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GENETICS AND BREEDING

NUTRITION

REPRODUCTION, PHYSIOLOGY, ANATOMY

TECHNOLOGIES OF ANIMAL HUSBANDRY

TECHNOLOGIES OF THE AGRO FOOD PRODUCTS PROCESSING

WILD LIFE MANAGEMENT, FISHERY AND AQUACULTURE

GENETICS AND BREEDING

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RESEARCH ON PRODUCTION PERFORMANCE OF CROSSBRED EWES PRODUCED FROM THE CROSSING WITH RAMS OF THE BREED TURCANA GERMAN BLACK HEAD INFLUENCE ON THE ECONOMIC EFFICIENCY OF FARM

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Abstract
The research aim was to highlighted the fact that by crossing the local sheep (Merinos) and meat production specialized breed there are obtained good quality meat production lambs with superior performances besides the local breed performances.
There were conducted two experimental groups, the control group consisting of 50 sheep were mated by the same breed rams and the experimental group consisting of 50 Merinos sheep mated by Ile de France rams. It was determined the body weight of lambs at birth, of one month age, two months age, three months age and at the age of delivery, at six months. 5 lambs were slaughtered in each group and there was calculated the slaughter yield
There conclusions were drawn:
1. The crossbred lambs, males and females had a larger weight than the lambs from the control group at all the ages the determinations were made.
2. The slaughter yield was higher in crossbred lambs comparatively the ones in the control group.
3. By industrial crossing there was achieved an improvement of the growth speed and the slaughter yield, obtaining superior quality carcasses, very well quoted in EUROP classifying system.

Keywords*:lambs, slaughter, carcasses*

INTRODUCTION

Romania is according to the latest European statistics, third place in a ranking made according to the number of sheep and goats exploited.
The European Community does not occur as long as sheep meat is consumed, with a permanent deficit that is covered by imports from countries like New Zealand and Australia.
Romania sheep breeders, can deliver meat on the European market and can cover a part of the current deficit, but is required to improve the quality of sheep meat produced in our country.
Industrial crossings is a quick way to enhance and improve meat production, biological and economic effects of this cross is used whereby the phenomenon of heterosis, half-breeds showing a higher growth rate, better use of feed through Specific consumption per kg gain less, compared with the products breeds paternal clues are sometimes even superior to those of ameliorative race (Calatoiu, 1986; Calatoiu and Vicovan, 1986).
The purpose of this paper is to conduct comparative research on improving production of meat, correcting housing defects, increase yield at slaughter etc., from simple industrial crossing between sheep breed and breed meat Turcana German Black Head.

MATERIALS AND METHODS

The research was conducted on a flock of sheep from a farm-bred Turcana in Sibiu, the two groups of sheep. Control group, consisting of 50 Turcana sheep were crossed with rams of the same race and the experimental group, consisting of 50 female Turcana were crossed with rams of the breed German Black Head.

All lambs were obtained for research use, except where the determinations of slaughter yield only five lambs were used in each batch.
Maintenance for sheep was 150-160 days and 205-215 days in grazing calves. During loose housing, sheep have been maintained properly furnished and provided with shelters paddocks. He pursued the creation of animal comfort, ensuring sufficient accommodation space of 1.5 m2 per head adult sheep and 2.55 shelters m2/head in the paddock, with a front feeding of 0.5 m / head.
To ensure the vital functions were provided in three sheep rations 3 -2.5 kg dry substance, 1.5 to 1.6 UNL, 70-75 PDIN / PDIE, 4.5 g Ca, 2.5 to 3 g P per 100 kg live weight, supplementing these amounts are 15 - 20% in periods of preparation for mating and breeding, and by another 25-45% in the first 1-3 months gestation and lactation.
Administered daily ration was balanced in minerals and vitamins, to prevent metabolic disorders. Vitamin requirements of green fodder was provided by, when possible, or add the concentrated fodder premixes, and minerals providing necessary.
Feeding calves during the maintenance was made of vegetables, 0.5 to 1 kg / head / day, with succulent forage, fodder beet, 1.5 to 2 kg / head / day, with corn silage, 1.5 to 2 kg / head / day, with a mixture of concentrated feed in the structure came in, on average 25-30% barley, corn 50-60%, 8-12% of sunflower and soybean meal, 1% salt, 2% chalk feed.
Using pasture was grown on a longer period, it has positive effects on animal health and productive level, avoiding long and tiring road.
Water was provided ad libitum at pasture and shady resting place. The need for water was 3-4 times greater than the amount of dry matter intake, 3-6 liters per day respectively. Special attention was given to lactating ewes, which were used to stimulate lactation feed, succulent fodder, corn silage, fodder beet or green mass (Jarige, 1990; Tafta, 2008).
During the grazing forage ration was supplemented with a fibrous filler fodder and concentrates.
Feeding was carried from youth aged 8-10 days, the lambs were provided in specially designed pens, good quality hay, vitamins and concentrates consisting of 50% corn, 40% and 10% oats or peas Oil cake, making the administration at their discretion. This feeding was continued until weaning of lambs, after which the system was performed semi fattening lambs, for a period of 180 days, alternating system maintenance, 105 days and 75 days grazing calves.
In the first period for calves lambs were feed with forage ration was 0.662 kg DS, 0.663 UNC, 62.20 g PDIE and 63.8 g PDIN the induction phase and 0.803 kg DS, 0.752 UNC, 72.3 g PDIN and 73.7 g PDIE, during growth and fattening. During the grazing lambs habituation phase were fed with a forage ration was 1.028 kg DS, 0.912 UNC, and 104.1 g PDIN ,112.2 g PDIE the induction phase and 1.495 kg DS 1 UNC 30, 143 g PDIN and 130 g PDIE, during growth and fattening.
In the second period for calves lambs were fed with forage ration was 1.30 kg DS, 0.749 UNC, 92.6 g PDIE and 100.6 g PDIN the induction phase and 1.59 kg DS, 1072 UNC , 130.3 g PDIE and 137.3 g PDIN the finishing phase. Benefited from grazing lambs seeded plots, which was used a mixture of 70-75% grasses (*Dactylis glomerata, Festuca pratensis, Lolium perenne*) and 25% perennial legumes (*Medicago sativa, Trifolium repens*). Fattening lambs was structured in three stages: 1 - Loose housing with an accommodation period of 15 days and a period of growth and fattening of 35 days; 2 - grazing, with an accommodation period of 15 days and a period of growth and 90 days fattening; 3 - Loose housing with an accommodation period of 10 days and finishing 35 days. Switching from one pasture to fatten the calves was achieved by an induction phase of 15 days, increasing the daily intake of feed concentrates and volume. During the calves were fed ad libitum lambs, with the unique blend of feed ratio was 30% and 70% concentrated fibber. Water and salt lumps were provided ad libitum, both at pasture and in calves. Monthly weighing of animals was done by the end of the fattening period.

RESULTS AND DISCUSSIONS

After registering births and weighing lambs were calculated main indices take the groups of sheep breeding (Table 1).

Table 1. The main indices of breeding

Group	Mounted sheep	Sheep have given birth	Lambs obtained	Fecundity (%)	Prolificacy (%)
Ţ*Ţ	50	46	49	92.00	106.52
Ţ*BHG	50	47	56	94.00	119.14
Average	100	93	105	93.00	112.90

From the table are playing a number of breeding indices determined. The batch of ewes with rams German black head cross was so high fecundity and prolificacy Turcana lot. If fertility is high with only two points, 94% in group Turcana the sheep rams were crossed with black head and 92% in German Turcana group, the sheep prolificacy in group Turcana rams were crossed with German Black Head was by 119.14% from 106.52% to just Turcana group.

Table 2. The daily average gain of lambs during lactation

Group	Average daily gain (g / day)			
	n	0-30 days	30-60 days	0-60 days
		X ± sx V%	X ± sx V%	X ± sx V%
Ţ*Ţ	49	181.66±7 20.22	170±6 17.16	175.83±7 23.16
Ţ*BHG	56	201±7 17.26	194.66 16.86	197.83±7 19.38

Table 2 plays an average gain of lambs weight, sex, birth to age one month and at weaning.
Ewe lambs from group Turcana rams crossed with German Black Head, as both males and females had higher weight gains in lambs produced from ewes Turcana group. Similar results in weight gain and Black Head Teleorman breed (Roşu, 2011).

Table 3. Evolution of body weight of lambs to weaning

Group	n	Weight at birth	weight group in 30 days	60 days weight
		X ± sx V%	X ± sx V%	X ± sx V%
Ţ*Ţ	49	3.31±0.08 3.6	8.76±0.15 17.32	13.86±0.24 17.08
Ţ*BHG	56	4.44±0.08 1.26	10.47±0.10 14.30	16.31±0.22 18.36

In Table 3 it is presented the body weight of lambs at birth, at age one month and two months of age (at weaning). Both males and females as the group of ewes from rams crossed with German Black Head, had higher body weight both at birth and age as one month and two months. Lambs from that batch of sheep rams crossed with German Black Head had an average birth weight of 4.44 kg body while lambs of group coming Turcana had at birth weight 3.31 kg. At weaning lambs from that batch of sheep crossed with German Black Head rams had an average body weight of 16.31 pounds, while lambs of group coming Turcana weaning had an average weight of 13.86 kg, 2.45 kg less.
In Table 4 is given weight recorded at the end of fattening period and average daily gain during fattening achieved. If lambs derived from crosses rams sheep with German black head Turcana achieved a mean increase 109.6 g / day, derived from group Turcana lambs were made only an average gain of 89.6 g / day. Thus at the end of fattening lambs derived from crosses rams german sheep with black head Turcana reached a mean body weight 36.04 kg, while lambs derived from ewes Turcana group achieved an average weight reached only 29.98 kg.

Table 4. Dynamics of growth in semi fattening period (180 days)

Group	n	Weight group in the early end of fattening	Fattening weight	Average daily gain (g / day)
		X ± sx V%	X ± sx V%	X ± sx V%
Ţ*Ţ	49	13.86± 0.22 17.02	29.98±0.40 19.62	89.6± 5 16.84
Ţ*BHG	56	16.31±0.24 17.92	36.04±0.36 20.16	109.6±4 13.08

Table 5. Slaughter yield

Specify	MU	Group	
		Ţ*Ţ	Ţ*BHG
Live weight	g	30.56±6.35	37.68±7.89
Chilled carcass weight	g	13.35±4.58	17.84±5.42
Slaughter yield	%	43.71±1.04	47.37±0.01

MU – measuring unit
Yield obtained from the slaughter of five lambs from each group, was 47.37% in lambs derived from the sheep Turcana group were German black cross with rams head and only 43.71% of lambs derived from the group of sheep Turcana.

CONCLUSIONS

1. Sheep crossing with rams Turcana German black head lead to an increase in prolificacy sheep Turcana with 12.62%, compared to the group of sheep Turcana.
2. Lambs, both women and male, coming from the group of sheep with rams Turcana German black head cross, higher weight gains realized throughout the life.
3. Average weight at the end of the fattening period was higher by 6.06 kg in lambs derived from crosses of sheep with rams Turcana German Black Head (36.04 kg) than lambs of ewes Turcana consignments coming from.
4. Return lamb to the slaughter of sheep coming from the group Turcana rams crossed with German black head, is higher by 3.66% compared to lambs derived from ewes Turcana group.
5. If you perform calculations in the two groups of sheep studied, the amount of meat in case they can sell more for each sheep is greater than 6.27 kg for sheep crossed with rams of the breed German Black Head, at a price selling 14lei/Kg of housing, resulting in a higher income per ewe 87.78 lei meat only, not taking into account the possible higher price can be obtained with improved meat quality and carcass conformation.

