at standardization of different fields (CT committees). For example, *CT 95 - Food products and methods of analysis* (EuroIMM) is the Romanian technical committee to perform standardization of:

– methods of microbiological analysis and sampling;
– terminology;
– guidelines for food and biological products in terms of production, processing, packaging, labeling and storage.

International technical committees on agriculture and food are ISO committees. In the area of foodstuffs there is TC 34 / SC 6 Committee to perform standardization for meat, chicken, fish, eggs products and their derivatives.

In order for a "Recommended International Standard" to be agreed it must pass through a complicated 10-step procedure. Thus, a typical standard document will include the following:

1. *Name of standard* - should be clear and concise, and should normally be the common name by which the commodity is known;
2. *Scope* - should contain a clear statement as to the food or foods to which the standard is applicable;
3. *Description* - should contain a definition of the product, with an indication of the raw materials, processing, types and styles, and form of pack;
4. *Essential composition and quality factors* - should give detailed quality specifications of all controllable quality factors, with tolerances where appropriate, e.g. odor, flavor, texture, size designation etc.;
5. *Food additives* - should give names of additives permitted and, where appropriate, maximum amounts permitted;
6. *Contaminants* - may highlight special problems. Should refer to the acceptable limits for contaminants;
7. *Hygiene* - the product should be prepared in accordance with the appropriate sections of the General Principles of Food Hygiene as recommended by the Codex Committee on Food Hygiene;
8. *Weights and Measures* - should give minimum total fill and minimum drained weight;
9. *Labelling* - should be in accordance with the "Recommenced International General Standard for the Labelling of Pre-packaged Foods";
10. *Methods of analysis and sampling* - all

methods should be endorsed by the Codex Committee on Analyses and Sampling.

STANDARDS REVIEW FOR FROZEN BEEF MEAT

1. Determination of total ash (ISO 936/1998)
Scope: the determination of the total ash;
Definition: total ash from meat and meat products mass of the residue obtained after incineration at a temperature of (550 ± 25)°C under the operating conditions specified in this International Standard, divided by the mass of the test portion;
Principle: a test portion is dried, carbonized and then incinerated at (550 ± 25) °C. After cooling, the mass of the residue is determined.

2. Determination of nitrogen content (ISO 937/1978)
Scope: determination of the nitrogen content of meat and meat products.
Definition: the quantity of nitrogen corresponding to the ammonia.
Principle: digestion of a test portion with concentrated sulfuric acid, using copper sulfate as a catalyst, to convert organic nitrogen to ammonium ions; alkalisation, distillation of the liberated ammonia into an excess of boric acid solution, titration with hydrochloric acid to determine the ammonia bound by the boric acid, and calculation of the nitrogen content of the sample from the amount of ammonia produced.

3. Determination of moisture content (ISO 1442/1997)
Scope: determination of the moisture content of meat and meat products.
Definition: loss in mass obtained under the operating conditions specified in the standard, divided by the mass of the test portion. Moisture content is expressed as a percentage of mass.
Principle: through mixing of the test portion with sand and drying to constant mass at 103°C ± 2°C.

4. Determination of total fat content (ISO 1443/1973)
Scope: determination of the total fat content of meat and meat products.
Definition: total fat content as a percentage of mass.
Principle: boiling of the test free the occluded the resulting mass, light petroleum, of portion with dilute hydrochloric acid to and bound lipid fractions, filtration of drying, and extraction with n-hexane or the fat retained on the filter.

5. pH value (ISO 2917/1999)
Scope: pH value of all kinds of meat and meat products, including poultry, applicable to products which may be homogenized and also to non-destructive measurements on carcass meat, quarters and muscles.
Definition: result of measurements performed in accordance with the procedure specified in this International Standard.
Principle: the potential difference is measured between a glass electrode and a reference electrode, which are placed in a sample or a sample extract of the meat or meat product.

6. Determination of chloride content (ISO 1841-1/1946)
Scope: determination of the chloride content, with sodium chloride contents ≥ 1.0 % (m/m).
Definition: total chloride content expressed as sodium chloride as a percentage of mass.
Principle: extraction of a test portion with hot water and precipitation of the proteins. After filtration and acidification, it adds of an excess of silver nitrate solution to the extract, and titration of this excess with potassium thiocyanate solution.

7. Determination of total phosphorus content (ISO 2294/1974)
Scope: determination of the total phosphorus content of meat and meat products.
Definition: the phosphorus content, expressed as a percentage of mass of phosphorus pentaoxide.
Principle: mineralization of a sample with sulfuric and nitric acids. Precipitation of the phosphorus as quinoline phosphomolybdate. Drying and weighing of the precipitate.

8. Determination of the sensorial characteristics of frozen beef meat (ASTM E1871/2010, ASTM E1885-04/2004)
Scope: sensory evaluation of beef meat (fresh, refrigerated and frozen).

Definition: standards show the protocol for sensory evaluation, sensorial descriptive analysis, standard test method for sensory analysis—triangle test.

STANDARDS REVIEW FOR FROZEN FISH

1. Standard for quick frozen blocks of fish fillet, minced fish flesh and mixtures of fillets and minced fish flesh (Codex Stan 165/1989)
Scope: applies to quick frozen blocks of cohering fish flesh, prepared from fillets or minced fish flesh or a mixture of fillets and minced fish flesh, which are intended for further processing.
Definition: quick frozen blocks are rectangular or other uniformly shaped masses of cohering fish fillets, minced fish or a mixture thereof, which are suitable for human consumption, comprising single or mixture of species with similar sensory characteristics. Fillets are slices of fish of irregular size and shape that are removed from the carcass by cuts made parallel to the backbone and pieces of such fillets, with or without the skin. Minced fish flesh used in the manufacture of blocks is particles separated skeletal muscle, free from bones, viscera and skin.
Principle: the freezing process shall be carried out so the temperature of maximum crystallization is passed quickly. The quick freezing process shall not be regarded as complete unless and until the product temperature has reached -18°C or colder at the thermal center after thermal stabilization. The product shall be kept deep frozen so as to maintain the quality during transportation, storage and distribution.

2. Standard for quick frozen finfish, uneviscerated and eviscerated (Codex Stan 36/1981)
Scope: frozen finfish uneviscerated and eviscerated.
Definition: frozen finfish suitable for human consumption, with or without the head, from which the viscera or other organs may have been completely or partially removed.
Principle: the product, after any suitable preparation, shall be subjected to a freezing process carried out in such a way that the range of temperature of maximum crystallization is passed quickly. The quick freezing process shall not be regarded as complete unless and until the product temperature has reached -18°C or colder at the thermal center after thermal stabilization. The product shall be kept deep frozen so as to maintain the quality during transportation, storage and distribution.

3. Standard for quick frozen fish sticks, fish portions and fish fillets (Codex Stan 166/1989)
Scope: quick frozen fish sticks (fish fingers) and fish portions cut from quick frozen fish flesh blocks, or formed from fish flesh, and to natural fish fillets, breaded or batter coatings, singly or in combination, raw or partially cooked and offered for direct human consumption without further industrial processing;
Definition: A fish stick (fish finger) is the product including the coating weighing not less than 20 g and not more than 50 g shaped so that the length is not less than three times the greatest width. Each stick shall be not less than 10 mm thick. A fish portion including the coating may be of any shape, weight or size. Fish sticks or portions may be prepared from a single species of fish or from a mixture of species with similar sensory properties.
Fillets are slices of fish of irregular size and shape that are removed from the carcass by cuts made parallel to the backbone and pieces of such fillets, with or without the skin.
Principle: the product shall be subjected to a freezing process carried out in appropriate equipment in such a way that the range of temperature of maximum crystallization is passed quickly. The quick freezing process shall not be regarded as complete unless and until the product temperature has reached -18°C or colder at the thermal center after thermal stabilization. The product shall be kept deep frozen so as to maintain the quality during transportation, storage and distribution.

4. Determination of total inorganic and organic mercury (Codex Alimentarius 7-1991, EC Regulation 221/2002)
Scope: fish and fishery products can be infested with mercury, from the pollution agents in oceans and seas.

Principle: The tissue sample is digested at 100°C using a 45 % NaOH solution containing cysteine as a mercury binder(EC, 12.05.2004). .

5. Determination of the organoleptic characteristics of frozen fish (ASTM, 1983, STAS 9736-85)

Scope: establishes the technical conditions that frozen fish (entire, eviscerated, non-eviscerated or portioned) must comply before putting on the market.

Definition: standard shows the protocol for evaluation of the aspect, color, smell, aroma, consistency and texture for frozen fish.

Sanitary veterinary norm that establishes further requirements on sanitary veterinary control of frozen fishery products intended for direct sale to the final consumer (SVN, 9.11.2004)

– The products weighing over 3 kg are frozen individually. The products weighing less than 3 kg are frozen in a form of coated briquettes. The packaging must bear a label stating assortment, species, and date of freezing. Fresh frozen fish can be kept for 6 months in cold storage rooms at -18 °C, and for 10-15 days at a temperature of -12 °C;

– When the frozen products are to be sold at a temperature that will not maintain the preservation, the label will state clearly and prominently that the product has been defrosted, with respect to the official labeling and advertising related to food (Seafood Edu-online).

CONCLUSIONS

Standardization is an important criteria for assessing the progress made by Romania in the integration process to the European Union. Although the compliance with standards is not mandatory (the standards are not laws but rules, guidelines and characteristics for activities and their results), "*being in compliance*" certifies that the products, services and processes "in compliance" have the best quality while delivering the highest possible safety levels to the consumers (EC Reg.1169/2011).

The acceptance of European standards as national standards in EU countries aims primarily to fade away of technical barriers to trade, so the countries will not deal with different requirements for the same products. The implementation of European standards helps to achieve the main principle of the European single market, namely the free movement of goods and services.

To meet these criteria, over 80% of European standards were adopted into the national legislation. Therefore, in Romania, in recent years, standardization activity was focused on the adoption of international ISO standards (especially the European standards) as EN Romanian standards.

While it is likely that national and European standards will always coexist in Europe, it is estimated that, over time, national standards of a Member State will be reduced to a share of 10% of all standards (EuroIMM).

ACKNOWLEDGEMENTS

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DIY WATER FILTERING MODULE FEATURING AN AUTOMATED C&C UNIT ON REVERSED AQUAPONIC SYSTEMS

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Abstract

The goal of the project was to design and build a self-sustaining herbs production system in an applied research environment based on an ornamental aquarium as a nutrient source for plants. The solution was to setup an ornamental aquarium, which, on the next stage of the project, became part of an aquaponic system where the required herbs are grown. The novelty emerged from the specific requirements of the project, which led to a build of a reversed aquaponic system, on which the fish tank is positioned above the grow bed. Designing a new Command & Control Unit surpassed the technical issues that emerged due to the lack of conformity with a "traditional" aquaponic system. A new Water Filtering Unit was also designed in compliance with project requirements. The paper will present the general DIY ("Do It Yourself") steps to build the Command & Control and Water Filter units for a reversed aquaponic system. The Water Filter Unit build requires no special tools or skills, so anyone can replicate it. On the other hand, the Command & Control Unit requires some special skills as automation electrician, so professional help may be required in order to replicate this particular unit.

Key words*: aquarium, command & control unit, DIY, reversed aquaponic system, water filter unit.*

INTRODUCTION

An aquaponic system is a symbiotic closed-loop recycling water system that combines the best of hydroponics and aquaponics in a way to create a closed, self-sustainable, dirt-free, weed-free and chemical-free grow system for fish and plants. In such a system the wastes generated by fish such as urine, ammonia and decomposed fodder (Nicolae, 2007) are converted by nitrifying bacteria into forms that plants can accept in their nourishment processes, thus, acting as biofilters by cleaning the water before being sent back to the fish tank (Figure 1).

The Recirculation Aquaculture Systems (RAS) started in the 1950s in Japan and was introduced experimentally in Europe in the 1970s. The commercial utilization of aquaponics started in Northern Europe, especially in the Netherlands, Denmark and Germany only as early as 1980s. A special note goes to the paper on aquaponics published by Watten and Busch in 1984 (Connolly and Trebic, 2010). Today, this new innovative agriculture technology is widely adopted in countries in America, Australia and Middle East, even if different issues are still raised, such as the use of fishmeal and oil as feed ingredients. Unfortunately, the number of aquaponic implementations is still scarce in Europe.

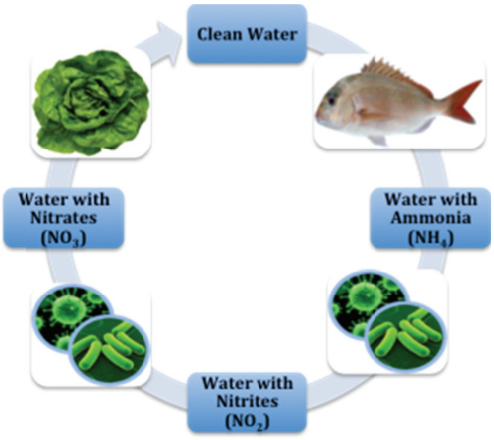

Figure 1. How aquaponic systems work

However, establishing a successful aquaponic system is less about fish and plants and more about "growing" the "good bacteria". It is so because the "good bacteria" will perform those

chemical reactions in the water in order to transform the compounds which are harmful for the fish and not usable by the plants into compounds which are harmless for the fish and usable for the plants, process known as "nitrification" (process which is part of the global nitrogen cycle). The process of luring the "good bacteria" and help it to establish viable colonies (widely known as "system cycling") is critical for any aquaponic system.

In terms of business, there are two major elements that define an aquaponic system: the fish and the plants. Fish can be grown in monoculture or in polyculture systems, based on its feeder habits: an algae feeder fish (as Tilapia), a benthic feeder fish (like Carp or Catfish), a zooplankton feeder fish (some Chinese Carp species), and so on. Natural and/or artificial food supplies may also be used. Based on the fish species and on the fish fodder, different growing scenarios will be available in order to identify which plants are most suitable to be grown on the specific water parameters sets generated by fish and fish fodder. Any existing aquarium can be easily upgraded to a self-sustaining herb production unit (Elia et al., 2014).

Regarding the fish, the most common grown species is tilapia *(Oreochromis niloticus).* However, any species of fresh water fish can be suitable for an aquaponic system as long as a proper fish tank is prepared (dimension wise) and the required environmental conditions are met. The most common cultivated plants are green leafy plants such as lettuce, basil, parsley and mint. There also have been cultivated tomatoes, cucumbers, cabbage, kale, celery, eggplant and okra but the income obtained from the herbs is much higher and therefore those are preferred (Rakocy et al., 2006; Connolly and Trebic, 2010).

In this context, the paper present the general DIY ("Do It Yourself") steps to build the Command & Control and Water Filter units for an ornamental aquarium turned into a full fledged *reversed* aquaponic system, an implementation with the fish tank suspended one meter above the floor AND above the grow bed (Nicolae et al., 2015).

MATERIALS AND METHODS

To assess the water parameters, the following indicators were used: water temperature, pH value and nitrogen concentration (ammonia, nitrites and nitrates).

During the period of system cycling, water temperature, pH value and nitrogen concentration (NH_3/NH_4^+, NO_2^-, NO_3^-) were daily assessed. While water temperature and pH value were determined by direct observation (using a thermometer and a commercial test kit), nitrogen concentrations were determined by using spectrophotometric analysis of water probes (Hodoșan, 2014).

This method requires measuring the intensity of light as a beam of light passes through the probe, knowing that each chemical compound absorbs or transmits light over a specific and known wavelength. The concentration values of nitrites and nitrates are then determined from the benchmark curves built out of the extinctions shown by spectrophotometer. The water assessment was carried out between December 2014 – January 2015.

The aquarium was built out of tempered glass, using silicone to harden and seal the joints. A sturdy aquarium stand was also built out of metal bars to withstand a total weight of 500 kg.

The Water Filter Unit build required no special tools or skills, so anyone can replicate it. On the other hand, the Command & Control Unit required some special skills as automation electrician, so professional help may be required in order to replicate this particular unit and to avoid electrical hazards (Figure 2).

Figure 2. Tools, C&C Unit and Piping elements

RESULTS AND DISCUSSIONS

The Water Filter Unit

The Water Filter (Figure 3) is an innovative system that acts like a two levels *whirl settlement tank* AND *biofilter*.

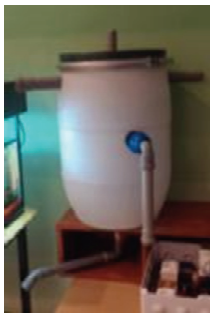

Figure 3. The Water Filter Unit

It was actually made from a 60 liters plastic barrel, provided with a custom piping system to allow water to flow in from the fish tank, flow out to the grow bed, and to collect the sediments. It also acts as housing for the biofilter, which was made out of a 12 liters plastic bucket. From the fish tank, the water with settlements is pushed by an electric pump, through a plastic hose, into the admission pipe of the Water Filter. The admission pipe release the water through a whirl system placed into the plastic bucket, located above its middle section (Figure 4).

Figure 4. The Whirl System

The water (while spinning around the round body of the bucket) eventually will gravity-fill up the lower section of the bucket, then, from bottom to top, through a sieve, will fill up the whole bucket. The larger solid particles in the water sink down to the bottom of the bucket and stay there. The residual water is collected once a day (Figure 5) through a pipe going from the bottom of the bucket, through the barrel, and fitted with a tap. *The residual water makes a very good fertilizer and is not to be disposed of.*

Figure 5. The Residual Water (left probe)

The sieve at the middle of the bucket holds the layers of inert plastic bio-ribbon which the nitrogen converting bacteria affix to, acting as biofilter (Figure 6).

It is the most important part of the aquaponic system, since most of the nitrification process occurs within it.

Figure 6. The Biofilter

The biofilter is held in place by a second filter for smaller solid particles (made out of sponge), and a lid with openings to let the water flow out of the bucket. The bucket is placed 10 cm above the bottom of the barrel (to

avoid some sealing issues), centered by a shaft made out of plastic pipes (Figure 7).
Flowing out from the bucket, the water starts to fill up the barrel until its overflow level is reached. Through the overflow and by a pipe system, the water reaches the grow bed. Upon filling the grow bed, the water flows into a collector tank. In the collector tank, when the water reaches a certain "High" level, a "smart" electric drain pump starts to push the water from the collector tank to the aquarium.

Figure 7. The Biofilter, with the lid on

The drain pump only stops when a preset "Low" level is reached. The "High" and "Low" levels are determined by using a floating micro-switch which, based on its horizontal or vertical alignment, closes or opens two electric circuits.

The Command and Control Unit

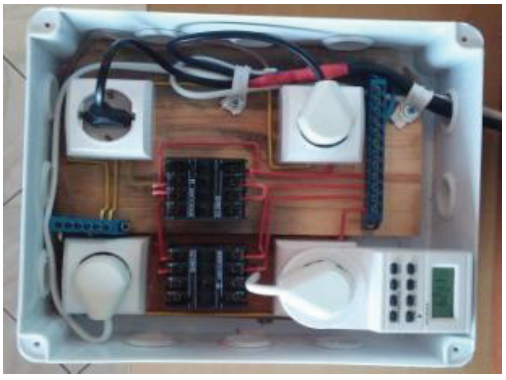

Figure 8. The Command & Control Unit

The characteristics of the C&C Unit controls (Figure 8) are:

- by means of a timer, the electric water pump placed in the fish tank;
- the electric water pump placed in the collector tank, powered according to the water level in the collector tank: as long as the water is below the "High" level, the pump is switched off. When the water reaches the "High" level, the pump starts to drain the collector tank and remains active until the water reaches the "Low" level. The "Low" level should be established is such a manner so the water pump will always be submerged.

CONCLUSIONS

The costs of such a system are very low and no special skills or tools are required. More than that, even the most expensive element of the system (the filtering unit) can be homemade.
This is a way for urban people to get closer to the nature, and, with virtually no production costs, to always have in their kitchen fresh herbs straight from the grow bed.

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COMPARISON OF BAYESIAN ESTIMATION AND CLASSICAL ESTIMATION OF BRUSHTOOTH LIZARDFISH (*Saurida lessepsianus* RUSSELL, GOLANI & TIKOCHINSKI 2015) GROWTH

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Abstract
The aim of this study is to compare Bayesian and Classical estimation for describing the growth curves of Brushtooth Lizardfish (Saurida lessepsianus Russell, Golani & Tikochinski 2015). Classical nonlinear regression method and Bayesian estimation method were used to obtain the estimation of the components of the von Bertalanffy growth model. The estimated parameters of the von Bertalanffy equation via two methods showed that Bayesian estimation is much better than Classical nonlinear regression in estimating growth parameters and reducing variation of growth model parameters.

Key words*: Bayesian inference, Saurida lessepsianus, Growth, von Bertalanffy.*

INTRODUCTION

In the fisheries study, fish growth data fitted by a suitable mathematical function to describe the growth, estimate parameters of growth, and compare the growth models between species or population. The shape of the growth line may change according to the genotype of living organisms, environmental factors and examined features. So, what is effect of statistical method on expression of the growth that is affected by all these factors? Answer of this question is the most important topic of finding best fitting model.

Historically, the von Bertalanffy growth equation (VBGE) has been the most common growth functions applied to fish growth in fisheries science (Ricker, 1975; Pauly, 1978; Chen et al., 1992; Helidoniotis et al., 2011). The VBGE is usually used in population dynamics and fisheries management to model individual growth of a species. The VBGE was obtained by thinking the growth of an animal because of the difference between catabolic and anabolic processes of an animal's metabolism (von Bertalanffy, 1957; Ursin, 1967; Sainsburry, 1980; Pilling et al., 2002). Generally, the VBGE is fit to length-age data using classical nonlinear least square techniques. Classical nonlinear regression assumes that there are enough measurements to say something meaningful. This somehow affects the assumptions of the VBGE. In the Bayesian approach, the data are supplemented with additional information in the form of a prior probability distribution. The prior belief about the parameters is combined with the data's likelihood function according to Bayes theorem to compute the posterior (Box and Tiao, 2011; Congdon, 2003; Siegfried and Sanso, 2006; Link and Barker, 2010; Akar and Gundogdu, 2014).

For this reason Bayesian inference provide a quantitative concept and obvious language in so as to analyze and express growth procedures. Logically, Bayesian inference is the clearest way of analyzing and interpreting growth models in light of data.

In this study we used Bayesian approach and classical approach to estimate VBGE parameters. Estimates of the von Bertalanffy growth parameters are compared with estimates Classical method. By this way, we tried to explain biological plausibility of parameters estimated by both methods.

MATERIALS AND METHODS

Length and age data of Brushtooth Lizardfish (*S. lessepsianus*) were collected between 2012 and 2013 from Iskenderun Bay of the Northeast Mediterranean Sea (Figure 1). The materials were collected by seasonally sampling using commercial bottom trawl. The

fork length (FL) was measured to the nearest 1 mm. The sagittal otoliths were examined under the stereo microscope for the age determination.

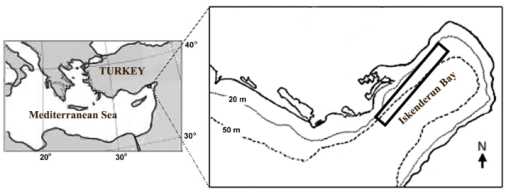

Figure 1. Study Area

Total of 400 individuals were sampled, ranging in size from 13 to 28.8 cm FL. Overall mean FL was calculated as 17.83 cm. Length-frequency distribution was given in Figure 2. As can be seen in the figure 2, the dominant length classes were 13-17 cm. Length-frequency distribution, minimum, maximum length, standard error sample size, mean length and its confidence interval values of *S. undosquamis* for each age class are listed in Table 1. As it can be seen, the age of *S. undosquamis* ranged from I to VI age classes and the most dominant age class was 2 with a value of 35.2% and age class 3 ranks second with a value of 25.2%.

The form of VBGE described by many researchers is the following;

$$L_t = L_\infty\left(1 - e^{-K(t-t_0)}\right) + \varepsilon_t$$

where t_0, L_∞ and K are the VBGE parameters, and the ε_t are assumed to be normally distributed error. The growth parameters t_0, L_∞ and K were estimated using the Classical Least Squares Method as recommended by Sparre and Venema, (1998). This produced least-squares estimates of the three von Bertalanffy growth parameters.

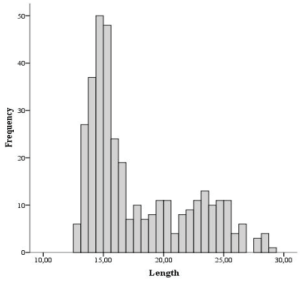

Figure 2. Length-frequency distribution

Table 1. Length-frequency distribution, minimum, maximum, standard error, 95% confidence interval and mean fork length values for each age class for Brushtooth Lizardfish

Age Class	N	Mean	Std. Error	95% Confidence Interval		Minimum	Maximum
				Lower	Upper		
1	80	13.71	0.04	13.63	13.81	13	14.2
2	141	15.29	0.05	15.17	15.41	14.4	16.8
3	101	20.53	0.21	20.11	20.95	17	23.9
4	32	24.47	0.06	24.34	24.61	24	25
5	28	25.91	0.14	25.61	26.21	25.1	26.8
6	18	28.26	0.18	27.83	28.69	27.5	28.8
Total	400	17.83	0.22	17.39	18.27	13	28.8

Bayesian approach fits the VBGE to the length at age data using Markov Chain Monte Carlo (MCMC) methods (Hastings, 1970; Gelman et al., 2004). These methods has four steps; i) Finding likelihood of the data, ii) Defining priors for all parameters, iii) Defining conditional probabilities for all parameters, and iv) Using the Bayesian method to estimating the posterior distribution for parameters (Gelman et al. 2004; Siegfried and Sanso 2006). Thus, our likelihood is as in the following:

$$L_t \sim dnormal(\mu_i, \tau)$$
$$\mu_i = L_\infty\left(1 - e^{-K(t-t_0)}\right)$$

We used informative priors for t_0, L_∞ and K based on published estimates of the same parameters in the FishBase (Froese and Pauly 2012) for *S. lessepsianus*

$$L_\infty \sim normal(40, 64.10)$$
$$-(t_0) \sim gamma(0, 265.10)$$
$$K \sim gamma(0, 83.10)$$

We used uninformative priors for τ, giving the full power of estimation to the data:

$$\tau \sim gamma(0.0001, 0.0001)$$

OpenBUGS (**B**ayesian inference **U**sing **G**ibbs **S**ampling; Thomas et al., 1992; Spiegelhalter et al. 2007; http://www.openbugs.net) was used to fit the model. The estimates of parameters were evaluated based on 1000000 samples, from Markov chain Monte Carlo (MCMC) simulation of the joint posterior distribution. We used a burn-in period of 10000 chains and generated posteriors for the parameters of the VBGE with the remaining chains.

RESULTS AND DISCUSSIONS

The growth parameters calculated by classical nonlinear regression were L_∞: 48.86 cm, K: 0.107 year^{-1} and t_0: -1.733 year (Table 2). The back-calculated lengths were determined by using von Bertalanffy growth parameters and both the observed and calculated growths in fork length are listed in Table 3. Growth curve was fitted to lengths-age for *S. lessepsianus* is showed in Figure 3. The growth parameters calculated by Bayesian nonlinear regression were L_∞: 30.62 cm, K: 0.3086 year^{-1} and t_0: -0.3046 year (Table 2). Growth curve was fitted to lengths-age for *S. lessepsianus* is showed in Figure 3.