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GENOTYPIC ASSESSMENT OF KARAKUL RAMS BY FUR SKIN QUALITIES OF PROGENY

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Abstract

The research purpose was the comparative appreciation of different evaluation systems for fur skin characters and features at Karakul lambs and methods of rams testing by the progeny fur skin qualities, by emphasizing of those advantages and disadvantages. Research has been done on breeding sheep from experimental farm of INZMV Maximovca, Anenii Noi district, and CAP „Agrosargal" from Hânceşti district. As a result of research has been found, that, the category of rams amelioration obtained as a result of the application of the terminological system of fur skin characters and features assessment at lambs appreciation and of simple testing method by the progeny fur skin, will not be afterwards confirmed, in proportion of 85%, when the decimal system of scoring is applied in order to appreciate the characters and features of lamb fur skins and will be used the biometric method of scoring, by testing the rams after the progeny fur skin qualities. Moreover, some rams that were qualified as reducing (worsen), as a result of testing after the progeny fur skin qualities by the simple method, have become ameliorator breeders, as a result of their testing after the biometric scoring system of progeny fur skin qualities and, reverse, other rams, which were qualified as enhancers in testing result by simple method, became reducers, as a result of their testing by biometric scoring method. It was concluded, that only application of terminological system assessment of Karakul lambs and of the simple method of rams testing after the progeny fur skin qualities are not enough for an objective evaluation of breeders amelioration category. The conclusions made as a the result of testing with simple method application are offen unreliable and contradictory, The use of decimal scoring system at Karakul lambs assessment and of biometric scoring method at testing rams after the progeny fur skin qualities, permits objective, genotypic evaluation of breeders amelioration category by determining the certainty criterion of the conclusions regarding amelioration degree of each ram apart. As a result of research was proposed a classification of Karakul rams by the amelioration value in following categories: I degree ameliorator – whose progeny exceeds by score ranking, the flock level with $t_d \geq 3.3$ ($B \geq 0.999$). II degree ameliorator - whose progeny exceeds by score ranking, the flock level with $t_d \geq 2.6 – 3.2$ ($B \geq 0.99$). Ordinary ameliorator - whose progeny exceeds by score ranking, the flock level with $t_d \geq 2.0 – 2.5$ ($B \geq 0.95$). Relative ameliorator - whose progeny exceeds by score ranking, the flock level with $t_d \geq 1.6 – 1.9$ ($B \geq 0,90$). Neutral – whose progeny doesn't have a certain positive difference comparing to flock level. Certain reducer – whose progeny surrenders by score ranking, the flock level with $t_d \geq 1.6$. Relative reducer - whose progeny surrenders by score ranking, the flock level up to $t_d < .1.6$.

Keywords: *testing, genotype, rams, Karakul, method, biometric, score.*

INTRODUCTION

The assessment of Karakul rams genotype after the progeny fur skin qualities, has some advantages comparing to other races, since these qualities can be measured in a relatively short period (150-155 days after the fertile insemination of the sheep) and at a relatively early age (1-2 years) of the ram (Каримов, 2007). Taking into account the fact that, some ram lambs, with a good body development, can be used at the mount at an early age of 6-8

months, the term of their genotype determination, after the progeny fur skins qualities, can be reduced up to one year of age. In this case, the interval of the flock generations succession will decrease, also with an year. Therefore, profiting of results of an early definition of Karakul rams genotype after the progeny fur skins qualities, the coach can enhance the selection effect and accelerate the rate of the flock genetic amelioration.

Despite the fact that, according to instructions of the evaluation marc of Karakul sheep, with amelioration principles in Republic of Moldova (1996), testing the rams after the progeny fur skin qualities is mandatory only for breeding farms, we consider that this test is appropriate to be done at all farms, both the livestock breeding farms as well those for the goods production, whereas about genetic breeding true value on the fur skin qualities of the breeders can be deemed not only according to the own fur skin qualities of lambskin (at birth), or to ascendants or collateral relatives, indicated in the individual form, or the breeding certificate of the purchased animal, but after the progeny fur skin qualities. The purpose of genotypic testing of Karakul rams after the progeny fur skin qualities consist in identification of the most valuable breeders with (prepotent) predominant amelioration genotype for their intensive use at reproduction and removal from the flock of non-valuable rams, reducers of fur skin qualities.

In karaculture are known several methods of rams testing, after the progeny fur skin qualities (Buzu et al., 1994; Buzu, 2000; Iliev, 1992; Pascal, 2007; Дъячков, 1980; Дюсегалиев, 2010; Нел, 1975; Рахманов, 1978). The main of these methods contain comparing the progeny fur skin qualities of the ram, which will be tested, with other ram groups, as are:

-comparing the fur skin qualities of the tested rams progeny to contemporary progeny qualities;

-comparing the fur skin qualities of the tested rams progeny to mothers fur skin qualities of same progeny;

-comparing the fur skin qualities of the tested ram's progeny to the race standard;

-comparing the fur skin qualities of the tested rams progeny to fur skin qualities of all new-born lambs in the flock.

Given that, the latter method is more thorough and practical in application, it was included in the official instructions (1996) as the basic method of genotypic testing of Karakul rams after the progeny fur skin qualities.

At the same time, regardless of comparing method of the progeny fur skin qualities with other progeny groups, an important problem remains the assessing of genotypic value, on the basis of statistical analysis of the data obtained in the test. In the old instructions (1967), of testing the Karakul rams after the progeny fur skin qualities, statistical processing of data was done by a simple method, which provided only percentage calculation of the share of valuable classes progeny (elite + I class). In case when this share at the tested ram was higher comparing to flocks progeny, this ram was considered as ameliorator. The conclusions done on the basis of these calculations not always confirmed in subsequent practical mating of the ewes with this ram, because the conclusions done on the basis of simple method of appreciation did not have a support of genetic certainty. Subsequently, some researchers (Кошевой, 1975; Нел, 1975) tried to undertake some tentative to apply the scoring system of appreciation of some fur skin characters and features, taken apart, without generalizing these into a synthesis system. Therefore, these attempts have not succeeded their real implementation in practice of breeders testing after their progeny qualities.

For these reasons, further, we have proposed in the new Instruction (1996), the use, at evaluation marc, of decimal scoring system for fur skin qualities assessment (characters and features), generalized in a synthetic character, as the lambs ranking at evaluation is, and the application of the biometric statistic method of processed data, obtained in testing the Karakul rams coats after the progeny fur skin qualities, as well as the determination of the certainty coefficient of the conclusion regarding the amelioration value of the ram.

However, the decimal scoring system of fur skin characters and features assessment at Karakul lambs evaluation marc and biometric statistics method of processing data obtained by testing Karakul rams after the progeny fur skin qualities, have not gained spread among

Karakul sheep keepers, in our country, partly due to insufficient scientific information and technology transfer in this field and, partly from lack of importance awareness of these methods by the Karakul sheep keepers specialists, both from the breeding farms, and, even more so, those from production farms.
In this context, we proposed a comparative assessment of different test methods for Karakul rams after their progeny fur skin qualities, of fur skin characters and features evaluation through a synthetic character at lambs evaluation marc, as well as various statistical processing techniques of obtained data, as a result of lambs evaluation marc and rams testing, highlighting the advantages and disadvantages of these methods.

MATERIALS AND METHODS

Research has been carried out on the Karakul sheep from agricultural farms: Experimental Section of the National Institute of Zootechny and Veterinary Medicine, from Maximovca, district of Anenii Noi and agricultural cooperative "Agrosargal" Hancesti district.
To the genotypic testing, after the progeny fur skin qualities, have been submitted main ram breeders, especially, breeders which proved, in previous testing, neutral amelioration results and required repeated testing to confirm the amelioration value, as well as some younger rams of 1.5 years, recently purchased or grown up in own household for breeding purpose. To do this, in mount season, were applied, the method of artificial insemination of females (at farms with a number of over 1000 sheep jellies), or their controlled mount (at farms with a number of under 1000 sheep jellies). The average load of insemination at a ram was around 50-70 sheep, but not less than 15-20 sheep.
Determination of lambs fur skin qualities was performed at their evaluation marc by applying both terminological system, according to the old instructions (1967), as well as decimal scoring system, according to the new evaluation instructions of Karakul sheep with amelioration principles in Republic of Moldova (1996).
Terminological system of Karakul lambs characters appreciation at evaluation marc, at the age of 1-2 days after birth, is to express the development value of the character in words (for example, very good, good, not so good and insufficient). Lambs class shall be determined according to the development degree of the fur skin characters and features on the whole, and is expressed in following classes: class I, class II, defect. Decimal scoring system, along with the characters and features of lambs fur skin appreciation, in the above mentioned terminology, provides also the application of the scoring system from 1 to 10, including: lambs of the elite class can be appreciated, at the sight of evaluator, with 8, 9 or 10 points, class I - with 5, 6 or 7 points, class II - with 3 or 4 points, defect category (brac)- with 1 or 2 points.
The genotypic testing of Karakul rams after the progeny fur skin qualities was done, both by simple and biometric scoring methods.
The first method - the simple one, is less objective, and consists in reporting of obtained indices at progeny evaluation marc of each breeder taken apart, as a percentage comparing to the totals obtained from all flock. According to this method, if the tested ram exceeded the flock level after the share of high class lamb descendants (elite + class I), it was considered ameliorator, and, reverse, if the yield was below the level of the flock, the ram was considered a reducer (worse). In case when the share of high classes' descendants was at the level of the flock, the breeder was considered neutral. The second method - biometric scoring, consists in appreciation of lambs' fur skin qualities by decimal scoring system and data processing, according to biometric statistics, with the determination of certainty coefficient (td), of the difference (dd-t) of the average value of the descendant characters of the tested ram, compared to the average of the flock. Evaluation of the ram genetic value, according to this method, was done according to the average score of the descendants ranking, compared to the average of flocks ranking. In case of need to specify, the evaluation of breeders genotype features is done on any characters or traits of fur skins taken apart. In order to determine the rams genetic value (the amelioration category) after the progeny fur skin qualities, were calculated the main genetic parameters of lamb descendants of the breeder

and of the flock, as a whole as follows: M_d - the arithmetic average of ranking score of the lamb descendants; M_t - the arithmetic average of the ranking score of the lambs for entire flock; σ - the average of square deviations of the score (characters variability); m - arithmetic average error; d-the difference between the arithmetic average and rams descendants scoring and flocks average; t_d - the certainty coefficient of the difference between the arithmetic averages;
The data obtained as a result of research, were statistically processed using computer software „STATISTICA – 6" and appreciated their certainty, according to variation biometric statistics, the methods of Плохинский Н. А. 1969.

RESULTS AND DISCUSSIONS

Analysing the results of Karakul rams testing after their progeny fur skin qualities, through the simple method, we have found (Table 1), that, after the share of higher class rams (elite + Ist class) in the progeny, the most valuable breeder is the ram with registration number 6356, which has the highest share of progeny (88.9%) of this kind of lambs and, after this point, exceeds the average level of the flock with 22.6%.

Table 1. Karakul rams testing after the progeny fur skin qualities by simple method

N. o/o	Rams registration no.	Progeny, N	inclusive:									
			Elite class		Ist class		IInd class		Brac (defect)		Elite + Ist class, %	Amelioration category
			head	%	head	%	head	%	head	%		
1	7823	50	10	20.0	30	60.0	10	20.0	-	-	70.0	Ameliorator
2	8144	35	2	5.7	18	51.4	14	40.0	1	2.9	57.1	Reducer
3	1668	30	3	10.0	17	56.7	9	30.0	1	3.3	66.7	Neutral
4	9125	50	1	2.0	35	70.0	10	20.0	4	8.0	72.0	Ameliorator
5	6502	13	1	7.7	8	61.5	2	15.4	2	15.4	68.2	Ameliorator
6	3745	10	-	-	7	70.0	1	10.0	2	20.0	80.0	Ameliorator
7	4907	10	2	20.0	5	50.0	3	30.0	-	-	70.0	Ameliorator
8	6356	9	1	11.1	7	77.8	1	11.1	-	-	88.9	Ameliorator
9	6218	12	2	16.7	7	58.3	-	-	3	25.0	75.0	Ameliorator
10	3982	29	2	6.9	8	27.6	16	55.2	3	10.3	34.5	Reducer
11	5422	36	10	27.8	11	30.6	15	41.7	-	-	58.4	Reducer
12	0073	25	8	32.0	8	32.0	8	32.0	1	4.0	64.0	Reducer
etc	. . .	. . .	. .	. .	. .	. .	. .	. .	. .	. .	. .	. . . . .
Total on the flock		**652**	**67**	**10.3**	**365**	**56.0**	**200**	**30.7**	**20**	**3.0**	**66.3**	**x**

However, this finding cannot be considered properly correct as the testing of this ram was done on a reduced number of progeny (9 heads). The old instructions for rams testing after their progeny qualities by the simple method, has shown, that the testing result can be considered valid, only if it has been obtained on a flock of at least 15-20 descendants. Thus, due to insufficient number of descendants, in our example, these cannot be considered real results obtained from testing the rams with registration number 6502, 3745, 4907 and 6218. Despite the fact, that all of these breeders are appreciated by the simple method as ameliorators, their testing cannot be considered as one performed.