Table 2. Parameter estimations of both methods

Method	Parameter	Mean	Std dev	Credible Interval	
				%2.5	%97.5
Bayesian Approach	K	0.3086	0.0880	0.1724	0.4599
	L_∞	30.620	5.2520	25.160	40.000
	t_0	-0.3046	0.2796	-0.8674	-0.0051
	$1/\sigma^2$	80.500	2.9500	24.460	82.400
	σ^2	0.0121	0.0195	0.0012	0.0408
Classical Approach	L_∞	48.868	11.757	25.763	71.792
	K	0.107	0.035	0.017	0.156
	t_0	-1.733	-1.719	-3.135	-1.103
	RSE	2.800	0.851	-	-

Estimates of L_∞ of Bayesian approach for *S. lessepsianus* were much closer to observed maximum length than Classical approach. The coefficient of K in result of both approaches was between 0 and 1. However estimate K of Bayesian approach higher than Classical approach and estimate of t_0 of Bayesian approach much closer to zero than Classical approach. As we see in Fig. 3 Growth is fast until the age class II and with growth in length is slightly reduced beyond the age class II. Correlation between parameters was found $cor(L_\infty, K) = -0.78$, $cor(L_\infty, t_0) = -0.18$ and $cor(K, t_0) = 0.57$ in Bayesian approach. Note that K and t_0 decrease as L_∞ increases.

Performance of the Bayesian growth model was verified based on 1000000 samples generated from MCMC simulation. A "burn in" sample of 10000 was initially rejected and the remaining 1000000 iterations from the chain sequence thinned at a rate of 1 sample in 10 (for removing autocorrelation of MCMC). Autocorrelation of the chain diminished after a lag of about 10. Since shape posterior density affects interpretation of parameters, it is necessary to take into account of shape of density graphs (Box and Tiao, 2011). The posterior distribution plots of each parameter given in Figure 4, L_∞ and K is distributed symmetrically around mean, estimates of t_0 close to zero with a long tail to the left.

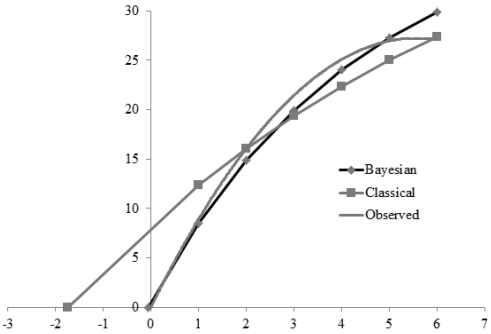

Figure 3. Bayesian and Classical von Bertalanffy length-at-age growth curve for *S. lessepsianus*

Since the growth function may vary, the shape of the growth curve for fishes may vary between populations or species also. Therefore it is essential to assess the goodness of fit in any comparison among approaches. The biggest difference in growth curve between this two approaches and the result of the estimation of VBGE parameters indicate that Bayesian approach biologically more plausible than Classical approach. According to many authors (Pauly, 1978; Chen et al., 1992; Sparre and Venema, 1998), L_∞ should be reasonably close to the maximum fish length in observed data, t_0 should be smaller or equal to zero and, K might vary between 0 and 1.

Results of Bayesian approach shows that estimate of L_∞ much more closely than Classical approach to maximum observed length (28.8 cm) and estimate of t_0 almost equal to zero (Table 2, Figure 4).

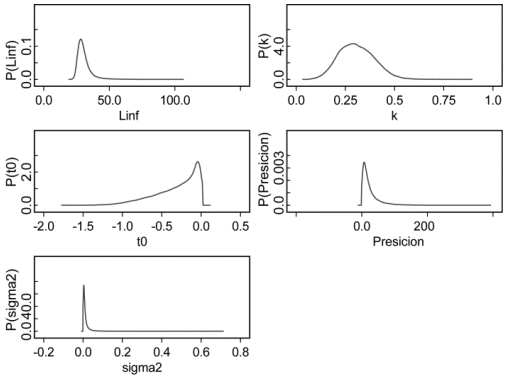

Figure 4. Kernel density histogram for the von Bertalanffy growth parameters L_∞, K, t_0, variance and precision and drawn from 100000 MCMC samples of the Bayesian brushtooth lizardfish growth model

When it is compared Bayesian estimation to previous studies, in this study, Bayesian approach produce biologically more plausible estimations (Table 3).

Table 3. Growth parameter estimates of *Saurida lessepsianus* from previous study

L_∞(cm)	K(year^{-1})	t_0(year)	Autor(s)
22.43	0.597	-1.365	Türeli and Erdem (1997)
41.27	0.118	-1.895	Çiçek (2006)
42	0.51	-0.29	Gökçe et al. (2007)
38.05	0.124	-1.680	Çiçek and Avşar (2011)
41.57	0.118	-1.895	Manaşırlı et al. (2011)
30.62	0.308	-0.304	This Study (Bayesian)
48.86	0.086	-2.119	This Study (Classical)

According to Pauly, (1978) and Sparre and Venema, (1998), estimated parameters of VBGE should be related to biological characteristics of inspected species. Since *S. lessepsianus* is a demersal fish, it is necessary to have lower K value. As it can be seen in Table 3, there are no similarities for the estimated K values between this study and reported by other studies from Turkish coast. When we consider the estimated value of t_0, in this study, Bayesian estimation of t_0 much closer to 0 than all other previous studies given in Table 3. It is identified with the nature of Bayes theory. Since Bayesian approach takes into account of prior knowledge, this minimizes difference between real value and estimation (Box and Tiao, 2011; Lee, 2004). According to Sparre and Venema, (1998), because of the growth begins at hatching when the larva already has a certain length, biologically, t_0 has no meaning. For this reason, value of t_0 should be zero or so.

The usage of prior knowledge in Bayesian approach may have effect on precision. When we consider that precision parameter shows the possibility of deviation of estimation from real value, low variance of Bayesian approach (0.01) makes it more preferable than classical approach (Helser and Lai, 2004; Helser et al., 2007). We can also compare with Bayesian and classical approach with considering variance. As it can be seen in Table 2 variance of Bayesian approach is lower than Classical approach.

Since growth has correlation to reproduction and survival of fish and its wide usage in fisheries population dynamics, it is one of the most important life history traits of fishes (Beverton and Holt, 1957; Ricker, 1975; Beverton, 1992; Helser and Lai, 2004). Von Bertalanffy growth model is most often estimated using the Classical approach. Comparatively fewer studies have reported quantitative comparisons of various statistical procedures. Generally, this is because popularity and simplicity of statistical methods. The methods presented in this study based on a nonlinear Bayesian growth model of brush tooth lizard fish growth clearly demonstrate that analysis of two methods simultaneously is not a limitation as discussed below.

CONCLUSIONS

Main conclusion from this study of Brushtooth Lizardfish growth is that suitable statistical methods can be used to assess growth parameters. Our use of nonlinear analysis using Bayesian inference for fish growth has the advantage of biological plausibility. If new sample are collected in the future, results of this study can provide an informative and plausible prior.

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FATS IN PRODUCTS FEED SUPPLEMENTS AND EFFECT ON RETENTION OF PROTEIN IN NILEM FISH (*Osteochilus hasseltii*)

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Abstract

Nilem fish (Osteochilus hasseltii) has a great potential as product of baby fish (three months old), and fish eggs (caviar) and that is now introduced in the floating cages started nursery phase. Optimization of cultivation through input-protein energy efficient as well as enrichment efforts fatty acids in the diet is very urgent. This study aims to get the kind of fat supplement of alternative materials which added to the feed to improve growth and protein retention in nilem fish reared in Cirata. The research was conducted in two stages: (1) Phase 1, scale up alternative sources of fats from hazelnut and meal worm (Tenebrio molitor); added in the feed formulation; (2) Phase 2, growth, feed conversion, and retention of protein on fish meat. The experimental design in biological assay using a completely randomized design (6x3); with standard ration treatment (Rs), then Rs with the addition of animal fat supplements (meal worm) and vegetable oils (hazelnut) in the form of flour and oil extraction, as well as a commercial feed controls. The results showed that: 1) Yield of pure oil of hazelnut and meal worm results flouring solvent extraction followed respectively by 27.3% and 12.15% of the fresh weight. 2) Feeding for four weeks showed that growth rate between 3.72 g - 5.82 g, with meal worm feed supplements 2% was highest absolute growth rate, and no significant with high protein on control, and so was hazelnut 2%. 3) Retention of protein obtained in supplement worm oil and hazelnut oil 1%, respectively 33.42 and 33.46%.

Key words: *fat supplements, growth rate, retention of protein, nilem fish.*

INTRODUCTION

The main feed materials are often used in fish feed is fish meal which has undergone the process so that the pressing and drying fish oil out. Until now, fish oil is a major supplier of the fatty acids ω3 good for fish cultivation and for human consumption of the fresh especially fish herbivore / omnivore like fish nilem able to synthesize omega-3 (EPA and DHA) fatty acids C-18, so it does not depend on the fish oil and fish meal in feed.

Sources of fat used as a supplement in unsaturated fatty acids other than fish oil is beef tallow, linseed oil and corn oil. Because these oils are generally imported it is necessary to look for another alternative feed ingredients based on local ingredients. Alternative sources of fatty acids that potentially are pecan seeds, and larvae of *Tenebriomolitor*. Pecan seed (*Alleurites mollucana*) is known as one of the original Indonesian spice recommended as a source of unsaturated fatty acid linseed oil substitute for part of the fruit (seed) oil content of 55-65%, and oil content in the shell by 60%.Based on this background, it is very important to do research on the utilization of fat supplements exploration results from the fatty acid source alternative materials in order to improve the growth performance, feed conversion of nilem fish and their effects on fish meat protein retention.

The purpose of this study is to determine the type of fat (vegetable and animal fat) best in fish feed formulations on fingerling of nilem fish in the floating net Cirata effect on growth, feed conversion, and protein retention in meat of nilem fish.

MATERIALS AND METHODS

Materials research was pecan seed and mealworm (*Tenebrio molitor*), nilem fish, feed raw materials and commercial feed,and floating net cages. The chemicals used include chemicals for extraction and chemicals for proximate analysis (protein, fat components, energy, ash, crude fiber). The treatment is based on the addition of a fat source (flour and oil) from animal and vegetable fat source in artificial feed. The feed material consists of fish meal, soybean meal and white bran.

The study consisted of two phases: separation and multiplication of feed supplements of fat extraction, analysis of nutrient composition, and formulation of nilem fish feed. The second stage was the Feeding Trial on nilem fish to obtain type supplement(flour and oil extract). Fat supplement added to low protein feed (20%), to see the value of the benefits and efficiency by measuring the growth, feed conversion continued the third phase measurement of protein retention in nilem.

Research procedure

The process of making fat supplements includes the following stages:

1) Preparation of materials including water level mealworm and hazelnut oil, performed with the following stages: solving or downsizing, then drying, followed by immersion in an organic solvent, then extraction and evaporation. Mealworm turn off the process by means of watering in hot water of 80°C is also intended as steaming process (provision of hot steam), then performed the drying process at a temperature of about 80° C and to reduce discoloration done by flipping back the biomass of mealworm as often as possible. Hazelnut is *Euperbiaceae* plant seeds, which initially had a hard seed should undergo the process of solving (crushed) shell beans, in order to obtain a yellow pecan seed round and intact.Formulation and manufacture of fat-supplemented feed for fish nilem stadia enlargement. The composition of the basal feed ingredients were Soybean 10%, rice bran 40%, fish meal 8% flour 5%; White bran 34%, and vitamin mix and mineral mix, respectively 1.5% . The addition of sources of fat in the form of flour by 2%, while in the form of oil by 1%, respectively mixed into the basal ration of raw materials, and made into pellets.
2) Biological Test Phase (feeding trials) include:
   a. Stage adaptation of fish to the container trial and media maintenance,
   b. Stage adaptation of fish to feed the test, for 1 week, and the determination of the number of feeding.
   c. Stage of collecting and recording data growth during maintenance, carried out for a month (30 days). Measurement of feed consumption, sampling body weight gain and feed conversion is done weekly maintenance.
3) The fish meat from fish sampling dissected, separated and in fillets, then weighed and the fresh weight aside into wrap clipping and temporarily stored in the refrigerator, and then analyzed the protein content.

The study was conducted experimentally by using a completely randomized design of six treatments and three replications. The treatment is based on the addition of fatty acids (from vegetable oils and fat animal), namely:

Feed A: basal feed (standard ration) protein 20%;
Feed B: Feed basal + 2% hazelnut flour;
Feed C: basal feed + 2% flour mealworm;
Feed D: basal feed + 1% bv/bw hazelnut oil extraction;
Feed E: Feed basal + 1% bv/bwmealworm oil extraction;
Feed F: commercial feed (ration control).

Treatment effect was tested by F test and statistical analysis to determine the differences of each used Duncan Multiple Range Test (Steel and Torrie, 1980).

RESULTS AND DISCUSSIONS

The results of the crude protein and extract ether analysis were 19% and 55% for hazelnut meal and 55.11% and 15.51% for mealworm. The results obtained by the mechanical extraction of materials for seed yield hazelnut 60%, while the mealworm 70% from fresh

weight. The reduced weight of the material ingredients of feed supplement is caused mainly by the loss of moisture, lost in the drying process and flouring. However, pressing and flouring still needed to gain fat. The components of macromolecules nutrients, valuable biological contents of cells located inside the plant so that treatment to remove the contents of a cell of the plant cell walls necessary, by breaking or damaging the cell walls so that the desired components can be taken. Cooking on mealworm through flouring and extraction is also intended to improve the digestibility of theshell mealworm and should be done peeling shell/skin from the body parts. The analysis showed that the fat content is greater hazelnut flour at 55%, compared with 15.51% fat mealworm. Protein content of mealworm greater than fat's. Inseparability of fat in the extraction process either mechanically or chemically called leaching (washing). In extraction plants, extraction or leaching is a process that is preceded by contact between the phases followed by diffusion of solution phase (solute) from the solid phase and liquid phase, so that the components are dissolved.

From the research results to the manufacture of mechanical extraction of hazelnut oil do Estrada (2011), walnut oil in the process of flouring do pressing and generate yield too little hazelnut oil. So pressing mechanical extraction results in this study was not done. To get more fat components followed by solvent extraction process. The results of the analysis of the fat content of grains hazelnut (hazelnut flour) large enough that 55%, with a calorific value of 6260 kcal/ kg., But the yield obtained from the flouring by 60%, so the content of dry hazelnut flour drying by 33%. After going through the stages of mechanical extraction with flouring, followed by solvent extraction stages or chemically using a solvent n-hexane with immersion techniques. According to Hartadi (1986) Steep extracted (extraction immersion) is the treatment of the material (raw material) resources containing fat in water or solvent hexane to remove dissolved materials. The materials are expected extracted substances such as oils, fats, or fatty acids and others more quickly and perfectly.

Table 1. Yield of Oil and Fat after extraction solvents of Hazelnut and Mealworm

	Hazelnut	Mealworm*
Extract ether (%)	55	15.51
Yield flour	60	70
Solvent : material ratio	2 : 1	2 : 1
Yield of extraction	30%	35%
Yield of oil/100 g raw material**)	27.3%	12.15%

Note: *) The results of solvent extraction with hexan solvent using techniques of maceration (soaking 24 hours) the hazelnut flour and mealworm
**)Yield of oil = (fat content (BK100%) x yield x preparation stage solvent extraction yield) of the initial weight (fresh).

Extraction of the fat that comes from animal should be preceded by a process called Rendering, a process that begins with the destruction of body tissues of animals such as bone, fat deposits by heating vacuum wet method, and then to separate the oil component by way of centrifuge (Gurr, 2002). The effectiveness of the process of dissolving fat can be determined from the agitation process, the large number of solvents and solvent type. According to Estrada (2011), on the whole seed extraction, leaching process efficiency is determined by the contact between the solvent and solute-containing solids to be separated. Leching Speed shows the speed rate leching depending on: the size of the particles, the type and speed of solvent flow, and temperature. Solvents are chosen to be selective for the separation of solutes concerned and low viscosity makes it easier to circulate.

The molecular weight was86.18 g/gmol, density 0.6548 g / ml, the freezing point of -95°C, and the boiling point of 69°C. These solvents are not polar, inert so it does not react with the components of oil and the price

is cheap, not flammable and non-toxic. In this study treatment with pressing (result precondition with flouring) continued extraction of oil cake. Hazelnut fatty acids are predominantly oleic which is a component of omega 9 fatty acid (C18: 1W9) similarly mealworm. Although the fat content of hazelnut greater than, the results of this study saphonification number (KOH) fat hazelnut higher, indicating smaller molecular weight. The higher the saphonification of vegetable fat has also been confirmed from the results of research Mathyazhagan (2011) that are generally non-food vegetable oils such as Jathropa, pongamin, containing volatile fatty acids that are quite high. The high content of constituent soap VFA will increase, thus increasing the numbers saponification and susceptible to oxidation. Although the variation of the fatty acid component is quite a lot but the fatty acids they need to be purified and further bindering, as well as additional treatment added antioxidants to improve stability, so that the molecules can be bound in glycerides and not easily evaporate or turn into free fatty acids.

The ratio of the use of polar solvent used in this study is a 2: 1 or 50g / 100ml n-hexane. The ratio of oil cake mass/volume n-hexan conducted to determine the yield (fat produced). It obtained from the extraction of oil produced by flouring stage followed by solvent extraction with a maceration technique using a solvent n-hexan. According to Estrada (2011), to produce a higher oil yield required processing techniques with expeller on the precondition phase and continued with chemical engineering. From the results showed that the yield resulting from stage expeller less but produce better quality oil than the immersion technique. This is because the use of solvent immersion technique more and can be repeated so as to produce greater oil yield.

Oil extraction solvent undergo a process of separation by heating to a temperature of about 70°C, which can cause the oil is oxidized to form aldehydes and ketones and free fatty acids. Besides oxidation also causes the breakdown of the double bonds that lack saturated degree decreases and the iodine numbers to be down. The decline in the degree of unsaturation causes the refractive index decreases. The disintegration of the oil with the oxidation process has caused the amount of triglycerides is reduced, which causes the saphonification is reduced. The time required for the extraction process takes quite a long time so that the solvent can dissolve the solute to reach a point of equilibrium.

In the extraction of fat hazelnut, pressing method only by mechanical means, resulting in a higher oil quality but lower the net yield of oil (yields) than pressing a mechanical way, followed by extraction with a solvent (oil cake) (Estrada, 2011). The decline in oil quality is shown by an increase in free fatty acid content (free fatty acid) and colors as well as a decrease in the number of iodine and saphonification.

Nilem fish used comes from local farmers with the initial weight of about 5 grams. Growth is defined as the change of fish in weight, size and volume in line with the changing times (Effendie 1997). According Djajasewaka and Djajadireja (1980), the growth rate will be different levels depending on the ability to digest and utilize the feed as optimally as possible.Analysis of variance showed that the growth rate of fish nilem treated feed supplements increase by 1-2% indicates real. Feeding with the addition of flour feed supplements mealworm 2% highest absolute growth rate that is equal to 5.82 grams of 4 weeks of the study. Daily growth in each treatment has a value that is not much different and it ranged between 3.72 g-5.82 g.

According to Gurretal (2002) proximate composition of the body can be affected by the fatty acid composition because its permeability properties support the overall cell metabolism. The cell membrane is not only covers the outside of the cells that wrap around the cell, but also covers the outside of the cell organelles such as ribosomes. Cell membrane permeability was influenced by the lipid active role influenced by unsaturated fatty acids compounds such phospholipids (Bellet al.,1986). Changes in the permeability of cell membranes can interfere with the activity of the enzymes o that it does not directly interfere with protein synthesis in cells.

Table 2. Growth absolute and Protein Retention

Treatment	Growth Absolute (g)	Protein Retention (%)
A (Basal Feed)	3.72 a	22.96 a
F (Control)	4.61 b	27.75 b
B (Hazelnut 2%)	4.75 bc	28.73 b
C (Mealworm 2%)	4.80 c	33.19 c
D (Hazelnut oil 1%)	5.28 cd	33.42 c
E (Mealworm oil 1%)	5.82 d	33.48 c

In Table 2 it appears that produced the lowest protein retention in treatment without the fat supplement (Feed A), while the retention of the protein with the addition of hazelnut oil and oil supplements mealworm higher.

Retention of higher protein with the addition of oil showed a positive effect on protein synthesis. The cell membrane permeability of phospholipid that is influenced by lipid active role influenced by unsaturated fatty acids compounds such phospholipids (Bell et al., 1986).

Then were changes in cell membrane permeability that can disrupt the activity of enzymes in mitochondria that are rich in unsaturated fatty acids (Fleischer et al., 1962 in Mokoginta, 1986). If this happens, then indirectly interfere with protein synthesis in cells. These events seen on treatment a deficiency in fatty acids n-3 and n-6, so that the protein content be low at 53.06%.

Ensminger et al. (1990), state that fish that lack essential fatty acids, free water content and body fat will increase, but the protein content will decrease.

The same thing happened in the feed is too high fatty acids, the body becomes low protein, high levels of body fat into a situation were allegedly closely associated with protein and body fat as a result of fatty acids in the diet. This happens because the protein is a molecule that is polar and can bind water molecules while the fat is non-polar and does not bind water.

Then, when the review of energy, allegedly part of the energy from fat in feed D used efficiently for growth in addition to metabolism, whereas most proteins utilized by the fish for growth well because fat is a protein sparing effect in the use of energy for the body.

CONCLUSIONS

The results showed that:

1) Yield of pure oil of hazelnut and meal worm results flouring solvent extraction followed respectively by 27.3% and 12.15% of the fresh weight.

2) Feeding for four weeks showed that growth rate between 3.72 g - 5.82 g, with meal worm feed supplements 2% was highest absolute growth rate, and no significant with high protein on control, and so was hazelnut 2%.

3) Retention of protein obtained in supplement worm oil and hazelnut oil 1%, respectively 33.48% and 33.42%.

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HARD TICK (ACARI: IXODIDAE) CO-INFESTATION OF ROE DEER (*CAPREOLUS CAPREOLUS* LINNAEUS, 1758) IN VOJVODINA HUNTING RESORTS (SERBIA)

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Abstract

Roe deer are important hosts for ticks, providing them constant and reliable source of food, as well as the mode for habitat distribution. The aim of this study was to determine the species diversity of ixodid ticks parasitizing on the roe deer and the level of co-infestation. Eight tick species sampled from the 72 carcasses of roe deer were identified: I. ricinus, D. marginatus, D. reticulatus, H. punctata, H. concinna, H. sulcata, R. sanguineus and R. bursa. The most abundant were the females of I. ricinus and the highest prevalence was obtained for I. ricinus and D. marginatus. The single-species infestation was not detected. The co-infestation of all identified tick species was found on 15 carcasses and equally high percentage was obtained for co-infestation of six tick species. Roe deer as the habitat generalist and polyphagous herbivore are exposed to a greater tick species diversity present at the same habitats. The roe deer co-infestation with ixodid ticks in Vojvodina region could be explained by both sets of factors that promote multiparasitism: the spatial distribution of the hosts and the host life quality.

Key words*: ticks, roe deer, co-infestation, Ixodes, Dermacentor.*

INTRODUCTION

The ecological niche is one of the most important and the hardest to define concepts in ecology. According to the competitive exclusion principle (Gause's principle), there are no means that two species that compete for the same resource could coexist at constant population values in the same niche of the same habitat for a long period of time if other ecological factors remain constant. According to Amarasekare (2000), there are theories that suggest that spatial dynamics may play a key role in maintaining both competitive and consumer resource interactions. Still, how to explain multiparasitism or other multi-species systems?

The multiparasitism is a condition in which different species of parasites parasitize a single host using it as a same ecological niche. According to Vaumourin et al. (2015) some parasites could occur more frequently than expected because the same factors promote their presence, not because they interacting synergistically. The most important factors are: environmental and climatic conditions, host density, host behaviours and host physiological conditions. All factors that promote multiparasitism could be grouped in two sets: 1) factors that influence host exposure – the spatial distribution of host, and 2) factors that influence host susceptibility are intrinsic to hosts – host life history traits (Vaumourin et al., 2015).

European roe deer (*Capreolus capreolus* Linnaeus 1758) are very common species all over Europe (Vor et al., 2010). This ungulate species is a habitat generalist and polyphagous herbivore. Therefore, it is able to feed on a variety of plants and thus live in the different habitats. In Vojvodina region, roe deer are usually found in the fragmented habitats: agroecosystems bordered by deciduous forest belts and shrubby vegetation. These habitats are also suitable for ixodid tick populations (Jurišić et al., 2011; Jurišić et al., 2012). All ixodid tick species are obligatory hematophagous parasites and their occurrence is strictly limited to the host presence. Consequently, roe deer are important hosts for ticks, providing them

constant and reliable source of food, as well as the mode for habitat distribution (Vazquez et al., 2011; Vor et al., 2010).
The aim of this study was to determine the species diversity of ixodid ticks parasitizing on the roe deer and the level of co-infestation.

MATERIALS AND METHODS

Ticks were collected during the five year study (2011-2015) from the hunted carcasses of roe deer (*Capreolus capreolus*) immediately after the hunt. Each carcass was systematically and thoroughly inspected using palpatory technic by three observers. The collected specimens were properly labelled and transported to the laboratory till examination and identification. Tick species were identified up to species level according to identification keys: Nosek & Sixl (1972) and Estrada-Pena et al. (2004).
The study was performed at 8 localities on the territory of Autonomous Province of Vojvodina: Bač (30.149 ha, N45°20`447`` E19°13`108``), Bačka Palanka (52.204 ha, N45°14`198`` E19°20`239``), Bačko Petrovo Selo (11.304 ha, N45°41`144`` E20°05`283``), Turija (5.343 ha, N45°32`509`` E19°51`239``); Novi Bečej (60.745 ha, N45°35`509`` E20°07`219``), Pančevo (69.565 ha, N44°51`530`` E20°37`453``), Novi Kneževac (30.539 ha, N46°01`456`` E20°04`598``) and Ruma (50.560 ha, N45°01`570`` E19°49`189``). All studied localities were described as agroecosystems with sporadic shrub and bush vegetation and wide belts of mixed deciduous forests.
The ixodid tick infestation was described using four parameters: the prevalence (P), the average infestation intensity (AII), the abundance (A) and the infestation index (II) (Petrović, 2015).

RESULTS AND DISCUSSIONS

The total number of 2623 ticks specimens were sampled from the 72 carcasses of roe deer (56 males and 16 females). Eight tick species from four genera were identified: *Ixodes ricinus* (Linnaeus, 1758) (63.44%), *Dermacentor marginatus* (Sulzer, 1776) (13.46%), *D. reticulatus* (Fabricius, 1794) (3.70%), *Haemaphysalis punctata* (Canestrini and Fanzago, 1878) (3.55%), *H. Concinna* (Koch, 1844) (2.71%), *H. sulcata* (Canestrini & Fanzago, 1878) (4.69%), *Rhipicephalus sanguineus* (Latreille, 1806) (6.21%) and *R. bursa* (Canestrini & Fanzago, 1878) (2.25%). The most abundant were the females of *I. ricinus* (10.98%). The larval stages of all identified species, as well as the nymphs of *D. marginatus*, *D. reticulatus*, *H. punctata*, *H. sulcata* and *R. bursa* have not been found on the hosts. The highest number of sampled ticks was at hunting resort Novi Kneževac (20.21%) and the lowest at locality Ruma (3.55%).
The highest prevalence was obtained for *I. ricinus* and *D. marginatus*, as they were found on all prospected roe deer carcasses. The lowest values of all four parameters were detected for *R. bursa*, as it was found in small number on only 34 prospected carcasses. Additionally, the high prevalence was obtained for *R. sanguineus*, as this species was found on 69 roe deer carcasses (Table 1).