Second place in rank of tested breeders was occupied by the ram with registration number 3745, whose progeny has a share of 80% of elite + I class lambs. According to this index, his progeny exceeds the average flocks level with 13.7%. At the same time, we specify, that in the progeny of this breeder completely are lacking he lambs of the highest assessment class, such as elite class, and there are, in a significant quantity (20%) individuals of the worst quality of assessment namely the brac.
The third place in the rank of tested breeders, is taken by the ram with registration number 6218 in whose progeny is 75% share of elite + Ist class lambs, which exceeds the average level of the flock with 8.7%. But in the progeny of this

ram persists the biggest share of brac-lambs, constituting 25%.

Continuing the description of ameliorators features of the ram-breeder from the fourth place, in the ranking of breeders mentioned in the table, we can observe that this male with registration number 9125, which has a sufficient number of descendants (50 lambs), does not distinguish a lot from first three breeders, but by a lower rate after the share of lambs of elite + I^{st} class, compared to the flock's average, what is only 5.7%.

And, only the fifth breeder (no. 7823) of the breeders ranks, tested after their progeny fur skin qualities, is really an ameliorator, because, despite the fact, that it doesn't exceed much (3.7%) the average flock after the share of elite + I^{st} class descendants share class I, has a significant share from elite upper class lambs (20%) and has no progeny with bad qualities of brac fur skin.

Analysing the amelioration value of breeders, determined by the simple method, just after the share of elite + I^{st} class lambs, we conclude, that male enhancers do not significantly differ between them, than by this share level.

This is explained by methodological imperfection statistical processing of testing data by this simple method, which consists in the fact that at the conclusion deduction regarding rams amelioration value after the fur skin qualities, will not be taken into account the value importance of Elite class lambs, as well as those from I, II and brac classes.

For example, after the share of elite + I^{st} class lambs, the ram no. 9125 (which has a sufficient number of descendants - 50 heads) is considered an ameliorator, because exceeds the flocks average with 5.7%. At the same time, comparing the indices of this rams progeny fur skin qualities with the progeny of the first breeder (no. 7823), we note that they are more inferiors, referring to the share of elite lambs. At the progeny of ram no. 9125, there is a share of lambs with unsatisfactory fur skin qualities (brac – 8.0%). But, these special features of progeny fur skin qualities are not taken into account at the general genotypic assessment of the breeder after the progeny qualities.

Such effects are produced over the rams, which have obtained, the category of neutral or reducer, tested by the simple method, because the reducer-rams can differentiate clearly one from each other, such as in our example reducer-ram no. 3982, in whose progeny are only 34.5 % share of elite + I^{st} class lambs, and rams reducers no. 5422 and 0073, which have in their progeny also 27.8 and 32.0%, of elite class lambs.

The simple evaluation method of rams genotype after the progeny fur skin qualities, is not taken into account the certainty degree of the difference (td) between the progeny value and the flock's average, which give to conclusions a suspicious aspect of low-probability.

To demonstrate the essential differences that occur between the simple and the scoring biometric methods at testing of breeding rams after the progeny fur skin qualities, we will examine the results of genotypic of same rams in the second version, compared to the first method. (Table 2).

As a result of obtained data analysis, we found that from those seven rams, that were tested by the simple method and have obtained the category of ameliorator, only one ram with the registration number 7823 confirmed this category as a result of genotypic testing after the progeny fur skin qualities by the second biometric scoring method.

Two other rams, with no. 4907 and 6356, were appreciated by the simple method as ameliorators, becoming just neutral, as a result of testing after the progeny fur skin qualities by second biometrical scoring method. This is explained by the fact, that, the positive difference between average score of the progeny of these two rams, taking apart, and the average score of the flock, were not certain (t_d = 0.77 and 1.61; P > 0.1).

Surprising is the fact, that the other 4 rams (no. 9125, 6502, 3745 and 6218) that have obtained the category of ameliorator, as a result of testing by simple method, became relative reducers as a result of genotypic testing after the progeny fur skin qualities, by second biometric scoring system. The name of relative reducer is linked with the fact that negative differences between the average score of the progeny of these 4 rams, taken apart, and the average score on the flock, were not certain (t_d = 0.46-1.33; P > 0.1).

Table 2. Genotypic testing of Karakul rams after the progeny fur skin qualities, according to biometrical scoring method

No. o/o	Rams registration number	Descendants, **N**	Arithmetical average **(M)**	Square deviation **(σ)**	Average error **(m)**	$M_t - M_d$ **(d)**	$t_d = d/\sqrt{m_t^2+m_d^2}$	Share of lambs **elite+I$_{st}$ class, %**	Amelioration category	
									Biometrical method	Simple method
1	7823	50	6.04	1.80	0.25	**+0.70**	**2.69**	70.0	Amel. II degree	Ameliorator
2	8144	35	4.94	2.02	0.34	-0.40	1.14	57.1	Relative reducer	Reducer
3	1668	30	5.37	1.87	0.34	+0.03	0.08	66.7	Neutral	Neutral
4	9125	50	5.02	1.64	0.23	-0.32	1.33	72.0	Relative reducer	Ameliorator
5	6502	13	5.08	2.06	0.57	-0.26	0.46	68.2	Relative reducer	Ameliorator
6	3745	10	4.90	2.13	0.67	-0.44	0.66	80.0	Relative reducer	Ameliorator
7	4907	10	5.80	1.89	0.60	+0.46	0.77	70.0	Neutral	Ameliorator
8	6356	9	6.00	1.22	0.41	+0.66	1.61	88.9	Neutral	Ameliorator
9	6218	12	4.58	2.21	0.64	-0.76	1.19	75.0	Relative reducer	Ameliorator
10	3982	29	4.17	1.65	0.31	**-1.17**	**3.65**	34.5	Relative reducer	Reducer
11	5422	36	6.14	2.29	0.38	**+0.80**	**2.11**	58.4	Ordinary amel.	Reducer
12	0073	25	6.16	2.30	0.46	**+0.82**	**1.78**	64.0	Relative amel.	Reducer
etc	. . .	. . .	. . .	. . .	. . .	. . .	. . .	. . .	. . . . .	. . . .
Total on the flock		**652**	**5.34**	**1.71**	**0.07**	**x**	**x**	**66.3**	**x**	**x**

where:
N – number of rams descendants;
M_d – arithmetical average of ranking score of lamb-descendant;
M_t – arithmetical average of ranking score of lambs on whole flock;
σ – average of square deviation of the score (characters variability);
m – arithmetical average error;
d – difference between arithmetical average of rams progeny score and flocks average;
t_d –certainty coefficient of difference between arithmetical averages.

Especially important and unexpected is the fact that other 2 rams with registration numbers 5422 0073, being from reducers category, as a result of testing by simple method, have become ameliorators, as a result of genotypic testing after the progeny fur skin qualities, by second biometrical scoring method, where first ram obtained the category of ordinary ameliorator, with the first threshold certainty of the theory of probability forecasts without error according to Student ($t_d = 2.11$; $P < 0.05$) and the second breeder obtained the category of relative ameliorator, because the certainty of the difference between the average score of the rams progeny fur skin qualities and average of the flock is to zero threshold ($t_d = 1.78$; $P < 0.1$).

If by determining the amelioration category of tested males after the progeny fur skin qualities, by the simple method, is influenced by only one key factor - the difference between the share of elite + Ist class descendants of the ram and average of the flock, then by determining the amelioration value of tested rams after the progeny fur skin qualities, by biometrical scoring system, are influenced by several factors, such as:

- **d_{t-d}**, difference between fur skin qualities of the tested rams lamb progeny, expressed by average ranking score and this index value at lambs from whole flock;
- **t_d**, difference certainty coefficient (d_{t-d}), which allows the formulation of a certain conclusion about amelioration value of the tested ram and which by its deduction formula depends on:
- **m_t** and **m_d**, the error of arithmetical average (M_t) and ranking score of the lambs over the flock and this characters of the tested rams progeny (M_d);
- **σ**, the average deviation of the lambs ranking score squares (characters variability);
- **N,** number of rams descendants, or of lambs on whole flock.

We would like to mention that, while genotypic determining of breeders amelioration category by the second processing method of test

materials after the progeny qualities, each descendant class value, taken apart, has influence in determining the average score of the tested rams descendants (M_d). Thus, the descendants of the elite class contribute to the formation of the average ranking and genotype value with 8-10 points, those of I^{st} class - with 5-7 points, those of II^{nd} class - with 3-4 points, and those of brac - category with only 1-2 points. Therefore, the data from examined examples (Tables 1 and 2), indicates that as the tested rams progeny contains several individuals of higher classes with high score, as thereof average score is bigger and, vice versa, as the breeder has as descendants less lambs of higher classes and more lambs of inferior classes, as more the average value of ranking score of ram's descendants is lower.

The characters variability (σ) of the ranking score influences directly the value of the arithmetical average error (M) of this character. In Table 2, we see that as more the ranking score variability is higher, as more the ranking average error increases, and vice versa, as less the ranking variability is, as less is the average error.

The progeny number of the tested ram (N), influences also, directly and inversely, the arithmetical average error by its determining formula ($m = \sigma / \sqrt{N}$). From the results examined above, we see that as bigger is the number of descendants, as lower is the arithmetical average error, and, vice-versa, with the decrease of the descendants number, the arithmetical average error of the ranking score increase.

And finally, all these factors up-nominated (d_{t-d}, m_t, m_d, σ, N), taken all together, influence the certainty coefficient (t_d) of the difference between the average score of the lamb descendant ranking and average of this character on the flock by its determining formula ($td = d_{t-d} / \sqrt{m_d^2 + m_t^2}$), based on which can be made a definite conclusion concerning the rams amelioration value tested after the progeny fur skin qualities by biometrical score method.

The analysis of research results (Tables 2 and 3) shows that, with the increase of the certainty coefficient value of the difference (M_d-M_t) of selected character value, increase respectively also the rams amelioration degree tested after the progeny fur skin qualities by biometric score method.

Thus, we have concluded that the category and amelioration degree of Karakul rams, tested after progeny fur skin qualities, by biometric score method, may be defined, depending on the certainty criterion size (t_d) of difference between arithmetical averages of lambs ranking score.

Based on this conclusion, as a result of rams testing after the progeny fur skin qualities by biometric score method, we assigned (classified, split) the rams in different value groups depending on the category and amelioration degree (Table 3).