Table 1. The prevalence (P), average infestation intensity (AII), abundance (A) and the infestation index (II) of identified tick species

Tick species	P (%)	AII	A	II
Ixodes ricinus	100.00	23.11	23.11	23.11
Dermacentor marginatus	100.00	4.90	4.90	4.90
Dermacentor reticulatus	66.67	2.02	1.35	0.90
Haemaphysalis punctata	52.78	2.45	1.29	0.68
Haemaphysalis concinna	54.17	1.82	0.99	0.53
Haemaphysalis sulcata	59.72	2.86	1.71	1.02
Rhipicephalus sanguineus	95.83	2.36	2.26	2.17
Rhipicephalus bursa	47.22	1.73	0.82	0.39

The most abundant tick species of roe deer in Vojvodina was *I. ricinus* (23.11%) which is similar to the results of Carpi et al. (2008), Skotarczak et al. (2008) and Vor et al. (2010). Although *I. ricinus* is the predominant tick species in Europe (Barandika et al., 2008; Mihalca et al., 2012), the high number of adults found on the studied hosts could be explained by the fact that the immature stages of *I. Ricinus* are capable of feeding on almost any vertebrate, but adults require the blood meals of more than 1 ml, especially from the animals that could provide it better, such as ungulates (Donzé et al., 2004).
In Vojvodina, *D. marginatus* follows the distribution pattern of *I. ricinus* as the second most abundant tick species (Jurišić et al. 2011; Jurišić et al., 2012, Petrović et al., 2015). This species prefers deciduous forests, pastures, meadows and all kinds of shrub vegetation along the river banks. Its presence is registered

at all prospected localities and on every carcass as well. *D. reticulatus*, was found at all prospected localities, but only on 48 carcasses. This species prefers river basins, wet/flooded forests, grassland and meadows, rich in bush and shrub vegetation, which is confirmed by similar conclusions of Mihalca et al. (2012).

H. punctata and *H. concinna* were found at six prospected localities with low abundance on the roe deer. According to Nosek (1971, loc. cit. Mihalca et al. 2012), these species prefer pastures, forest margins and forest-steppes which have been in decline over the past decade in Vojvodina. *H. sulcata* was the most abundant species of *Haemaphysalis* genus, but found only at five localities. Similarly to findings of Mihalca et al. (2012), this species prefers warm and humid areas in Vojvodina.

The high value of *R. sanguineus* prevalence and its presence at all prospected localities indicate that, although it is an endophilic, monotropic and three-host tick species, it is also able to survive and maintain its population in the outdoor environments (Dantas-Torres, 2010) and therefore parasite on cattle or wild ungulates (Estrada-Pena et al., 2004). *R. bursa*, on the other hand is a typical representative of tick fauna commonly found on ungulates, with preferences to areas well covered with bush and steppe vegetation (Fuente et al., 2004). This species was found at 6 prospected localities with low values of prevalence and abundance.

The single-species infestation was not detected. The co-infestation of all identified tick species was found on 15 carcasses (20.83%). Equally high percentage was obtained for co-infestation of six tick species. Oppositely the results of Vazquez et al. (2011), where 99.7% of examined roe deer had single-species infestation by *I. ricinus* and only one specimen of roe deer double-species infestation (*I. ricinus* and *D. marginatus*), this research indicates that the co-infestation of six and more tick species is characteristic for roe deer in Vojvodina (59.72% carcasses) (Figure 1). The average burden of parasitizing ticks of all developmental stages and at all prospected localities was 36.43 per a roe deer.

The results from other European researches state that on red deer (*Cervus elaphus* Linnaeus 1758) could be found up to 15 different ixodid tick species (Vazquez et al., 2011).

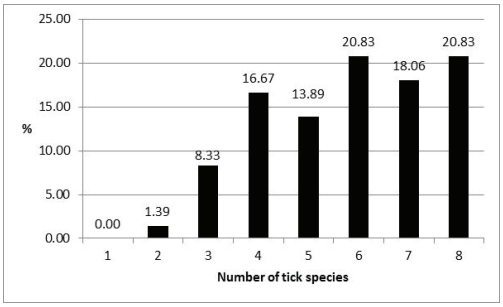

Figure 1. The co-infestation of tick species

Ruiz-Fons et al. (2006) found 10 different ixodid tick species parasitizing Iberian red deer (*Cervus elaphus hispanicus* Linnaeus, 1758), mainly *Hyalomma marginatum marginatum* (63.7%), *R.* (*Boophilus) annulatus* (7.9%) and *R. bursa* (7.5%). According to Vor et al. (2010), roe deer havea preference for dense vegetation and their diurnal rhythm of feeding and resting make them easily accessible hosts for questing ticks. Furthermore, roe deer have large home ranges (10 to more than 200 ha) (Vor et al., 2010). According to Vaumourin et al. (2015) hosts with larger distributions are more likely to become co-infested, as are the hosts that occupy ecological niches in which several parasites are present. Therefore, roe deer as the habitat generalist, which are adapted to a wide range of environmental conditions (including anthropogenic factors) and polyphagous herbivore that could exploit a large number of food resources are, consequently, exposed to greater tick species diversity present at the same habitats. The roe deer co-infestation with ixodid ticks in Vojvodina region could be explained by both sets of factors that promote multiparasitism: the spatial distribution of the hosts and the host life quality.

CONCLUSIONS

Eight tick species sampled from the 72 carcasses of roe deer were identified: *I. ricinus*, *D. marginatus*, *D. reticulatus*, *H. punctata*, *H. concinna*, *H. sulcata*, *R. sanguineus* and *R. bursa*. The most abundant were the females of *I. ricinus* and the highest prevalence was obtained for *I. ricinus* and *D. marginatus*. The single-species infestation was not detected. The co-infestation of all identified tick species was

found on 15 carcasses. Roe deer as the habitat generalist and polyphagous herbivore are exposed to a greater tick species diversity present at the same habitats. The roe deer co-infestation with ixodid ticks in Vojvodina region could be explained by both sets of factors that promote multiparasitism: the spatial distribution of the hosts and the host life quality. Due to consistent population densities, wide home ranges and seasonal migrations, most of the vertebrate species found at hunting resorts are responsible for the maintenance of the high tick density populations in certain habitats and therefore seasonal, spatial, transstadial and transovarial transmission of pathogens important for human and animal health.

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SELECTIVITY OF LESSEPSIAN FISH, RANDALL'S THREADFIN BREAM (*NEMIPTERUS RANDALLI* RUSSELL, 1986) IN THE GULF OF ANTALYA, EASTERN MEDITERRANEAN

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Abstract

Size selectivity of sorting grid was studied for Randall's threadfin bream in the Gulf of Antalya, Mediterranean Sea. Trawling's were carried out in October - December 2012, with a traditional bottom trawl net (600 mesh around the mouth). The fish totally retained in upper codend (catches) and bottom codend (escapes) were used to estimate the selectivity. Selectivity data analyzed as covered cod-end method by means of a logit function of Maximum Likelihood Method. The Kolmogorov – Smirnov test (KS – test) was applied in order to determine the difference between size groups of escapes and catches codends. The percentages of the total catch in terms of weight were 68% retained and 32% escaped. Mean L_{50} value of Randall's threadfin bream was calculated as 13.19 ± 0.48 cm. Total weight of Randall's threadfin bream caught in hauls was 12.996 kg. The results confirm that using sorting grid fixed mesh codend gave higher L_{50} value than size selectivity studies by using Hand-Woven Slack Knotted mesh codend.

Key words: *Size selectivity, Lessepsian, Randall's threadfin bream, Antalya gulf, Eastern Mediterranean.*

INTRODUCTION

Members of the family *Nemipteridae* are abundantly distributed in coastal waters, and occur mainly on muddy or sandy ground between 5 to 80 m depths usually in schools (Russell, 1990). They are carnivorous, feeding on small fish, crustaceans, molluscs, (mainly cephalopods), polychaetes and echinoderms.

Randall's threadfin breams *Nemipterus randalli* Russell, 1986 is widely distributed in the western Indian Ocean, especially off India, Pakistan, and Persian Gulf and in the Gulf of Aden. Additionally, the species is known off the east African coast and in waters surrounding Seychelles and Madagascar (Russell, 1990). *N. randalli* is reported in the Red Sea including the Gulf of Aqaba (Baranes and Golani, 1993; Golani and Bogorodsky, 2010). The first Mediterranean record of *N. randalli* was reported in the eastern Levantine Basin by Golani and Sonin (2006), but wrongly identified as *Nemipterus japonicas* (Bloch, 1791). At present, the species appears to be up to date successfully established in some areas of the eastern Mediterranean such as the Turkish marine waters (Erguden et al., 2010) and the close coast of Lebanon (Lelli et al., 2008).

Improved selectivity can be achieved in different ways, by modifying the gear design and/or operation and by using alternative fishing gears. In trawls mesh size and by inserting filtering grids in front of codend is a well-known measure to regulate the size of captured organisms. Successful separation of targets and non-targets species can also be achieved by using grid devices (Valdermarsen, 2005).

Mediterranean fisheries are remarkable for the large number and variety of commercially important species caught and the wide range of fishing methods employed, from artisanal to industrial (Stewart, 2002). Management of fishing stocks in the Mediterranean Sea is mainly based on defining closed areas and seasons, Minimum Landing Sizes (MLS), Minimum Mesh Sizes (MMS) and limiting effort (Sala and Luchetti, 2010; Tokaç *et al*., 2014). Trawl catches contribute 90% of the total landing in the Turkish demersal fishery (Metin et al., 2004). Presently Turkish Fisheries Regulations (TFR) defines a minimum codend mesh size of 40 mm for the Black Sea, 44 mm for the Aegean Sea and the Mediterranean for demersal trawls. Additionally, the use of 40 mm square mesh codend is left to fishermen's preference (Anonymous, 2012). Many studies published

during the last decade have clearly shown that the selectivity of commercially used codends are rather poor in Turkish demersal trawl fisheries (Tosunoğlu et al., 2007; Ateş et al., 2010; Tokaç et al., 2010; Aydın et al., 2011; Özbilgin et al., 2012).

Gulf of Antalya is predominantly overfished by vessel from Iskenderun and Mersin. Most of commercially fish species live at a depth of 30-200 m. *Mullus barbatus barbatus* Linnaeus, 1758 (Red mullet), *Mullus surmuletus* Linnaeus, 1758 (Surmullet), *Upeneus moluccensis* (Bleeker, 1885) (Goldband goatfish), *Saurida undosquamis* (Richardson, 1848) (Brushtooth lizardfish), *Boops boops* (Linnaeus, 1758) (Bogue), *Pagellus erythrinus* Linnaeus, 1758 (Common pandora), *Spicara flexuosa* (Linnaeus, 1758) (Picarel), are main demersal commercially important species of Mediterranean coast of Turkey.

This is first selectivity study for Randall's threadfin bream by using horizontal bar spacing from the eastern Mediterranean Sea. Although, there are many selectivity studies carried out by using different shape (diamond, square, hexagonal) and sizes (40, 44, 50 mm) of codends in the Mediterranean coast of Turkey (Ferretti and Froglia, 1975; Kınıkarslan, 1976; Livadas, 1988; Jukic and Piccinetti, 1988; Gurbet, 1992; Stergiou et al., 1994; Gurbet et al., 1997; Lök et al., 1997; Tokaç and Tosunoğlu, 1997; Tosunoğlu et al., 1997; Tokaç et al., 1998; Tosunoğlu, 2000; Akyol et al., 2000; Kınacıgil et al., 2001; Fiorentini and Leonori, 2002; Özbilgin and Tosunoğlu, 2003; Tosunoğlu et al., 2003), there are no selectivity study by using sorting grid in the Mediterranean coast of Turkey.

MATERIALS AND METHODS

In total 14 towing were conducted in the Gulf of Antalya (northeastern Mediterranean Sea), at depths of 30 – 200 m (36.840724°N 30.935353°E, 36.827437°N 31.072305°E – 36.887438°N 30.887438°E, 36.816852°N 31.048630°E), in October - December 2012 with a traditional bottom trawl net (600 mesh around the mouth).

The towing speed varied between 2 and 3 knots. The mean effective towing duration was 106 min (range 90 – 120 min). The research vessel (R/V Akdeniz Su) has a length overall of 26.5 m and 2 x 450 HP engines. Sorting grid was attached aft belly of the net. After each haul, fishes caught from both codends were sorted by species and were separately weighed. The fish totally retained in upper codend (catches), and bottom codend (escapes) were used to estimate the selectivity (Figure 1). All taxa were determined to the species level whenever possible and the respective weights recorded from the codends.

Selectivity data was collected by using commercial demersal trawl gear of the research vessel with the trouser codend technique for Randall's threadfin bream. All individuals (without using subsamples) were immediately weighed (total wet weight) to the nearest 0.1 g and measured to the nearest cm in the laboratory of the research vessel.

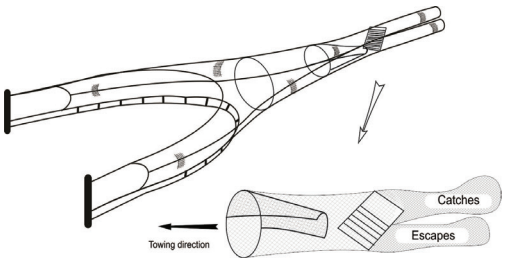

Figure 1. General view of the sorting grid and codends

Selectivity curves of the individual hauls were obtained by fitting the logit function: $r(l) = \exp(v_1 + v_2 l) / [1 + \exp(v_1 + v_2 l)]$ by means of the maximum likelihood method as given in Wileman et al. (1996), where the parameters v_1 and v_2 are the intercept and slope of the linear logistic function, respectively. The mean selectivity curves were estimated from the individual hauls which were fitted taking into account the between haul variation. The Kolmogorov – Smirnov test (K - S test) was applied in order to determine the difference between size groups of escapes and catches codends (Aydın et al., 2007).

RESULTS AND DISCUSSIONS

Selectivity of sorting grid for lessepsian fish species was studied in the gulf Antalya. The present study is first selectivity study by using

horizontal grid bars for Randall's threadfin bream in the gulf of Antalya, Turkey.

Total 14 hauling were carried out during the trials and in total, 317 for Randall's threadfin bream entered the codends (total weight 12.996 kg) Randall's threadfin bream was caught (Table 1).

Table 1. Weights, numbers, and percentages of total numbers of Randall's threadfin bream retained in codends

Species	Amount
Randall's threadfin bream	
Weight in catches codend (kg)	8.816 (68%)
Weight in escapes codend (kg)	4.180 (32%)
Number in catches	152 (48%)
Number in escapes	165 (52%)

Table 1 shows the weight and number of the investigated species. Other species usually present in the catch were *Solea solea* (Linnaeus, 1758), *Chelidonichthys lucerna* (Linnaeus, 1758), *Trachurus mediterraneus* (Steindachner, 1868), *Trachurus picturatus* (Bowdich, 1825), *Pagrus pagrus* (Linnaeus, 1758), *Nemipterus randalli* (Russell, 1986), *Merluccius merluccius* (Linnaeus, 1758), *Citharus linguatula* (Linnaeus, 1758), *Chelidonichthys lastoviza* (Bonnaterre, 1788), *Spicara flexuosa* (Linnaeus, 1758), *Boops boops* (Linnaeus, 1758), *Serranus hepatus* (Linnaeus, 1758), *Serranus cabrilla* (Linnaeus, 1758), *Diplodus annularis* (Linnaeus, 1758), *Dentex maroccanus* (Valenciennes, 1830), *Pagrus caeruleostictus* (Valenciennes, 1830), *Sillago sihama* (Forsskål, 1775), *Trichiurus lepturus* (Linnaeus, 1758), *Bothus podas* (Delaroche, 1809) and *Zeus faber* (Linnaeus, 1758) from the Gulf of Antalya.

Kolmogorov - Smirnov test showed that differences between length groups of catches codend and escapes codend for Randall's threadfin bream wasn't significant (*K - S* test, D: 0.1765; $P = 0.930$) ($p > 0.05$).

Selectivity parameters of Randall's threadfin bream

The total number of 317 are taken to be evaluated for Randall's threadfin bream and 152 (48%) of these were retained on the catches codend; 165 (52%) of these were retained on the escapes codend. The length distribution was between 6 and 22 cm and showed a peak at 13 cm. While fish size groups retained on the catches codend were ranging 10 – 22 cm, escapes codend groups were 6 – 15 cm (Figure 2). L_{25}, L_{50}, L_{75} selection ranges and selection factor are calculated for Randall's threadfin bream. Lengths at fifty percent retention (L_{50}) values for Randall's threadfin bream was found as 13.19 ± 0.48 cm. Selection range (SR) and selectivity factor (SF) were found as 2.9, 3.77, respectively (Table 2).

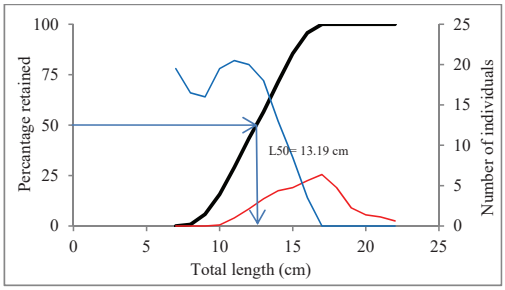

Figure 2. Selectivity curves and length distribution (red line: catches; green line: escapes) of Randall's threadfin bream

Table 2. Selectivity parameters of sorting grid system for Randall's threadfin bream

Catches	152
Escapes	165
SR	2.9 cm
SF	3.77
L_{25}	11.72 cm
$L_{50} \pm$ SE	13.19 ± 0.48 cm
L_{75}	14.66 cm
$v_1 \pm$ SE	9.85± 0.47 cm
$v_2 \pm$ SE	-0.74 ± 0.11 cm
MLS/SFM	(There are no published studies yet)

L_{25}, 25% retention length; L_{50}, 50% retention length; L_{75}, 75% retention length; SR, selection range; SF, Selection factor; v_1 and v_2, regression parameters, SE, standard errors; SFM, size at first maturity; MLS, minimum landing size

There was found only one study for Randall's threadfin bream by using Hand-Woven Slack Knotted mesh codend in the Mediterranean coast of Turkey and there isn't any size for the first maturity length of *N. randalli* in the Mediterranean Sea and no minimum landing size for Turkish Fisheries Regulations (TFR).

Although there wasn't found any reproduction study on estimating minimum size at maturity or the size of the smallest mature of *N. randalli* in the Mediterranean Sea, in total length 10 cm was found in Kerala, India (Mohamed et al., 2014). Eryaşar et al. (2014) was found the lengths at fifty percent retention values for Randall's threadfin bream as 2.01 ± 1.32 cm for standard mesh codend (300 meshes around its circumference) and 5.92 ± 0.11 cm for narrow mesh codend (300 meshes around its circumference) by using hand-woven slack knotted codend, in Mersin Bay. Both values are smaller than minimum size at maturity. L_{50} value found in present study as 13.19 ± 0.48 cm is higher than minimum size at maturity and mesh codends by using hand-woven slack knotted codend.

It is considered that these differences include the location of the research, differences in net materials, different hang-in ratios, and using different mesh size in codends. Besides these, the differences between L_{50} sizes may be occurred fishing the populations of different size groups in localities or a lesser intensive fishing pressure on this species may have occurred in the gulf of Antalya.

Also a different size at first maturity can be found for lessepsian fish, Randall's threadfin bream in the Mediterranean Sea.

It is clear that selectivity study by using hand-woven slack knotted codend, in Mersin Bay gave lower L_{50} size than present study by using sorting grid bar fixed 44 mm codends. According to Sarda et al. (2004) and Aydın, (2008); size selectivity studies made with sorting grids are more successful than size selectivity studies with different mesh size and shapes.

In the successfully selectivity studies, it is waiting that number of fish individuals larger than minimum length size or size at first maturity retained in the catches codend would be expected low. Only 1 (1%) individual larger than L_{50} size was retained in catches codend. This show that sorting grid fixed trawl codend gave high selectivity results.

A total of 17 individuals smaller than L_{50} size were retained in catches codend. The rate of fish groups belong Randall's threadfin bream should be retain in escapes codend but retained catches smaller than L_{50} value were found as 10%. It is considered that some very large pet bottles, garbage bags, twigs, tires, and very large fishes such as stingrays and groupers may have caused to off the selectivity grids during the hauls.

CONCLUSIONS

For a sustainable fishing management, fishing immature individuals and discard species should decrease. Because of this reason, size selectivity of trawl nets should improve. This can apply only by using different shape and size of mesh or by using sorting grids for. There are no published studies for *N. randalli* yet. The L_{50} size found in this study can be used for minimum length size.

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SIZE SELECTIVITY OF SORTING GRID FOR COMMON PANDORA (*PAGELLUS ERYTHRINUS* (LINNAEUS, 1758)) IN THE GULF OF ANTALYA, EASTERN MEDITERRANEAN

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Abstract

Size selectivity of sorting grid was studied for Common pandora in the Gulf of Antalya. Trawling's were carried out in October - December 2012, with a traditional bottom trawl net (600 mesh around the mouth). The fish totally retained in upper codend (catches) and bottom codend (escapes) were used to estimate the selectivity. Selectivity data analyzed as covered cod-end method by means of a logit function of Maximum Likelihood Method. The Kolmogorov – Smirnov test (KS – test) was applied in order to determine the difference between size groups of escapes and catches codends. The percentages of the total catch in terms of weight were 56% retained and 44% escaped. Mean L_{50} value of common pandora was calculated as 14.47 ± 0.27 cm. Total weight of common pandora caught in hauls was 29.937 kg. The results confirm that using sorting grid fixed mesh codend gave higher L_{50} value than size selectivity studies with different mesh size and shapes.

Key words: *sorting grid, size selectivity, Common pandora, Antalya gulf, Eastern Mediterranean.*

INTRODUCTION

The common pandora, *Pagellus erythrinus* (Linnaeus, 1758), is a member of family Sparidae and an important commercial food fish in the Mediterranean region. It is distributed in waters of the continental shelf of the northeastern and central-eastern Atlantic Ocean, from Norway to Guinea-Bissau including the Mediterranean Sea and the Madeira, Canary and Cape Verde Islands (Coelho et al., 2010). This species is caught with bottom trawls, beach seines, on line gear, traps (Canary Islands), trammel nets, gill nets, bottom long lines and hand lines (Russell, 2014). The minimum landing size for this species is 15 cm in Turkey and in the Mediterranean (Özbilgin et al., 2012). It can grow up to 60 cm but its usual length in catches is 10 to 30 cm (Bauchot and Hureau, 1986). During the MEDITS expedition, the range was from 4 to 27 cm (average value 14.72 cm) (Vrgoč, 2000), in the same area. Generally, longer fish were caught in deeper water (Adriamed, 2010).

According to FAO statistics, the global production of *P. erythrinus* was 15.000 tonnes/per year in 1963 - 1968. After that date, it was subsequently decreased significantly since 1987 to 2007 to about 4.500 tonnes per year. (Coelho et al., 2010)

Improved selectivity can be achieved in different ways, by modifying the gear design and/or operation and by using alternative fishing gears. In trawls mesh size and by inserting filtering grids in front of codend is a well-known measure to regulate the size of captured organisms. Successful separation of targets and non-targets species can also be achieved by using grid devices (Valdermarsen, 2005).

Gulf of Antalya is predominantly overfished by vessel from Iskenderun and Mersin. Most of commercially fish species live at a depth of 30-200 m. *Mullus barbatus barbatus* Linnaeus, 1758 (Red mullet), *Mullus surmuletus* Linnaeus, 1758 (Surmullet), *Upeneus moluccensis* (Bleeker, 1885) (Goldband goatfish), *Saurida undosquamis* (Richardson, 1848) (Brushtooth lizardfish), *Boops boops* (Linnaeus, 1758) (Bogue), *Pagellus erythrinus* Linnaeus, 1758 (Common pandora), *Spicara flexuosa* (Linnaeus, 1758) (Picarel), are main

demersal commercially important species of Mediterranean coast of Turkey.

This is first selectivity study for Common pandora by using horizontal bar spacing from the eastern Mediterranean Sea. Although, there are many selectivity studies carried out by using different shape (diamond, square, hexagonal) and sizes (40, 44, 50 mm) of codends in the Mediterranean coast of Turkey (Ferretti and Froglia, 1975; Kınıkarslan, 1976; Livadas, 1988; Jukic and Piccinetti, 1988; Gurbet, 1992; Stergiou et al., 1994; Gurbet et al., 1997; Lök et al., 1997; Tokaç and Tosunoğlu, 1997; Tosunoğlu et al., 1997; Tokaç et al., 1998; Tosunoğlu, 2000; Akyol et al., 2000; Kınacıgil et al., 2001; Fiorentini and Leonori, 2002; Özbilgin and Tosunoğlu, 2003; Tosunoğlu et al., 2003), there are no selectivity study by using sorting grid in the Mediterranean coast of Turkey.

In this study, size selectivity of sorting grid for common pandora in the gulf of Antalya, eastern Mediterranean.

MATERIALS AND METHODS

A total of 14 hauls were conducted in the Gulf of Antalya (northeastern Mediterranean Sea), at depths of 30 – 200 m (Figure 1), with a traditional bottom trawl net (600 mesh around the mouth).

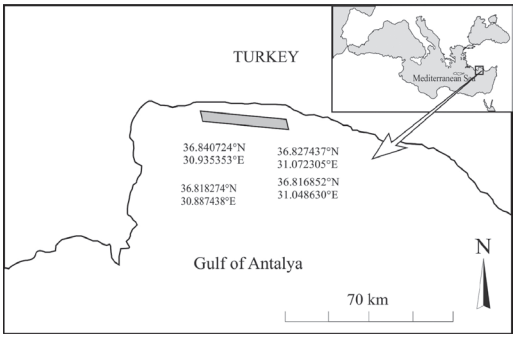

Figure 1. Study area for selectivity study conducted in bottom trawl fishery

The towing speed varied between 2 and 3 knots. The mean effective towing duration was 106 min (range 90 – 120 min). The research vessel (R/V Akdeniz Su) has a length overall of 26.5 m and 2 x 450 HP engines. Sorting grid was attached aft belly of the net. After each haul, fishes caught from both codends were sorted by species and were separately weighed. The fish totally retained in front of the grid and collected upper codend (catches), and bottom codend (escapes) were used to estimate the selectivity (Figure 2, 3). All taxa were determined to the species level whenever possible and the respective weights recorded from the codends. All individuals (without using sub-samples) were immediately weighed (total wet weight) to the nearest 0.1 g and measured to the nearest cm in the laboratory of the research vessel.

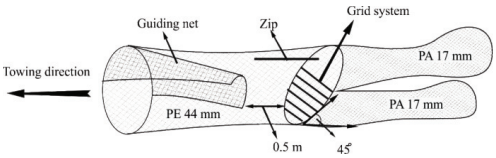

Figure 2. General view of the sorting grid and codends

Figure 3. Sorting grid fixed bottom trawl net used in the study

Selectivity curves of the individual hauls were obtained by fitting the logit function: $r(l) = \exp(v_1 + v_2 l) / [1 + \exp(v_1 + v_2 l)]$ by means of the maximum likelihood method as given in Wileman et al. (1996), where the parameters v_1 and v_2 are the intercept and slope of the linear logistic function, respectively. The mean selectivity curves were estimated from the individual hauls which were fitted taking into account the between haul variation. The Kolmogorov – Smirnov test (K - S test) was applied in order to determine the difference

between size groups of escapes and catches codends (Aydın et al., 2007).

RESULTS AND DISCUSSIONS

Selectivity of sorting grid for commercial fish species was studied in the gulf Antalya. The present study is first selectivity study by using horizontal grid bars for Common pandora in the gulf of Antalya, Turkey. Total 14 hauling were carried out during the trials and a total weight of 29.937 kg Common pandora was caught (Table 1).

Table 1. Weights, numbers, and percentages of total numbers of common pandora retained in codends

Species	Amount
Common Pandora	
Weight in catches codend (kg)	19.087 (67%)
Weight in escapes codend (kg)	9.442 (33%)
Number in catches	358 (56%)
Number in escapes	279 (44%)

Table 1 shows the weight and number of the investigated species. Other species usually present in the catch were *Solea solea* (Linnaeus, 1758), *Chelidonichthys lucerna* (Linnaeus, 1758), *Trachurus mediterraneus* (Steindachner, 1868), *Trachurus picturatus* (Bowdich, 1825), *Pagrus pagrus* (Linnaeus, 1758), *Nemipterus randalli* (Russell, 1986), *Merluccius merluccius* (Linnaeus, 1758), *Citharus linguatula* (Linnaeus, 1758), *Chelidonichthys lastoviza* (Bonnaterre, 1788), *Spicara flexuosa* (Linnaeus, 1758), *Boops boops* (Linnaeus, 1758), *Serranus hepatus* (Linnaeus, 1758), *Serranus cabrilla* (Linnaeus, 1758), *Diplodus annularis* (Linnaeus, 1758), *Dentex maroccanus* (Valenciennes, 1830), *Pagrus caeruleostictus* (Valenciennes, 1830), *Sillago sihama* (Forsskål, 1775), *Trichiurus lepturus* (Linnaeus, 1758), *Bothus podas* (Delaroche, 1809) and *Zeus faber* (Linnaeus, 1758) from the Gulf of Antalya.