Table 3. Distribution of rams tested by the amelioration category

No. o/o	Rams registering No.	Descendants **(N)**	Arithmetical average **(M)**	Square deviation **(σ)**	Average error **(m)**	Difference $M_t - M_d$ **(d)**	Certainty coefficient **(td)**	Certainty threshold **(B)**	Amelioration category of the ram
1	9031	79	6.14	1.98	0.22	+0.86	3.74	0.999	**I_{st}** degree ameliorator
2	8235	117	5.85	2.19	0.20	+0.57	2.71	0.99	**I_{st}** degree ameliorator
3	8148	60	5.90	2.06	0.27	+0.62	2.21	0.95	Ordinary ameliorator
4	9115	55	5.80	2.07	0.28	+0.52	1.79	0.90	Relative ameliorator
5	8071	143	5.36	2.02	0.17	+0.08	0.44	-	Neutral
6	9235	88	4.48	2.00	0.21	-0.80	3.64	0.999	Certain reducer
7	2683	43	5.09	2.21	0.34	-0.19	0.54	-	Relative reducer
8	3372	12	6.58	2.02	0.58	+1.30	2.24	0.95	Ordinary ameliorator
9	8705	18	5.61	1.91	0.45	+0.33	0.71	-	Neutral
etc.		. . .	. . .	. . .	. . .	. . .	. . .	. . .	. . . . .
Total on the flock		**1226**	**5.28**	**2.02**	**0.06**	**x**	**x**	**x**	**x**

As the basic argument, or landmark, for this classification, have served the four thresholds of probability forecasts without error after Student (zero-threshold - $B_0 = 0.90$ or $P < 0.1$; $B_1 = 0.95$ or $P < 0.05$; $B_2 = 0.99$ or $P < 0.01$; $B_3 = P < 0.001$). Depending on the classification of certainty coefficient of arithmetical average (M_t-M_d) of the ranking score of the descendant lambs and lambs on whole flock, Karakul rams were classified by the amelioration value in following categories:

Ist degree breeder - is conferred to rams whose progeny exceeds, by the average ranking score, the flocks level, with the highest certainty level of forecasts without error ($t_d \geq 3.3$; $B \geq 0.999$). As a rule, the difference between the descendants value and average on the flock is very high. These rams are most valuable by the genotype of fur skin qualities and submit constantly their qualities through heritability to their progeny.

IInd degree breeder - is conferred to rams whose progeny exceeds, by average ranking score, the flocks level, with second degree of certainty threshold of forecasts without error. The coefficient value of the difference certainty (t_d) is located within the limits of 2.6-3.2 ($B \geq 0.99$). The difference, between the progeny value and the average on the flock, is also quite large. This kind of rams is also valuable and transmits by heredity constantly their fur skin qualities to descendants.

Ordinary breeder - is conferred to rams whose progeny exceeds, by the average ranking score, the flocks level with first degree certainty threshold of forecasts without error. The value of the certainty coefficient of the difference (t_d) is located within the limits of 2.0-2.5 ($B \geq 0.95$). The difference between the progeny value, and the flocks average is moderate, but definite.

This kind of rams is quite valuable and transmits through heredity to their progeny the fur skin qualities.

Relative ameliorator – is conferred to rams, whose progeny has a pattern above, by average ranking score, to exceed those of the flock, with certainty of zero thresholds of forecasts without error. The coefficient value of difference certainty (t_d) is located within limits 1.7 – 1.9 ($B \geq 0.90$). The difference between the progeny value and flocks average is not quietly sure, that's why it is considered that it has only a pattern above. Relative ameliorator rams are less valuable and certainly do not transmit through heredity to their progeny the fur skin qualities. These breeders need a repeated testation to check their amelioration value.

The neutral category - is assigned to rams, whose difference between the progeny average ranking score, and the flock's level is positive, but not significant, and the value of the certainty coefficient (t_d) is located within the limits of 0.0-1.6. This kind of rams do not represent a genotypic value, that's why, they cannot be used for breeding within the flock where were tested, than as triers. These rams may be tested by progeny fur skin qualities in other flocks.

The category of certain reducer - is assigned to rams, whose progeny yields, by the average ranking score to the flock's level, with the certainty coefficient of the difference, at least $t_d = 1.6$. Such rams represent a genetic danger for the flock, having a genotypic value lower than that of the flock, that's why, is recommended to be sent to the slaughter for meat.

The category of relative reducer - is assigned to rams, whose progeny yields, by the average ranking score, the flocks level, but the certainty coefficient of the difference is less than 1.6. Such rams also represents a genetic danger to the flock, having a genotypic value lower than that of the flock, so it is recommended to be removed from the flock.

From above mentioned example, is obvious, that the largest amelioration category has the ram no. 9031, whose progeny exceed the arithmetical average value of the lambs ranking score on whole flock with 0.86 points, or with 16.3% ($P<0.001$). The certainty coefficient of the difference is quite big - $\mathbf{t_d} = 3.74$. Therefore, the deduced conclusion is, that, to this breeder will be conferred the category of I_{st} degree ameliorator, and it is certain with highest probability threshold of forecasts without error.

The breeder no. 8235 has a progeny, which exceeds, by fur skin qualities score, the flocks average with 0.57 points, or 10.8%, with the certainty coefficient of this difference of $t_d = 2.71$ ($P < 0.01$) is, also, of high genotypic value, with the certainty of second degree

threshold probability of forecasts without error, and has the II[nd] degree breeder's category.

It is interesting the example of ram no. 8148, whose progeny exceeds, by fur skin qualities score, the flocks level with 0.62 points or 11.7%. At first view, this breeder, has a difference of average score of the progeny fur skin qualities, compared to the flock, bigger than that of ram no. 8235, it would seem that it has a better amelioration value, but due to a lower certainty coefficient of this difference (t_d = 2.21; P < 0.05), this ram is assigned in a lower category of ordinary breeder.

The ram with registration no. 9115 has a progeny that exceeds, after fur skin qualities score, the flocks average with 0.52 points or 9.8%, with the certainty coefficient of this difference of only t_d = 1.79, has a lower genotypic value, compared to its fellows. This breeder is assigned in the category of relative ameliorator, which means that after the testing of progeny fur skin qualities it is required an iteration to specify the amelioration category.

At the same time, from the same table, we can observe that the ram no. 8705, although exceeds the flocks average by progeny average ranking score with 0.33 points, or 6.3%, cannot be considered an ameliorator, because the difference by this index is not definite according to the probability threshold of forecasts without error (t_d = 0.71; P > 0.1), therefore, he was assigned to the neutral category. In case if, the data processing of tested rams by progeny fur skin qualities, would be done, according to the first method, this breeder would be considered ameliorator.

The breeder no. 9235 of this table, is an example of a true certain reducer, because its progeny yields, by fur skin qualities score, the flocks average with 0.80 points or 15.2%. The certainty coefficient of this difference constitutes t_d = 3.64 (P < 0.001). Therefore, the conclusion regarding the amelioration category of this ram is certain with the highest probability threshold of the forecast without error.

And the most interesting example of this table is the ram no. 3372, which managed to get into the category of ordinary ameliorator, with a low number of progeny (only 12 lambs), which exceeded the flocks average by ranking score with 0.58 points or 24.6% (t_d = 2.24; P <0.05). The example demonstrates that by applying the scoring biometric method at Karakul rams testing by progeny fur skin qualities, in some cases, can be obtained certain results, on a small number of descendants (10-12 lambs).

These aspects of rams genotypic testing by progeny fur skin qualities have a major importance in identifying the real amelioration breeders, which contributes to the genetic amelioration of the flock. Only real ameliorators, with a high certainty coefficient of the value difference of progeny fur skin qualities, compared to the flocks average, can be used as founders, or genealogical lines continuers of high performance, for the genetic amelioration of the flock, for the improvement of the genetic structure of interracial type or of the breed as a whole.

CONCLUSIONS

1. Only the application of the terminological system of Karakul lambs assessment and of simple method of testing the rams after the progeny fur skin qualities, are not sufficient for an objective evaluation of breeders amelioration category. The conclusions made on the basis of the test results with the implementation of this system and this method is often unreliable and contradictory.

2. The use of decimal scoring system at Karakul lambs assessment and of scoring biometric method by rams testing after the progeny fur skin qualities allows the objective genotypic evaluation of breeders amelioration category with the determination of the conclusions certainty criterion regarding the amelioration of each ram, taken apart.

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SELECTION OF MOLDOVAN KARAKUL SHEEP BY THE BODY WEIGHT

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Abstract

The research purpose was the factors which determine the variability of Karakul sheep body weight, heritability degree identification and repeatability of this character, determining the selection efficiency of the sheep by this important character. The research has been made on the Moldovan Karakul sheep flock INZMV, village of Maximovca, Anenii Noi district. The body weight of the sheep was determined with technical weighing scales: at birth, 20-90 days, at 6-18 months, adult age, annually. The research has shown that the body weight of Karakul sheep has a decisive impact on meat production and on fur skin surface obtained from new-born lambs. Thus, from the rams of 6 months, which were intensively fatted, with body mass 35-36 kg, it were obtained carcasses of 16.6 ± 0.3 kg, of R and U categories, according to UE classification scale. The slaughter yield of these ram lambs was 47.5 %. From reformed sheep, which were intensively fatted, with body mass of 64-65 kg, were obtained massive carcasses, with weight of 32.3 ± 1.0 kg, reported to R and U categories, according to UE classification scale. The slaughter yield of these sheep was 54.8 %. It has been found that, as bigger are the lambs at birth, that bigger is the standard surface of the fur skins. With increasing of lambs body weight at birth from 3.9 up to 4.4 kg, the share fur skins with big surface (> 1400 cm^2),increase from 28.2 % up to 63.8 % or 2.3 times (P < 0.001). With further increase of lambs body weight up to 5.4 kg, increase substantially the share of fur skins with very big surface (> 1800 cm^2), from 15.3 % up to 91.4 % or with 6.0 times (P < 0.001). Phenotypic correlation coefficient (r_{xy}) between body weight of Karakul lambs and standard surface of fur skins is: $r_{xy} = 0.64 \pm 0.04$ (n_{st} = 10-15-22; P < 0.001). From corpulent parents it were obtained progeny with a big body weight. The rams with big body weight 86-100 kg have given progeny with high body weight at birth of 5.16 ± 0.03 kg, rams with average body weight of 71- 85 kg, have given progeny with average body weight of 4.78 ± 0.04 kg, and rams with low body weight of 60 – 70 kg, have given progeny with lowest body weight – 4.45 ± 0.04 kg. The progeny of corpulent rams exceeded, after the body weight at birth, the progeny of average rams with 0.38 kg or 7.9 % (P < 0.001) and of the small ones with 0.71 kg or 16.0 % (P < 0.001). This shows that between body weight of ram fathers and body weight of lambs progeny exist a positive genotypic correlation. In good years with sufficient vegetation on the pasture, the body weight of the sheep achieved a high level, and was on average at breeding batch: at adult rams – 92.6 ± 3.0 kg, at sheep jelly – 57.8 ± 0.3 kg, at rams of 18 months – 63.6 ± 5.5 kg, at sheep of 18 months - 53,1 ± 0,4 kg, being significantly higher, than in bad years, respectively, with 11.7% (P < 0.05), 14.8% (P < 0.001), 16,1% (P < 0.05) and 7.6% (P <0.001). Based on the research results, it were made following conclusions: body weight of Moldovan Karakul sheep is one of the most important morph productive selection characters, because it has a direct impact on both, the meat production and on fur skins surface obtained from lambs at age of 2-5 days after birth; the internal factors which treat the phenotypic variability of body weight, the most important is heredity (genotype), and of external factors – nutrition and feeding (forage base); sheep of Moldovan Karakul type are more precocious, comparing to sheep of Asian Karakul, which represents one of the interior biological particularities of this interracial type of sheep. This type of sheep continue to grow in body until the age of 2.5-3.5 years. Selection of Moldovan Karakul sheep after the body weight is modestly efficient, thanks to heritability and moderate repeatability of this character. The coefficient of body weight heritability is not very high ($h^2 = 0.3$), but quiet ($t_r = 2.6$; P < 0.01). The coefficient of body weight repeatability (r_w) at different age of young sheep and adult sheep varies within the precinct of 0.23 – 0.47.