Kolmogorov - Smirnov test showed that differences between length groups of catches codend and escapes codend for common pandora wasn't significant (*K - S* test, D: 0.1875; $P = 0.912$) ($p > 0.05$).

Selectivity parameters of Common pandora

The total number of 637 are taken to be evaluated for common pandora and 358 (56%) of these were retained on the catches codend; 279 (44%) of these were retained on the escapes codend. While fish size groups retained on the catches codend were ranging 11 – 22 cm, escapes codend groups were 7 – 19 cm (Figure 4). L_{25}, L_{50}, L_{75} selection ranges and selection factor are calculated for common pandora. The results were found as L_{50} = 14.5 ± 0.27 cm, SR = 2.9, SF = 4.14 (Table 2).

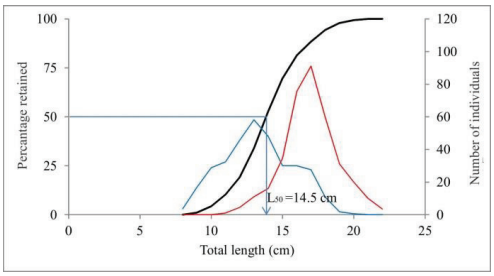

Figure 4. Selectivity curves and length distribution (red line: catches; green line: escapes) of common pandora

Table 2. Selectivity parameters of sorting grid system for common pandora . L_{25}, 25% retention length; L_{50}, 50% retention length; L_{75}, 75% retention length; SR, selection range; SF, Selection factor; v_1 and v_2, regression parameters, SE, standard errors; SFM, size at first maturity; MLS, minimum landing size

Catches	358
<MLS	41 (11%)
≥MLS	317 (89%)
Escapes	279
<MLS	210 (75%)
≥MLS	69 (25%)
SR	2.9 cm
SF	4.14
L_{25}	13.03 cm
L_{50} ± SE	14.5 ± 0.27 cm
L_{75}	15.92 cm
v_1 ± SE	10.9± 0.27 cm
v_2 ± SE	-0.76 ± 0.03 cm
MLS/SFM	15 cm

There were found three selectivity studies for common pandora by using different size and material of mesh codends in the Mediterranean coast of Turkey and 4 studies in Atlantic (Table

3). Tokaç et al. (1995) was calculated L_{50} value as 10.07 cm (by using 20 mm codend) and 13.27 cm (24 mm codend) in Aegean Sea. Ozbilgin and Tosunoğlu (2003) were estimated as 10.50 cm (by using 22 mm codend) in same research area. Both studies the hooped covered codend method were used. Çiçek (2015) was estimated L_{50} value as 7 cm by using the covered cod-end technique in Karataş, coast of Adana.

Table 3. Selectivity parameters of common pandora selectivity studies conducted in Atlantic and Mediterranean Sea

References	Mesh/Grid size (mm)	L_{50} (cm)	SF	Locality
Tokaç et al., 1995	40	10.8	-	Mediterranean Sea
	36	12.4	-	
	44	13.8	-	
Özbilgin and Tosunoğlu, 2003	22	10.5	-	
Çiçek, 2015	22	7.20	3.18	
Present Study	**35**	**14.5**	**4.14**	
Levi et. al., 1971	40	11.8	0.89	Adriatic Sea
Vrgoč, 1995	40	11.30	-	
Jukić and Piccinetti, 1987	40	11.8	0.89	
	41	11.8	0.89	
	51	12.2	0.95	
	55	16.4	0.90	
	65	20.5	0.91	
Joksimovic, 1999	13.5*	7.60	-	
	16.1*	8.68	-	
	17.5*	10.82	-	
	22*	15.00	-	
	35*	15.26	-	

* knot to knot.

The L_{50} that we determined was 14.47 cm for a net with sorting grid fixed 35 mm codend mesh size in the gulf of Antalya, intermediary between three previously reported values in the Mediterranean Sea. Possible reasons for these differences include the location of the research (different seas), differences in net materials, and different hang-in ratios (Tosunoğlu and Tokaç, 1997; Tokaç et al., 2004). Besides these, the differences between L_{50} sizes may be occurred fishing the populations of different size groups in localities or a lesser intensive fishing pressure on this species may have occurred in the gulf of Antalya.

It is clear that third studies by using hooped and covered codend methods gave lower L_{50} size than present study by using sorting grid bar fixed 35 mm codends. According to Sarda et al. (2004) and Aydın, (2008); size selectivity studies made with sorting grids are more successful than size selectivity studies with different mesh size and shapes.

Generally, it is waiting that number of fish individuals larger than MLS retained in the escapes codend would be expected low, in the size selectivity studies. A total of 41 (11%) individuals of common pandora larger than L_{50} size were retained in catches codend. It is considered that some materials having very large size such as pet bottles, garbage bags, twigs, tires, and very large fishes such as stingrays and groupers may have blocked the selectivity grids during the hauls and individuals smaller than L_{50} couldn't pass the bar and changed the direction and retained in catches codend.

CONCLUSIONS

In order to improving the size selectivity of trawl nets, square mesh codend and fixed sorting grid bar together may give better results for size selectivity of immature individuals and discard species.

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ARTIFICIAL FERTILIZATION OF THE OHRID TROUT AND THE PRESENCE OF ITS SUMMER FORM IN THE LAKE

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Abstract

Management and conservation of Ohrid trout, the most important endemic species of this lake, is one of the main issues of collaboration between the two transboundary states. The decline of fishing amount during the years is improved by the artificial fertilization of koran applied both in Macedonia and Albania. Thereby, lake Ohrid reinhabited with about one million offsprings of Salmo letnica per year, only from the Albanian part. The technical enhancement of artificial fertilization makes possible the considerable increase of the koran amount in the hunting period. Meanwhile, the koran summer form, Salmo letnica aestivalis has resulted in a decrease of the fishing amount according to data fishermen. The main purpose of this paper is to show that the management of koran artificial fertilization is directly connected with the fishing amount. So the enlargement of artificial fertilization even in summer time, for the Albanian part, will result in the fishing summer form increased.

Key words: *artificial fertilization, conservation, Ohrid trout, Salmo letnica aestivalis.*

INTRODUCTION

Lake Ohrid, a lake of tectonic origin is localized in Balkan region, in the transborder area between the two countries; Macedonia and Albania. According to many authors the period of its existence exceed 2-3 million years (Satankovic, 1960), making it one of the long-lived lakes in the world, that for more than 1 million years has provided a freshwater habitat for its species. Despite the fact that some biotic groups are poorly studied or not studied at all, approximately 1200 native species are known from the lake, including 586 animals and at least 212 are endemic. With these endemic species and a surface area of 358 km^2, Lake Ohrid is probably the most diverse lake in the world, taking surface ares into account (Albreht & Wilke, 2008). Ten of 17 fish species and 34 of 38 kinds of mollusks that populate the lake, thought to be endemic (Stankovic, 1960). Among salmonoids, Lake Ohrid trout, *Salmo letnica* (Karaman, 1924) or Koran, is an important element of the freshwater fauna and as it is placed clearly on the phylogenetic Adriatic group *S. trutta* mtDNA (*S. trutta*, Bernatchez, 2001 dhe Apostolidis, et al. 1997, *S. letnica*, Sell & Spirkovski, 2004). The so-called Ohrid trout or Koran, *Salmo letnica*, by different authors (Stefanovic, 1948; Stankovic, 1960; Dimovski, Grupce and Spirkovski, 1992) has been considered as a polymorphic specie regarding to the taxonomy and ecological characteristics. According to the authors above, based on various data there are four forms of Ohrid trout that can be distinguished by the different intralacustrine environments, by the time and the different substrates that they use for spawning. There is an absence of the studies that determine the genetic differences of these forms, however Sell and Spirkovski have shown that *Salmo letnica typicus* and *Salmo letnica aestivalis* are two genetically distinct forms based on the sympatric differentiation results. To preserve and protect natural resources and biodiversity of Lake Ohrid, the Lake Ohrid Conservation Project is applied (LOCP). The realization of the project objective has developed and supported effective cooperation between Albania and Macedonia for joint environmental management of the watershed. The project began in late 1998 and early 1999, with an expected duration of three years. One of the most important points of Lake Ohrid Monitoring Program has been the conservation of the most important species of this lake. But while the Koran or Lake Ohrid trout, is also subject to important fisheries, in order to maintain the natural lake population in stable conditions, and to allow fishing at the same time, since 1935 is established the process of

artificial fertilization and the repopulation of the lake, already in Macedonia and Albania respectively. Since then, the lake reinhabited each year with about 1 million fingerlings from Macedonian and Albanian side as well. The lake restocking with fingerlings from Koran artificial fertilization is significantly reflected by the increasing of the fish's number in the fishermen catches and at the other side this avoid the endangerment of a particular fish specie such as *Salmo letnica*. But this artificial fertilization, mainly is realized for the Koran winter form in November- February, its reproduction period. Years ago, in Struga is realized the artificial fertilization of the Koran summer form, but in a small amount. The main purpose of this paper is to show that the artificial fertilization of *Salmo letnica aestivalis* would help the conservation and protection of the Koran summer form as one of two genetically distinct forms of Ohrid trout.

MATERIALS AND METHODS

The realization of this paper is based on data collected over the amount of fishing for several years and the cultivation techniques of Koran on the Lake Ohrid Albanian side. We provide quantitative data through the inspectorate office of fisheries in Pogradec, on Koran catches from 1947 to 2013. The processing in Microsoft Office Excel of catches quantities in quintal [metric] (1 quintal = 100 kg) over the years gave us a development trend in the graphical representation of these measurements. These data are collected by licensed boats that accurately respect the allowed fishing periods for Ohrid trout. It is important mentioning here that in addition to licensed individuals there are unlicensed fishermen that for several years have done abusive catches who have influenced in the quantity mismatches of Ohrid Trout in the lake. In contact with the Specialist of Ohrid trout growing economy, in Lin, Pogradec, we have followed the work realized in different development stages and the growth environments until the fishes reached the fingerling stage. From the data collected we have estimated the eggs quantity losses in different cultivation stages and the offspring effectiveness which released in the lake, in the period of September - October. The laying eggs apparatus in dark rooms, the constant water flow and the monitoring of temperature, pH, water purity showed optimal development conditions of eggs in the same way and as well as the tanks premises where the fingerlings growed until they weigh about 3 gram. Based on the observations and calculations we have done, we predicted the possible conditions of artificial fecundation, cultivation and conservation of Koran summer form, *Salmo letnica aestivalis*, that follows the same development stages as Koran winter form. In our work, a very important information has been obtained from several meetings with the old fishermans, to create a final screen of the Koran summer form catches. The fish catches declining of the *Salmo letnica aestivalis* amount, are a strong appeal to establish coordination and management mechanisms in its intensive conservation through the artificial fertilization and cultivation techniques that till now are used for the winter form cultivation.

RESULTS AND DISCUSSIONS

Figure 1 is a graph showing the quantity of Koran catched from licensed fishermen over the years. The Inspectorate of fishing in Pogradec possesses data from 1947 to 2013.

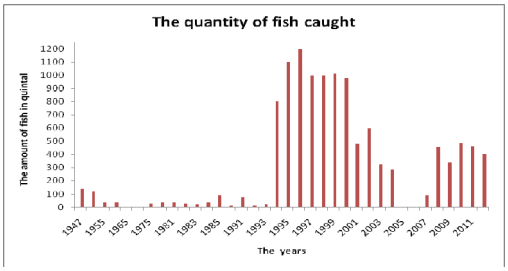

Figure 1. The annual quantity of Ohrid trout catched by the lincensed fishemen in Albanian side

According to figures refer we notice a lower pace of the amount of fishing during the years 1947- 1993, ranging from 16 quintal up to 140 quintal koran per year. For 1965, 1970 and 2005 and 2006 there are no data on Koran catches amount for albanian side. If we see further in 1994 noticed a trend of increased amount of the Koran catches, which continues until 2000 and where the amount of fish caught varies from 800 to 1200 quintals per year. This

period, which discerns with the increased of Koran catches quantity, coincide with the important activity of the Koran cultivation impiant in Lin economy. So, the improving of technological equipment and eggs cultivating environments in this economy associated with a ranked production of 180 000 -700 000 fingerlings annually. In the following years and nowadays the amount of fingerling stage produced annually by this economy goes about 1 million fingerlings. Despite this, in the years 2002-2012 the amount of Koran catches comes declining with an annual quantity account of about 300- 480 quintal. The main reason for this decrease in the quantity of fish catches is the overfishing or abusive fishing of Koran. The number of unlicensed boats in recent years has come increased and consequently the activity of undeclared fish catches gives us lower values of the Koran catches amount . If we compare the data of the Koran captured in the Ohrid and Struga area, in recent years appear a decrease of fish caught amount (Spirkovski & Talevski, 2002). The highest quantity catches appear in the years 1987-1990 which coincides with an intensive activity on Ohrid trout cultivation by the Hydrobiological Institute of Ohrid. The followed years presented the need for a cross-border water management plan of the lake and the Lake Ohrid Conservation Project (LOCP) was the result of specialists discussions from both countries; Albania and Macedonia.

Following the artificial fecundation and cultivation steps of Ohrid trout in Lin economy, in Pogradec, we concluded on the effectiveness of breeding techniques used by specialists. So, the embryonic mortality during the fertilized eggs incubation and the next steps is even lower depending on the apparatus used for laying eggs. The values vary from 5.5% in the groove form apparatus, 6.8% in the William apparatus and 8.3% in the Mc Donald apparatus. So the survival of the fertilized eggs until the fingerlings stage is 91.7% - 94.5%, that in the number language translates into about 1 million fingerlings issued annually in the lake. This Ohrid trout artificial cultivation economy, only proceed with his winter form with a considerable effectiveness that not only helps in the Koran conservation but also creates significant opportunities for fishing quantities. Contacting the fishermans that have fished for decades in Lake Ohrid, we took the unanimous opinion of the reduction trend in the Koran summer form caught amount. The specialists of offspring cultivation in Lin economy, now with perennial experience, have the necessary potential to realised the possibility of applying the artificial cultivation also for the Koran summer form, *Salmo letnica aestivalis*.