Keywords: *body weight, sheep, Moldovan Karakul, heritability, repeatability.*

INTRODUCTION

The Karakul race sheep has a number of biological morpho-productive features, one of which is basic and refers to the unique, very beautiful fur skin of the new-born lamb, slaughtered at 1-5 days after birth. For its value, it is considered a luxury fur skin, located in the same row as the noblest natural furs (sable, mink, fox). This is explained by the superior aesthetic ornamental qualities of the loops, excellent silky aspect of follicle sheath, perfect thermal features, as well the durable resistance to exploitation of fur skin apparel. For these reasons, the lambs fur skin in first days after birth is considered, so far, primary and important main production of Karakul race sheep, reared in Central Asian countries, South-West Africa and in other geographical worlds regions.

Multiple research in this area (Adametz, 1927; Bosânciuc and Taftă, 1997; Hundt, 1954; Ильев, 1957; Васин, 1971; Pascal, 2007; Ştefănescu et al., 1973; Иванов, 1914; Ursu and Romanescu, 1997; Абдиваитов, 1978; Абуов, 2010; Алимбаев, 2011; Аралбаев, 2011) demonstrate that the sheep selection of this breed in nominated areas, is done, mainly, according to fur skin qualities, not paying attention to the other animal morph-productive characters. Subsequently, other countries in other regions of the world (Kechawartz, 1957;

Nicov, 1936; Taftă et al., 1997; Бастаев, 2010) were carried out research also for other morph-productive characters of this sheep breed, especially for the milk and meat production.

The research accomplished by some notorious scientists (Дюсегалиев, 2010; Ескара, 2011), at the beginning of the XXth century, have shown, that, at this stage of spreading Karakul race sheep in Europe and other geographic regions of the world, the relative economic value of the fur skin, occupies the first place by share (56.4 – 60.4%) in total income, obtained from a sheep per year. Of this share, results the conclusion that the fur skin production was indeed, by then, primary and very important.

Comparing the results of our current research (Иванов, 1914), to scientific research from a century ago, we ascertain that the situation has changed entirely, since the economic value of fur skin production, taken together with the lamb, as related production in the mixed version of the sheep exploitation, occupies a share of only 11,8% in the total income, obtained from a sheep per year. In this context, the fur skin production being one of the selection characters of Moldovan Karakul race, although it remains paramount and cannot be considered principal. At the same time, we observe that, in this period of time, the meat production of Karakul sheep, according to the share of economic value, in the total income obtained from a sheep per year, has been moved from the last place - with the share of 2.6%, to second place (by milk production) - with a share of 27.1%, exceeding, thereby, with 2.3 times the economic value of the fur skin character. According to the economic value, the fur skin of Karakul lamb (the price of the fur skin), currently, in the Republic of Moldova, is equivalent with just 2.33 kg of meat in the carcass. Such transformations of economic value of Karakul race selection characters had happen, over time, also in other geographic regions of the world. In order to compare, we specify that, according to research from Kazakhstan (Бастаев А.У., Онкуляев М.А., 2003; Бастаев, 2010), the price of one Karakul fur skin, in this country, is equivalent with 2.0 kg of carcass meat.

In these economic conditions, the body mass of Karakul sheep, which determines the meat production, becomes one of the main characters, which are currently selected.

At the same time, in the special literature of this field (Плохинский, 1969; Шефер, 1977; Юлдашбаев, 2009), there is not sufficient information on body weight at Karakul race sheep and selection results according to this feature of the sheep populations. Therefore, the research and study of the factors that treat the body weight variability of Karakul sheep, identification of heritability and repeatability degree of this morph-productive character, determination of the efficiency of the sheep populations selection after this important character, is a quite actual issue.

MATERIALS AND METHODS

The research has been carried out on the livestock of Moldova Karakul sheep from Experimental Section of the National Institute for Animal Science and Veterinary Medicine (INZMV), v. Maximovca, district of Anenii Noi.

The body weight of the sheep was determined permanently, in accordance with the Assessment Instructions of Karakul sheep, with amelioration principles in Republic of Moldova (1996).

Youth sheep was weighed several times per year, at different ages, with different technical scales. The lamb was weighed at birth with hand scale, according to the new system, recommended by us (Buzu, 2012). At the age of 20 days, the lambs were weighed individually, with the technical medical scale for weighing children, with capacity up to 15 kg and accuracy gradation of 0.01 kg. From the age of 3 months, the youth sheep, and adult sheep, were weighed individually at the technical scales with capacity up to 150 kg and with accuracy gradation of 0.1 kg.

In order to carry out the sheep weighing, on the scale shelf, was installed and attached a narrow cage with two doors (incoming and outgoing). The cage dimensions were provided, to fit a tightly closed hornless ram (horned rams were weighed separately in a bigger cage). This sheep cage was entered voluntary at the door, after it was closed immediately.

In order to ensure the conditions for voluntary entry of the sheep in the cage, in front of the

scale was arranged another enclosure which fit 50 sheep with pen and hall to entry. At the cage exit was arranged another enclosure with a capacity of 50-200 sheep. At the simultaneous opening of the doors, the sheep from the entry hall, seeing through the cage the sheep from the opposite enclosure (outgoing), enters voluntary the cage, after which the doors would be closed immediately. After weighing, at the opening of the exit door, the sheep went out freely of the cage. This weighing system allowed the examination of appearance and constitution of the animal, the animals weight registration, without its injury, contributing to relief the staff work and increase its productivity.

According to the instructions in force, in addition to weighing the youth sheep, whole sheep population of all ages, was weighed in fall, at the end of October, after which, was started the season of sheep insemination, according to the elaborated mating plan. The data of sheep weighing and notes of their appearance and constitution defects identifications, were recorded in the Register body weight evidence of Karakul sheep (F-10K).

The data obtained, as a result of investigations were statistically processed, using computer software „STATISTICA – 6" and evaluated their certainty, according to variation biometric statistics, by methods of Плохинский, 1969.

RESULTS AND DISCUSSIONS

Results of the research have shown that body weight of Moldovan Karakul sheep, presents one of the most important morpho-productive character, given that it has a direct impact, both on the meat production, derived from the slaughtered animals carcasses, as well as on the fur skins surfaces obtained from the lambs slaughtered at the age of 2-5 days after birth.

Despite the fact that, according to the communications of Дъячков, 1980, the body weight of Karakul sheep of Asian type, compared to other breeds, is relatively low (live weight of the ewes constitute 40-45 kg, of the rams - 50-60 kg), the meat production cannot be neglected, because it complements significantly the food ration of human population.

In the Republic of Moldova, the meat production of Moldovan Karakul sheep comes from raw lambs carcasses slaughtered for fur skin (3.3-3.8 kg per carcass non-beheaded), from youth sheep carcasses (with unsatisfactory fur skin qualities) grown and fattened for meat, until the age of 6 or 18 months, as well from adult sheep carcasses, recast and removed, for various reasons, out of reproductive cycle of the flock (Table 1). Due to the fact that Moldovan Karakul sheep have a more massive development, compared to Asian type, from them will be obtained also a quite good meat production. The research has shown that, since the youth sheep of six months, with body weight of 28-32 kg (ewes) can be obtained carcasses with average weight of 12.7 ± 0.24 kg, of C category, according to EU classification grid (Pascal, 2007).

Table 1. Body weight and meat production potential of Moldovan Karakul sheep

Specification	Youth sheep of **6** months		Recast sheep	
	ewes	fattened ram lambs	Non fattened	Fattened
Body weight before slaughter, kg	28.6 ± 0.46	35.8 ± 0.3	50.0 ± 0.12	64.6 ±1.1
Carcass weight, kg	12.7 ± 0.24	16.6 ± 0.2	22.3 ± 0.49	32.3 ± 1.0
Inner fat with kidneys, kg	0.1 ± 0.05	0.4 ± 0.1	2.0 ± 0.11	3.1 ± 0.4
Slaughter weight, kg	12.8 ± 0.33	17.0 ± 0.2	24.3 ± 0.57	35.4 ± 1.0
Slaughter yield, %	44.8	47.5	48.6	54.8

The slaughter yield of the animals is 44.7%. From ram lambs of 6 months, intensively fattened, with body weight of 35-36 kg, can be obtained good and very good carcasses, with a weight of 16.6 ± 0.2 kg, of R and U categories, fat and medium fat, according to EU classification grid. The slaughter yield of these ram lambs is 47.5%. Youth sheep carcasses, of C, R and U categories, are among the most requested for export, including for EU. From

the adult recast non fattened ewes, with body weight before slaughter of 50 kg, it may be obtained mediocre and quite good carcasses, with the weight of 22.3 ± 0.49 kg, reported to the types P and O, according to EU classification grid. The slaughter yield of these ewes is 48.6%.

From recast and intensively fattened ewes, with the body weight, before slaughter, of 64-65 kg, it may be obtained massive, good and very good, fat and very fat carcasses, with the weight of 32.3 ± 1.0 kg, reported to the types R and U, according to EU classification grid. The slaughter yield of these sheep is 54.8%. Recast sheep carcasses of category P, O, R and U are required for export, particularly, to Arab countries.

At the same time, the body weight of Karakul sheep has particular importance to obtain fur skins of large surface, because, on third equal terms, the fur skin's surface determines its commercial value.

The research demonstrates that the lamb body weight at birth has a direct and positive correlation with fur skin surface, obtained at slaughter (Table 2).

Table 2. Relation between body weight of Moldovan Karakul lambs at birth and standard surface of fur skins obtained from them

Body weight of the lambs at birth, kg	Number of lambs	Including fur skin surface (cm^2):									
		< 999		1000 - 1399		1400 - 1799		1800 - 2199		> 2200	
		pieces	%	pieces	%	pieces	%	pieces	%	pieces	%
> 5.5	14	-	-	-	-	-	-	-	-	14	100
5.0 – 5.4	35	-	-	-	-	-	-	32	91.4	3	8.6
4.5 – 4.9	57	-	-	-	-	34	59.7	19	33.3	4	7.0
4.0 – 4.4	47	-	-	5	10.6	30	63.8	12	25.5	-	-
< 3.9	39	6	15.4	15	38.4	11	28.2	6	15.3	1	2.6
Total	**192**	**6**	**3.1**	**20**	**10.4**	**75**	**39.1**	**69**	**35.9**	**22**	**11.5**

From the presented data, it is evident, that, as more the lambs at birth are bigger, the standard surface of the fur skins is larger. It was found, that the largest surface have had the fur skins obtained from lambs, with body weight, at birth of over 5.5 kg. The lambs of this batch had the fur skins over 2200 cm^2. Also, lambs with body weight of 5.0-5.4 kg had fur skins of very large surface (> 1800 cm^2). The fur skins with the surface less than 1800 cm^2, in these batches of lambs, were not at all. The lambs, with the body weight at birth of 4.5-4.9 kg have, mostly, (59.7%) fur skins with large surface (> 1400 cm^2) and 40.3% fur skins with very large surface.

The lowest surface of the fur skins was obtained from small lambs, with body weight at birth < 3.9 kg. Among fur skins obtained from lambs of this batch, it were 38.4% with middle surface (900-1400 cm^2) and 15.4%, with small surface (700-900 cm^2).

It was found that, with increasing of lambs body weight at birth, from 3.9 to 4.4 kg, the yield of fur skins with the medium surface decreases from 38.4% to 10.6% or 3.6 times (P < 0.001), and of fur skins with large area, increase from 28.2% to 63.8% or 2.3 times (P < 0,001). With further growth of lambs body weight at birth from 3.9 to 5.4 kg, substantially increase the share of fur skins with the very large surface, from 15.3% to 91.4% or 6.0 times (P < 0.001) and entirely disappears the share of fur skins with small and medium surface.

The phenotypic correlation coefficient (r_{xy}) between Karakul lambs body weight at birth, and the standard surface of fur skins obtained from these lambs is $r_{xy} = 0.64 \pm 0.04$ (n_{st} = 10-15-22). The certainty significance of the correlation coefficient has the highest probability threshold of forecasts without error (P < 0.001).

Proceeding from these considerations, the Moldovan Karakul sheep body weight was included in the genetic amelioration process of this type of sheep populations, as one of the most important selection characters at all stages, starting from assessment (1-2 days after birth), at the age of 20 days, 3 months (weaning), 6 months, 18 months and, annually, at the adult age.

The body development of Karakul sheep, as the other races, depends on both internal factors, especially, heredity (genotype, breed, line), as

well as external ones, especially on food factors (Ursu and Romanescu, 1997; Нел, 1975; Юлдашбаев, 2009).

Our research has shown that from corpulent parents (with a big body weight) are obtained descendants, with the body weight also big. Ewe and ram from ascendants with a big body development have heritable genetic capabilities of producing corpulent descendants. Mating the ewes with corpulent rams, contributes to obtain corpulent lambs (Table 3).

Table 3. Body weight of Moldovan Karakul lambs at birth, depending on body development of father rams, kg

Father-rams, kg			Lambs (descendants) at birth			
No. batch	n	Body weight., kg	n	M ± m	σ	C_v, %
1	7	86 - 100	581	5.16 ± 0.03	0.84	16.2
2	4	71 - 85	487	4.78 ± 0.04	0.86	17.8
3	6	60 - 70	517	4.45 ± 0.04	0.93	20.9

The research results have shown that, in one and the same flock, under equal conditions of growth and maintenance, from corpulent rams of Ist batch, with a mass of 86-100 kg, was obtained the most corpulent progeny with the body weight at birth of 5.16 ± 0.03 kg; from rams of IInd batch, with middle body weight, of 71 - 85 kg, was obtained progeny with medium body weight of 4.78 ± 0.04 kg and from rams of IIIrd batch, with body weight less than 60 - 70 kg, was obtained progeny with the lowest body development – 4.45 ± 0.04 kg.

The progeny of the corpulent rams of I_{st} batch exceeded, by body weight at birth, their fellows of IInd batch with 0.38 kg, or 7.9% ($P < 0.001$), and those of IIIrd batch with 0.71 kg, or 16.0% ($P < 0.001$). Lamb-descendants of the rams from IInd batch (with the body weight of 71 - 85 kg), exceeded, by body weight at birth, their fellows of IIIrd batch (descendants of rams with body weight of 60-70 kg) with 0.33 kg, or 7.4% ($P < 0.001$). It demonstrates that between body weight of father-rams and body weight of lambs-descendants at birth, there is a clear genotypic correlation. The heritability coefficient of body weight is not high ($h^2 = 0.3$), but quite significant ($t_r = 2.6$; $P < 0.01$).

The body weight of Moldovan Karakul lambs at birth has a quite evident repeatability at different ages and periods of development of youth sheep, and subsequently at the adult age. The repeatability coefficient value (r_w) of body weight, at different ages of the youth sheep, as well of the adult sheep, varies within the limits of 0.23-0.47 (Table 4).

Table 4. Genetic repeatability ($\mathbf{r_w}$) of body weight at Moldovan Karakul youth sheep

Age of youth sheep	Repeatability coefficient value, $\mathbf{r_w \pm m_r}$	Certainty coefficient, $\mathbf{t_{rw}}$	Certainty threshold acc. to Student
At birth – 20 days	0.47 ± 0.07	6.7	P < 0.001
At birth – 90 days	0.39 ± 0.09	4.3	P < 0.001
At birth – 6 months	0.26 ± 0.08	3.2	P < 0.01
At birth – 18 months	0.23 ± 0.09	2.6	P < 0.01
Adult age	0.25 ± 0.09	2.8	P < 0.01

This means that, as the youth sheep, is more developed at an early age, as bigger will be its body weight at the adult age. These parameters confirm that sheep selection by body weight of the youth, at different ages, as well at the adult age, is real and true. The selection effect, calculated by the classical formula **($E = h^2 \cdot d$)**, is not big, but quite significant. For example, if the average ewes body weight of the flock, would constitute 53 kg, and the average of the breeding batch would be 56 kg, then the selection differential would be 3.0 kg, and the selection effect in a generation would be:

$$\mathbf{E = 0.3 \cdot 3.0 = 0.9\ kg.}$$

Therefore, the selection of Moldovan Karakul sheep, by their body weight, is effective and

contributes to the genetic amelioration of this character in the flock and creation of sheep populations with big body development.

According to researches of Дъячков, 1980, the youth sheep of Asian type Karakul race, has a sufficient growth rhythm, reaching at the age of 4.5 – 5.0 months, the body weight of 20-25 kg. Our research has shown that, the body development of Moldovan Karakul sheep and their precocity are, under conditions of Republic of Moldova, much higher, compared to the sheep of Asian type.

The sheep body weight is closely related with environmental conditions, especially, with natural forage (pasture vegetation), because, in Republic of Moldova, the sheep are, from April - May, until November-December, depending, exclusively, on natural pasture vegetation and stubbles of harvest grain (Table 5).

In particular, the sheep have access, in the period of September-October, to stubble of harvested maize, of which reasons they reach, at the end of October, the biggest fattening condition, that is why, the weighting of Karakul sheep is recommended to be done at the end of October, when the animal's body development potential is fully realized.

Thus, in the favourable years with sufficient vegetation, the average body weight of adult rams is 81.9 – 92.6 kg. The highest value of the rams body weight is 107 kg. All the rams selected from breeding batches were quite corpulent, with the typical exterior and robust appearance.

The sheep body weight has reached the average on the flock of 55.0 ± 0.3 kg and 55.5 ± 0.3 kg, which exceed the standard level of Asian type Karakul breed, (43 kg) with 12.0 – 12.5 kg, or with 27.9 – 29.0 % ($P < 0.001$).

Table 5. Body weight of Moldovan Karakul sheep in different years

Total on the flock of INZMV			Selected breeding batch		
Age group	n	M ± m	n	M ± m	Max
2006 (favourable year, with sufficient vegetation)					
Rams	6	92.6 ± 3.0	6	92.6 ± 3.0	107
Ewes	366	55.0 ± 0.3	198	57.8 ± 0.3	73
Rams of 18 months	8	59.8 ± 3.3	4	63.6 ± 5.5	80
Ewes of 18 months	138	52.2 ± 0.4	102	53.1 ± 0.4	62
Ram lambs of 6 months	34	29.7 ± 0.8	10	30.7 ± 1.1	40
Ewe lambs of 6 months	139	27.2 ± 0.4	89	29.0 ± 0.4	39
Lambs of 3 months	178	22.7 ± 0.3	162	23.0 ± 0.3	28
Lambs of 20 days	190	8.5 ± 0.8	170	8.7 ± 0.8	12
2007 (drought year, with insufficient vegetation)					
Rams	8	82.9 ± 4.2	6	88.2 ± 3.0	102
Ewes	399	47.9 ± 0.4	119	49.9 ± 0.5	65
Rams of 18 months	17	51.5 ± 1.7	5	57.2 ± 3.7	70
Ewes of 18 months	113	48.5 ± 0.5	91	49.0 ± 0.5	62
Ram lambs of 6 months	68	29.3 ± 0.8	11	35.8 ± 1.5	45
Ewe lambs of 6 months	150	25.8 ± 0.4	83	28.1 ± 0.3	39
Lambs of 3 months	210	18.5 ± 0.3	150	19.5 ± 0.2	24
Lambs of 20 days	226	6.6 ± 0.1	170	7.4 ± 0.1	10
2008 (favourable year, with sufficient vegetation)					
Rams	10	81.9 ± 2.6	4	89.0 ± 2.2	95
Ewes	292	55.5 ± 0.3	163	57.2 ± 0.4	75
Rams of 18 months	8	56.0 ± 3.0	3	64.0 ± 2.0	66
Ewes of 18 months	118	50.6 ± 0.5	85	52.1 ± 0.5	62
Ram lambs of 6 months	10	33.6 ± 1.8	4	38.8 ± 2.8	46
Ewe lambs of 6 months	138	31.7 ± 0.4	66	34.4 ± 0.4	42
Lambs of 3 months	164	22.1 ± 0.5	160	22.0 ± 0.5	27
Lambs of 20 days	226	7.6 ± 0.1	210	7.7 ± 0.1	11

Some ewes from this flock have reached maximum values of body development up to 73 and 75 kg. Having such a wide variability of this character, in the flock, it were selected quite corpulent ewes from breeding batches, with the average body weight of 57.8 ± 0.3 kg and 57.2 ± 0.4kg.

The youth sheep, having a high precocity of growth at all ages, has a quite good body development. The rams able to remount, at the age of 18 months, have had an average body weight of 59.8 ± 3.3 kg and 56.0 ± 3.0 kg with maximum values, at some individuals, of 80 and 66 kg. In breeding batches, it were selected rams able to remount, with average body weight, at same age of 63.6 ± 5.5 kg and 64.0 ± 2.0 kg. Due to the high precocity, ewes of 18 months have, also, a good development. Their body weight was, in favourable years, in average on the flock 52.2 ± 0.4 kg and 50.6 ± 0.5 kg, which exceeds the standard level of Asian type Karakul race (36 kg) with 16.2-14.6 kg or with 45.0-40.5% ($P < 0.001$). Some ewes, from this flock, have reached maximum values of body development up to 62 kg. In breeding batches, were selected ewes, with an average body weight of 53.1 ± 0.4 kg and 52.1 ± 0.5 kg.

The youth sheep of 6 months, in favourable years with satisfactory vegetation, reaches, also, a good body development. Due to a quite fast growth speed, ram lambs have had an average body weight of 29.7 ± 0.8 kg and 33.6 ± 1.8 kg, with maximal values up to 40 – 46 kg. In favourable years, with good forage base, in breeding batches, were selected ram lambs, with average body weight of 35.8 – 38.8 kg. The ewe lambs of 6 months have had, during these years, an average body weight on the flock equal with 27.2 ± 0.4 kg and 31.7 ± 0.4 kg. In the breeding batches, were selected ewe lambs, with the average body weight of 29.0 – 34.4 kg. Some ewe lambs reached, at this age, maximal values of 39 and 42 kg. These indices show a very high potential of this kind of sheep, regarding their precocity and body development of youth sheep at this age.

The quite early precocity of Moldovan Karakul youth sheep manifests immediately after birth. Thus, at the age of 20 days, lambs (mainly ewe lambs) have, during favourable years, the body weight equal to 8.5 ± 0.8 kg and 7.6 ± 0.1 kg, with maximum values at some individuals of 12 - 11 kg. At the age of 3 months (weaning), they reach, in average, the body weight of 22.7 ± 0.3 kg and 22.1 ± 0.5 kg, which is equivalent to the body weight of the Asian type youth sheep, at the age of 4.5 – 5.0 months (Дъячков, 1980).

Therefore, the precocity and a good body development of the youth sheep, at all ages, as well the big body weight of adult sheep, is one of the most important biological features which distinguish the type of Moldovan Karakul sheep versus other interracial regional geographical types of Karakul race, known in the world.

According to the authors communications (Васин, 1971), the classic Karakul race sheep (asian) are latish animals, because their body development, continues until a quite advanced age of 6 years.

Our research has shown, that the body development of Moldovan Karakul sheep, continues up to the age of 2.5 – 3.5 years (Table 6).

Table 6. Body weight of Moldovan Karakul ewes, depending on their age, kg (n = 55)

Ewes age, years	M ± m	σ	C_v, %
1.5	50.62 ± 0.53	5.43	10.7
2.5	55.74 ± 0.61	5.69	10.2
3.5	55.96 ± 0.55	5.61	10.0
4.5	56.77 ± 0.60	5.76	10.1
5.5	55.15 ± 0.69	5.85	10.6

Thus, within the age range from 1.5 up to 2.5 years, body weight of the sheep, increased significantly with 5.12 kg, or 10.1% ($P < 0.001$). From the age of 2.5 years up to 4.5 years, the body weight of the sheep increased insignificantly (just with 1.03 kg, or with 1.8%, $P > 0.5$). This allows us to affirm that the body weight of Moldovan Karakul sheep, beginning with the age of 3.5 years and up to 4.5 years, basically, remains at a constant level, after that, starts a slight decrease tendency. From these results, we can see the fact, that the type of Moldovan Karakul sheep is slightly more precocious, compared to the type of Asian Karakul sheep, which is one of the biological interior characteristics of this interracial type of sheep.