CONCLUSIONS

By the quantitative data and the realised statistical processing we noted a link between the activity of Koran artificial breeding economy in Lin and the quantity of Koran caught by licensed fishermen. The development of fish conservation and cultivation techniques from this economy has led to increased efficiency of lake repopulation with fingerlings, which is presented by the growing trend of the fish caught amount.

By the fishermen data based on their activity for tens of years in the Lake Ohrid water, we conclude in the decrease of caught quantity of Koran summer form, *Salmo letnica aestivalis*. We suggest that the management and coordination of the artificial cultivation of *Salmo letnica aestivalis* will lead not only to the caught amount increase of this form, but also in its optimal conservation status.

The Koran cultivation impiant in Lin, Pogradec has the necessary specialists, experience and facilities in which it would could be realized the cultivation of Ohrid trout summer form, *Salmo letnica aestivalis* that today appears as a very important issue of its conservation.

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EVALUATION OF THE ORAL RABIES VACCINATION PROGRAM OF RED FOXES (*Vulpes Vulpes*) POPULATION IN ROMANIA IN 2014

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Abstract
Rabies is a fatal zoonotic viral disease produced by a Lyssavirus and is causing more than 70,000 human deaths each year. In Romania foxes are the main wildlife reservoir. Oral rabies (ORV) vaccination of this specie is the most effective method to control and eventually eradicate rabies. Supported by co-financing program between Romania and European Union, successive ORV campaigns were conducted. Monitoring of the effectiveness of oral vaccination campaign has been carried out continuously from 2011 and was based on: (i) post-mortem laboratory examination of brain tissue of foxes by fluorescent antibodytest (FAT), (ii) detection of antibodies against rabies virus in thoracic liquid by ELISA, (iii) detection of tetracycline biomarker in teeth for the evaluation of vaccine bait uptake and (iv) discrimination between wild and vaccinated rabies virus strains by PCR and sequencing techniques. The laboratory analysis of 2014 campaign revealed 31.18% seropositivity and detection of tetracycline biomarker in 55.4% of the foxes tested. No vaccine-induced rabies cases occurred and all positive foxes (1.33%) were infected with wild rabies strains present in Romania.

Key words: *rabies, oral vaccination program, foxes, vaccine, baits.*

INTRODUCTION

Rabies is a Central Nervous System zoonotic disease, with the causative agent Rabies virus, the negative-sense single stranded RNA viruses of the Lyssavirus genus within the family *Rhabdoviridae*, divided into seven genotypes. Genotype 1 viruses is distributed worldwide and generally is found in terrestrial mammals and causes between 37.000 and 87.000 human deaths annually (Virus taxonomy 9th report, 2012; WHOExpert Consultation on Rabies second report, 2013).In Europe canine rabies has been eradicated from developed countries by control measures such as dog movement restriction and mass vaccination and now the major reservoir of rabies was replaced by wild animals, especially red fox (*Vulpes vulpes*) (Cliquet, 2015). Extensive oral vaccination programs (ORV) with baits for red foxes have reduced the incidence of rabies in many Western European countries (Slate, 2009; Zienius, 2011). In Romania dog mediated rabies was predominant before and after World War II with dogs accounting for about 75% of all rabies cases in 1946. When the fox rabies epizootic that spread throughout most of Europe reached Romania, rabies cases in foxes increased over those in dogs (Avram, 20006; Turcitu, 2010).

A co-financed by the EU and the Romanian state budget oral vaccination trial of foxes has been conducted in 16 counties from the western part of Romania in spring and autumn 2011. From 2012 the OV programs were implemented throughout the Romanian territory.

The objective of this study is to assess the effectiveness of the ORV monitoring program in 2014.

MATERIALS AND METHODS

Study area

The vaccination area of this study involved of the entire Romanian territory (237.500 km2). Romania is situated in the East of Europe and it has borders with Moldova to the Northeast, Ukraine to the North and East, Hungary and Serbia to the West and Bulgaria to the South. The Black Sea forms the Eastern border of the country (Turcitu, 2010). According to the cen-

tral authorities statistics the population of foxes for the year 2014 is estimated at 62.707 foxes.

Vaccine

Lysvulpen vaccine (Bioveta®, Czech Republic) has been used for ORV program. According to the vaccine data sheet, it contains SAD Bern modified attenuated strain cells (min 1.8 × 107 TCID50-max1.8 × 108 TCID50/bait). In one bait, there is one vaccination virus dose (1.8 ml) closed in aluminum-plastic blister. Round, dark brown bait is made of feed mixture attractive for foxes and other target animal species. Each bait contains 150 mg of tetracycline HCl, which is intended as an indicator of ingestion by target animal species (Lysvulpen data sheet). Baits were stored in freezers at -20°C prior to use and during the entire vaccination campaign as well.

Baits distribution

For 2014 the co-financing program for surveillance, control and eradication of rabies in Romania approved by Commission Decision 2013/722/CE, submitted by Romania, provides application on the entire territory of Romania, in 41 counties. The bait distribution includes border with Serbia, Hungary, Ukraine, Moldova and Bulgaria. The vaccination has been performed in only one campaign, September-October, because of tendering problems related to the procurement and aerial distribution of vaccine baits. The vaccination of foxes was carried out by air distribution of baits from 8 aircraft (number of 5.325.200 baits with an approx. 25 baits/km2), with a distance between flight lines of 500 meters and 150 meters altitude, by avoiding the territories of localities, water surfaces, highways, etc. Estimated surface suitable for aerial vaccination is approximated at 213.375 square kilometers. It has been done a manual distribution around localities and areas difficult to reach by plane (number of 75.400 of baits, approximately 25 baits/km^2). The data were recorded on Geographical Identification System (GIS) using Geographical Positioning System (GPS).

Samples collection

At a 45 days following vaccination campaign, there shall be performed the hunting of foxes in order to assess the efficiency of vaccination, for this purpose, there shall be shot 4 foxes/year/100 km^2. Brain samples were collected as previously described (EFSA Scientific report, 2010). Field blood samples were generally collected from the shouted foxes thoracic cavity. Samples were stored at -20°C until use for ELISA testing. The foxes lower jaws were collected from each sample.

ORV program monitoring

Taking into account the high incidence of rabies cases in Romania, and the fact that some infected foxes do not show any clinical/nervous symptoms when there are shot, there has been decided that all foxes shall be tested by standard method such as fluorescent antibody test (FAT) used for the detection of rabies virus antigen on brain samples (OIE, 2014). Different commercial immunofluorescent conjugates (Bio-Rad, Fujirebio, Rabitest) were used in order to perform this test. FAT negative samples are sent to NRL for further investigations. The 150 μm sections throughout canine tooth with some alveolar bone tissue (using ISOMET Low Speed Saw) have been performed from each lower jaw. Samples of tooth and surrounding alveolar bone were tested at the NRL for rabies by specific fluorescence to detect tetracycline deposits (excitation filter 380-425 nm, barrier 460 nm). The age of all the foxes tested was determined on the basis of dental examination (cub, younger than 12 months of age, adult older than 12 months (Cliquet, 2012). Immune response was assessed using the indirect enzyme-linked immunosorbent assay *(ELISA)* method (Bio-Rad Platelia Rabies II Kit, France). Assays were done in a 96 wells microplate, coated with rabies virus glycoprotein according to the producer recommendations. 0.5 EU/ml or above antibody titers were expressed as positive. FAT positive samples were sent to NRL to discriminate between wild and vaccinated strains using molecular biology techniques as previously described (Turcitu, 2010).

Statistical analyses were performed using GraphPad Prism version 6.01 software.

RESULTS AND DISCUSSIONS

Out of 6965 foxes planed to be hunted, 5448 (78.2%) were collected by hunters (Figure 1).

Out of these, 8% (n=436) were juvenile (Figure 2). Seventy three (1.33%) were positive by FAT. In a total of 5048 thoracic liquid examined, 1574 (31.18%) were positive by ELISA. Out of 1574 tested thoracic liquid, 142 (9%) were juvenile. Fifty five percents (n=5375) samples reacted positive to biomarker (tetracycline) detection (Figure 3).

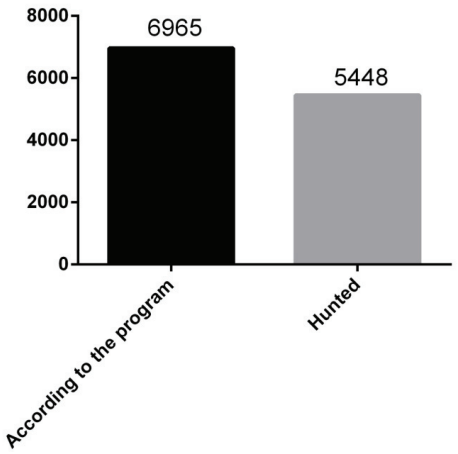

Figure 1. The number of the foxes planned to be hunted according to the program and the number of foxes achieved

Phylogenetic analysis demonstrated that all 73 field isolates from Romania found positive by RT-PCR belong to the classical rabies virus (genotype 1) and are all closely related. This shows that no vaccine-induced rabies cases occurred and all positive foxes were infected with wild rabies strains present in Romania (data not show).

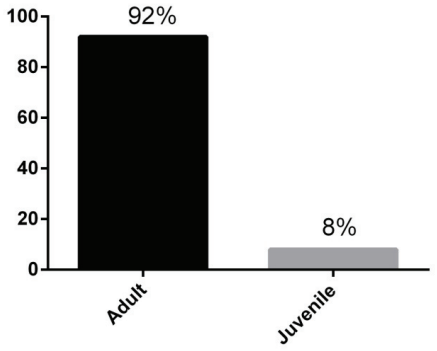

Figure 2. The age (%) of the hunted foxes

A foxes oral vaccination trial co-financed by the EU and the Romanian state budget has been conducted in 16 counties from the western part of Romania in spring and autumn 2011. From 2012 the OV programs were implemented throughout the Romanian territory according to the recommendations of the European Commission (European commission, 2002).

The efficacy of the OV campaigns in Romania was assessed by monitoring rabies prevalence in shot foxes, bait consumption and the immunisation rates. Oral vaccination campaigns were monitored from late July of the OV year to the following March by collecting head and serum samples from foxes. Autumn campaigns target adults and juveniles, while spring campaigns target mainly adults, because fox cubs are usually born from March to April, (Lloyd, 1980; Kauhala, 1996). We determined the age of all animals collected in 2014 in order to check the animal's effect on bait uptake and immunization levels as previously demonstrated (Bruyer, 2000).

Few studies have evaluated the effectiveness of OV according to the age of the target species (Bruyer, 2000; Rosatte, 2001). Tetracycline positivity rates were significantly higher than immunisation rates in both adults and young animals. There are several hypotheses may explain these discrepancies. First, the bait envelope may be ingested while the core containing the vaccine is not. Brochier et al. (1996) postulated that cubs may chew the baits without puncturing the vaccine capsule. When they are not hungry, foxes hide the baits in order to eat them later, which lead to inactivation of the live attenuated vaccine (Bachmann, 1990).

Fluorescence in teeth may be seen with an origin other than tetracycline from vaccine baits. Other possible sources of tetracycline include placental remnants from cows treated with tetracycline for infections associated with retained placentas, from chicken farm or from fish farms where tetracycline may be used (European commission, 2002). Foxes feed their cubs by regurgitation, and while regurgitated baits still contain tetracycline, the vaccine strain is destroyed by gastric acidity. Contact between the vaccine suspension and the oropharyngeal mucosa may sometimes be insufficient for immunization. The production of antibodies may be transient or absent, or

reach a low titer. In this last case, the sensitivity of the test used is a critical factor while the tetracycline gives life-long teeth deposits (European commission, 2002).

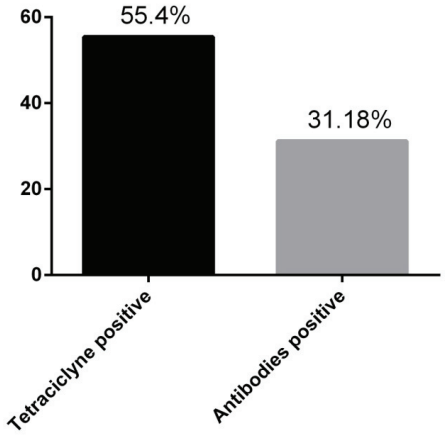

Figure 3. The percentages of the tetracycline positive samples and antibodies against rabies positive samples in hunted foxes

International organizations also recommend that ‘‘all rabies virus isolated should be typed in areas where attenuated rabies virus vaccines are used, in order to distinguish between vaccine and field virus strains’’ (European commission, 2002). Phylogenetic analysis demonstrated that all 73 field isolates from Romania found positive by hnRT-PCR belong to the classical rabies virus (genotype 1) and are all closely related. This shows that no vaccine-induced rabies cases occurred and all positive animals were infected with wild rabies strains present in Romania in 2014.

Vaccination will be succeeding in decreasing the outbreaks and eradicating the disease only if a sufficient percentage of population can be immunized. In a program aimed at free ranging wild animals, the proportion of the population which accept baits and the proportion of the individuals which will be protected from the rabies are essential features (Bachmann, 1990).

CONCLUSIONS

Romania has implemented a rabies control program since 2011.

Immunization rate and biomarker detection from the present ORV study suggest that distribution of vaccine in the field not have been too effective. Therefore additional research is needed to increase knowledge related the factors affecting the success or failure of wildlife oral rabies vaccination programs, such as effectiveness of the immune response in other species, like wild boars, jackals or even rabbits.

A continues monitoring and oral vaccination of foxes each year as well as the coordination and cooperation of vaccination programs between neighboring countries are very important.

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