On the basis of scientific research of correlative connections, of body weight heritability and repeatability, as well as the analysis of the results obtained during several years of sheep population selection, we developed the minimal

requirements of the standard breed, as well as the parameters of the standard-purpose, concerning this character's development of Moldovan Karakul sheep at different ages. The developed parameters are used at animals assessment, their selection in breeding batches and their admission to reproduce (Table 7).

Table 7. Parameters of standard body mass Karakul sheep of different age groups

Age group	Known standards		Developed standards	
	Karakul, Romania, Elite class requirements (Pascal, 2007)	Asian Karakul, assessment instructions, Ist class requirements	Moldovan Karakul, Ist class requirements	Moldovan Karakul, standard-purpose
Rams	55	55	75	85 – 100
Ewes	40	43	48	50 – 55
Rams 18 months	41	42	55	65 – 70
Ewes 18 months	35	36	43	44 – 49
Ram lambs 6 months	-	27	32	35 - 40
Ewe lambs 6 months	-	25	29	30 - 35
Ram lambs 3 months	-	16	19	20 – 22
Ewe lambs 3 months	-	15	17	18 - 20
Ram lambs 20 days	-	7.4	8.0	8.5 – 9.0
Ewe lambs 20 days	-	7.0	7.5	8.0 – 8.5

The principle of these parameters development is based on general rules, known in animal livestock, where any animal breed standard is established and recognized at the level of the minimal requirements to enclose them into the Ist class category.

Compared to the breed standard, the standard-purpose represents a model of animals, which enclose within the parameters searched by selectors, starting from the initiation stage of the creation process of a breed, type, lines, up to the final stage of creation and assimilation of the realized selection.

It should be noted, that the body weight parameters, developed for Moldovan Karakul sheep, are much higher, compared to the breed standard for other types of Karakul sheep. Thus, the minimal requirements of body weight of Moldovan Karakul lambs, at the age of 20 days, to be assigned to the race standard, are higher, compared to Asian type Karakul sheep, at ram lambs - with 0.6 kg and ewe lambs - with 0.5 kg, or with 8.1 and, respectively, 7.1%.

At the age of 3 months, the standard parameters of the Moldovan Karakul youth sheep body weight are higher, compared to Asian type Karakul sheep, at ram lambs - with 3.0 kg and at ewe lambs - with 2.0 kg, or with 18.7 and, respectively, with 13.3%.

A bigger difference of the standard body weight of Moldovan Karakul youth sheep, compared to Asian type Karakul, remains also at the age of 6 months. Thus, the standard body weight parameters at ram lambs is bigger with 5.0 kg and at ewe lambs - with 4.0 kg, or with 18.5 and, respectively, 16.0%.

With age advancing, the difference between the standard body weight parameters of Moldovan Karakul sheep and Asian Karakul sheep will emphasize. Thus, at the age of 18 months, the standard body weight parameters at ram lambs is bigger with 13.0 kg and at ewe lambs - with 7.0 kg, or with 31.0 and, respectively, 19.4%.

At the adult age, the minimal body weight standard parameters of Moldovan Karakul rams, exceed the standard of Asian Karakul breed with 20.0 kg and, the ewes - with 5.0 kg, or 36.4 and, respectively, with 11.6%.

Finally, generalizing the standard-purpose parameters of Moldovan Karakul sheep body weight, we ascertain, that they are, at all gender and age groups, detached larger, compared to the parameters of Asian Karakul race. The above mentioned results, obtained during the process of body weight amelioration at the sheep population of INZMV, demonstrates that the progressive directed selection according to this character had secured, practically, the reaching of planned parameters and the achievement of intended purpose to create the new type of Moldovan Karakul sheep.

CONCLUSIONS

1. The body weight of Moldovan Karakul sheep is one of the most important morpho-productive selected character, since it has a direct impact, on both, the meat production, derived from the slaughtered animals carcasses, as well as on the surface of fur skins, obtained from lambs at the age of 2-5 days after birth.
2. The phenotypic variability of body weight is influenced by a number of internal and external factors. Main internal factors is heredity (genotype), and of the external factors - nutrition and feeding (forage base).
3. The Moldovan type Karakul sheep are more precocious compared to Asian type Karakul sheep, which is one of the internal biological particularity of this interracial sheep type. This kind of sheep continues to develop in body growth (body weight) up to the age of 2.5-3.5 years.
4. The selection of Moldovan Karakul sheep, by body weight is modestly effective, thanks to a moderate heritability and repeatability of this character. The body weight heritability coefficient is not high ($h^2 = 0.3$), but quite significant ($t_r = 2.6$; $P < 0.01$). The body weight repeatability coefficient (r_w) of the youth sheep at different ages, as well as of the adult sheep, varies within the limits of 0.23-0.47.

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STUDY ON THE HERITABILITY OF SOME MORPHO-PRODUCTIVE CHARACTERS OF APIS MELLIFERA CARPATICA BEE FAMILIES POPULATED IN THE ZONE OF CENTER OF THE REPUBLIC OF MOLDOVA

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Abstract

The aim of the research was to identify the degree of heritability of main biological morph-productive characters of Apis mellifera carpatica bees family to streamline the process of genetic improvement of bee populations. The research was performed on the population of experimental bee apiary of the Institute of Zoology of the Academy of Sciences located stationary in a forest glade in the center of the country. It was studied heritability of major morpho-produtive characters such as queen prolificacy, family strength, brood viability, resistance to disease and the amount of honey gathered in the nest. The heritability coefficient of morpho-productive characters was calculated by the method h^2 *based on determining the genotype correlation of the above mentioned characters at 20 pairs of mother-daughter families. Research results have shown that between homologous characters of mother families and daughter families exist a genotypic correlation at different levels, from below average until strong, depending on selected character and the amplitude of the phenotypic variability of character. It was found that the genotypic correlation between mothers and daughters characters is enough strong at characters with narrow phenotypic variability, such as: disease resistance, queen prolificacy and family strength, and lower at characters with wider phenotypic variability, such as brood viability and the amount of honey gathered in the nest. The amount of genotypic correlation coefficients* (r_{mf}) *of characters from the first group varies within the limits of* $r_{mf} = 0.78 \pm 0.05$ *and* $r_{mf} = 0.85 \pm 0.03$*. The coefficient of heritability* (h^2) *of these characters being also at a high level, in the range of 0.61 to 0.72 having a certitude of the highest threshold of the theory of probability forecasts without error after Student (P<0.001). At the morpho-productive characters from the second group, such as, the brood viability and the amount of honey gathered in the nest, the genotypic correlation had an average level, comprised within the limits of 0.60 ± 0.09 and 0.56 ± 0.09 with enough high certitude, and the coefficients of heritability of these characters were at below average level, in the range of 0.36 0.31 (P< 0,01). The obtained results of researches of heritability of principal biological morpho-productive characters have been used to elaborate the plan for genetic improvement of bee families and prediction selection effect, calculated by the formula* $E_s = h^2 \cdot d$, *where:* E_s *- selection effect of selected character obtained in a generation;* h^2*- heritability coefficient of selected character;* d *- the differential of selection, calculated by the formula:* $d = M_{lp} - M_{st}$*, where:* M_{lp}*- the average value of selected character on families of bees from the breeding stock;* M_{st}*- the average value of the selected character on the bee families from whole apiary. The selection effect of bee families by morph-productive biological characters from first group, with the heritability coefficient* (h^2) *over average, was bigger than the effect achieved in selection of bee families by the characters in the second group, the coefficient of heritability* (h^2) *below average.*

Keywords**:** *honeybee, morpho-productive characters, phenotypic correlations, heritability.*

INTRODUCTION

According to heredity legalities, the genetic information movement, determines all heredity elements: information inheriting from parents, the genotype formation in zygote, basic protein synthesis, organism development under certain life conditions (ontogenesis), its basic reactions to the exterior actions (the reaction norm), the formation of sexual cells and fusion of male and female gametes in the descendant's zygote, (Плохинский Н.А., 1969). The measure (degree), in which parents heredity can be accomplished (transmitted) to the descendant, has held concerned the researchers since a long time. Multiple researches, in this field (Boeking, 2000; Collins, 1984; Ridnerer, 1986; Siceanu, 2005; Siceanu A., 2012; Билаш, 1991; Ильев, 1984), have shown that some morpho-productive characters of bee families

have a greater heredity (inheritance) degree, and others - a lower inheritance degree. This phenomenon in the genetics of populations gave rise to the concept of heritability. The absolute value of the heritability is expressed by coefficient of hereditary transmission, or hereditary coefficient (h^2), which is nothing as the share (measure), which occurs the genotype in formation of given character (Iliev, 1992), or the variability share, determined by heredity (genotypical variability) in the total phenotypic character's variability (Билаш and Кривцов,1991). Given the fact, that the heritability coefficient determines, through calculation formula, directly, the selection efficiency ($Es = h^2 \cdot d$) according to a certain, concrete character, it (the coefficient h^2) becomes one of the most important criteria of population genetics. As bigger the heritability coefficient of a morpho-productive character is, so more efficient its selection will be, and, conversely, as lower the heritability value is, so the selection efficiency by this character will be tougher. Depending on its size, some researchers (Борисенко, 1967) classify the heritability coefficient in: the high level coefficient $h^2 > 0.6$; the middle level coefficient (medium) $h^2 = 0.4 – 0.6$; the low level coefficient $h^2 < 0.4$. Other researchers (Bucătaru, 1993) divide the characters, by the heritability coefficient value (h^2), into three categories: intense heritable characters, where $h^2 > 0.4$; middle heritable characters, where $h^2 = 0.2 – 0.4$ and weak heritable characters, where $h^2 < 0.2$.

Some notorious researchers in animal livestock (Борисенко,1967) reported that, giving a significant importance to heritability as an index of a possible efficiency of the selection , follows, but it must be taken in account, that, the heritability coefficients of the same characters, calculated by the same method, at the different animal breeds, populations, herds, are not the same.

For these reasons, actually, to the heritability coefficient is assigned a major role in the genetic amelioration of livestock population, being determined the h^2 values for almost all main characters and traits of the animal breeds and populations.

In beekeeping, the determination of heritability coefficient of main morpho-productive characters becomes a technological selection procedure of bee families populations, in the works of Кривцов (1980), Collins (1984), Rinderer (1986), Билаш (1991), Boeking (2000).

Researches, concerning the determination of heritability degree of morph-productive characters at *Apis mellifera carpatica* bee families populations, inhabited in the Republic of Moldova, basically, have not been carried out.

In this context, taking into account, the above mentioned, we proposed the research purpose, to identify the heritability degree of some main biological morph-productive characters of *Apis mellifera carpatica* bee colonies, in order to make more efficient the genetic amelioration process of their population.

MATERIALS AND METHODS

The work was carried out, within the institutional applied project: code 11.817.08.17A "Elaboration of performance technology of diversified growth and exploitation of *Apis mellifera carpatica* bee families".

The research has been done on the bee colonies population of experimental apiary of Institute of Zoology of Science Academy from Moldova, located at the stationary, in a forest glade, at the canton no. 9 of Ghidighici forest district, Chisinau. The main melliferous sources from this area were: white acacia (*Robinia pseudacacia*), wide leaf linden (*Tilia cordata Mill*) and spontaneous polyfloral. It has been investigated the heritability of some main morpho-productive characters, such as, the queen's prolificacy, the bee family strength, the brood's viability, the resistance to disease, and the honey quantity gathered in the nest during the linden harvest.

Determination of bee families morpho-productive characters was carried out, according to the methodology developed by us (Cebotari, 2010), for livestock norm, concerning bee families assessment, raising and certification of beekeeping genitor material (Standard livestock for bee families, growth and certification of genitor beekeeping material, 2011).

The heritability coefficient of morpho-productive characters, was calculated by the method h^2, based on the genotypical correlation determination of the characters mentioned above, at 20 pairs of mothers-daughters families (r_{mf}).

The queens prolificacy (eggs/24 hours) was determined during the revision, in late spring (May 28), by dividing the number of cells with capped brood in the nest, by 12 (lifecycle of capped brood development, days), resulting from the eggs number, laid in 24 hours.

The bee family strength has been assessed, by determining the amount of the bees in the nest, at the evaluation moment. By multiplying the number of intervals between frames, filled, uniformly, with bees, for the coefficient of 0.25 for the standard frame Dadant (435x300 mm), was determined the bees amount in kilograms. The strength evaluation of mothers bee families, have been carried out, three times, during the active season. After these three feedback, was determined the average strength of the bee family. The strength of bee families daughters was evaluated only one time, before linden harvest.

The broods viability, as at mothers families, as well at daughters families, was determined by the average of second evaluation, carried out in June, by reporting the number of cells with survived larvae at 4^{th}-5^{th} day after laying, by the total number of cells from the marked surface on the honeycomb, layed compactly with 400 cells (10 x 10 cm), expressed as a percentage. The assessment has been done, twice a year, in every bee family, settling on the average of first two evaluations.

The disease resistance was determined by evaluating the bee families hygienic behaviour, according to the standard test, whereby the brood, on a compact surface, was killed artificially, in order to establish the speed and the accuracy, wherewith the bees identify and remove the dead brood. The evaluation was carried out, during May-July, twice, for the same bee families, under the same environmental conditions and at equal intervals of time. The brood was killed, in the capped stage (stern), by pricking with a fine needle, through the cells cap, on a honeycomb portion from the family's nest, on a square surface of 5 x 5 cm (100 cells), marked, in the corners, with matches. After 24 hours, since the introduction of the comb, into the nest, was evaluated the cells number, from which has been removed the brood. The ratio, between the removed brood and initially killed brood, on the marked honey comb surface, expressed as a percentage, represented the resistance to disease.

The honey quantity, gathered by bees in the nest, was determined during the linden harvest, by weighing frames with honeycombs and diminishing (from their total weight) the total weight of standard combs with frames without honey: for the frame type Dadant (435x300 mm) – 0.6 kg, for the frame type Langstroth (435x230 mm) – 0.5 kg.

The data obtained from research, was processed statistically, using computer software "STATISTICA–6" and was evaluated their certainty, according to variational biometric statistics, by the methods of Плохинский, 1969 (Iliev, 1992).

RESULTS AND DISCUSSIONS

The correlative links research results of the biological morph-productive characters of *Apis mellifera carpatica* bee families from apiary of Institute of Zoology of the ASM, showed that between homologous characters of mothers families and daughters families, there is a genotypical correlation of different levels, from medium to strong, depending on the selected character and amplitude of character's phenotypic variability (Table 1).

Thus, it was found, that the genotypic correlation, between mothers and daughters characters, is strong enough at the characters with low phenotypic variability, such as: resistance to disease, queens prolificacy, family strength, and weaker at characters with wide phenotypic variability, such as brood's viability and the honey quantity gathered in the nest.

Table 1. Genotypical correlation of some morph-productive characters of the *Apis mellifera carpatica* bee colonies from apiary of Institute of Zoology of ASM

No.	Selected character	N	$r_{mf} \pm m$	N_{st}	*P*	h^2	*Cv%*
1	Queens prolificacy, eggs/day	20	0.80 ± 0.04	12	0.001	0.64	5.8
2	Family strength, kg	20	0.78 ± 0.05	13	0.001	0.61	6.3
3	Broods viability, %	20	0.60 ± 0.07	17	0.01	0.36	8.4
4	Resistance to disease, %	20	0.85 ± 0.03	10	0.001	0.72	3.2
5	Honey quantity gathered in the nest, kg	20	0.56 ± 0.09	20	0.01	0.31	22.1

The genotypical correlation coefficients value (r_{mf}) of characters from the first group, varies within the limits contained in the r_{mf} = 0.78 ± 0.05 and r_{mf} = 0.85 ± 0.03. The heritability coefficient (h^2) of these characters is, also, of high level, within the range of 0.61 – 0.72, with a certainty of the highest threshold of probability theory of forecasts without error according to Student (P<0,001). For the morpho-productive characters of the second group, such as, the broods viability and the honey quantity gathered in the nest, the genotypical correlation was of average level, comprised within the limits of 0.60 ± 0.09 and 0.56 ± 0.09, with a quite high certainty, and the heritability coefficients, of these characters, were of bellow average level, comprised within 0.36 – 0.31 (P<0.01).

We have found that the strongest genotypical correlation was registered for the character of resistance to disease, which reflects, in fact, the hygienic instinct of bee families. This correlation is obviously reflected in Table 2 and Figure 1.

Table 2. Resistance to disease of mothers families and daughters families

Classes of resistance to disease, %	**Resistance to disease, %**			
	Mothers families		**Daughters families**	
	N	**M ± m**	**N**	**M ± m**
92 – 94	8	93.0 ± 0.2	11	93.1 ± 0.3
89 – 91	11	90.1 ± 0.2	7	90.3 ± 0.3
86 – 88	1	86.5 ± 0.0	2	86.0 ± 0.2
Average	**20**	**91.1** ± 0.4	**20**	**91.2** ± 0.4

The research has shown that, once with the increasing of resistance to diseases of mothers families, it is noted, almost directly proportional, the increase of resistance to diseases in daughters families. Thus, once with the increasing of resistance to diseases of mothers families from 86.5 ± 0.0 % up to 90.1 ± 0.2%, or with 4.2%, the value of this character at the daughters families increases from 86.0 ± 0.2%, up to 90.3 ± 0.3%, or 5%.

The genotypical correlation coefficient r_{mf} = 0.85 ± 0.03 indicates the fact that this selection character is not an alternative one, but it is one qualitative-quantitative, with a very high genetic determinism. In fact, we sustain the opinion of researchers (Крушинский, 1993), who affirm, that the hygienic behaviour (resistance to diseases) of bee families, "is not a native instinct, not even an obtained character, but it is formed as a result of the mutual interaction between genes and environment".

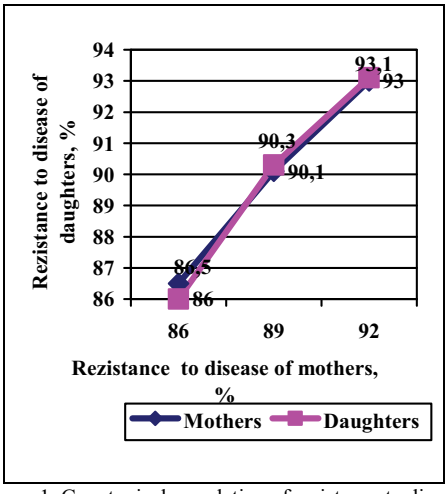

Figure 1. Genotypical correlation of resistance to disease of mothers families and daughters families

The high heritability coefficient (h^2 = 0.72) shows that, the variability share, determined by the genotype in the total phenotypic variability, constitutes 72%, and only 28% of this character's phenotypic variability is determined by environmental conditions. In order to compare, we mention, that Collins et al. (1984) have established such a high heritability coefficient (h^2 = 0.83 – 0.93) at the bee families

defence behaviour, which can be made as a parallel to the hygiene behaviour.

A quite high genotypical correlation, between mothers families and daughters families, was recorded as well, for the queens prolificacy character (Table 3 and Figure 2).

The dependence of daughters queens prolificacy, on mothers queens prolificacy is also quite pronounced, as well as the character of resistance to diseases.

Table 3. Prolificacy of mothers queens and daughters queens

Prolificacy de classes, eggs/day	**Queens prolificacy, eggs/day**			
	Mothers families		**Daughters families**	
	N	**M ± m**	**N**	**M ± m**
1800 – 1899	2	1833 ± 0	6	1837 ± 8
1700 – 1799	7	1761 ± 12	7	1774 ± 12
1600 – 1699	5	1643 ± 12	7	1649 ± 8
1500 – 1599	6	1556 ± 17	0	0 ± 0
Average	**20**	**1677 ± 23**	**20**	**1749 ± 19**

Thus, once with increasing of mothers queens prolificacy from 1643 ± 12 eggs/day up to 1761 ± 12 eggs\day, or with 7,2%, while the daughters queens prolificacy raised from 1649 ± 8 eggs/day until 1774 ± 12 eggs\day, or with 4,6%.

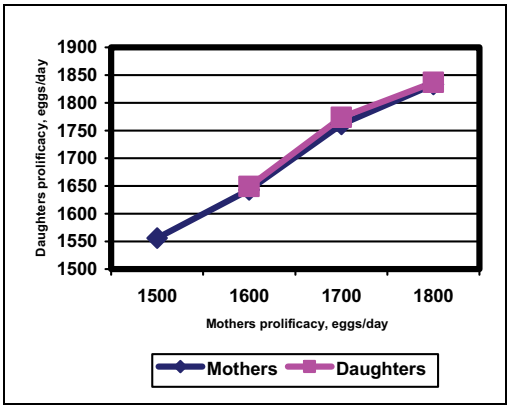

Figure 2. Genotypical correlation of prolificacy of mothers queens and daughters queens

From the graph illustration, we can see that the lines, which reflect the prolificacy of mothers queens and daughters queens raise almost simultaneously to the respective prolificacy levels.

The research results of prolificacy genotypical correlation, prove that, this morpho-productive biological character , of an extraordinarily high importance for the bee family, is also, one quantitative, with a very high genetic determinism. Both the genotypic correlation coefficient (r_{mf} = 0.80 ± 0.04) and the heritability coefficient (h^2 = 0.64), being quite raised, denotes, that the genotypical share of variability value of this character in the total phenotypical variability is quite big - 64%, and the paratypical variability rate, determined by environment, constitutes only 36%. In order to compare, we can mention that Кривцов (1976) had obtained in his research such a heritability coefficient of prolificacy high enough (h^2 = 0.57). This can be explained, primarily, by the genetically determined prolificacy potential of the queen, and, secondly, by the fact that working bees, with their inexhaustible diligence of the queens care, maintain the environmental variability share in the total variability of this character at the minimum possible level.

The bee family strength is a character directly determined by the amount of capped brood in the nest. Given the fact, that the amount of capped brood is obtained, as a result of laying (prolificacy) of the queen, and subsequently, determines, decisively, the quantity of the bee in the nest, the correlative connections between these morph-productive characters are pretty tight. Therefore, the genotypical correlation of the bee family strength (Table 4, Figure 3) has a similar tendency as the queens prolificacy.

It was found, that once with the increasing power of mothers families strength, occurs a significant increase of the daughters families strength.

Table 4. The strength of mothers queens and daughters queens

Strength classes, kg	**Families strength, kg**			
	Mothers families		**Daughters families**	
	N	**M ± m**	**N**	**M ± m**
3.00 – 3.24	14	3.13 ± 0.03	-	-
2.75 – 2.99	6	2.87 ± 0.02	-	-
2.50 – 2.74	-	-	-	-
2.25 – 2.49	-	-	2	2.27 ± 0.02
2.25 – 2.49	-	-	18	2.13 ± 0.02
Average	**20**	**3.06 ± 0.04**	**20**	**2.14 ± 0.02**

So, the quantitative level of the daughters families strength, composed of swarms in spring, is lower, compared to the strength of

mothers families, the genotypical correlation of this character is pretty close, being on average r_{mf} = 0.78 ± 0.05. Hence, also the heritability coefficient, of the bee family strength, is high h^2 = 0.61 (P<0.001) and, significantly, with the highest certainty threshold of the probability theory of forecasts without error according to Student.

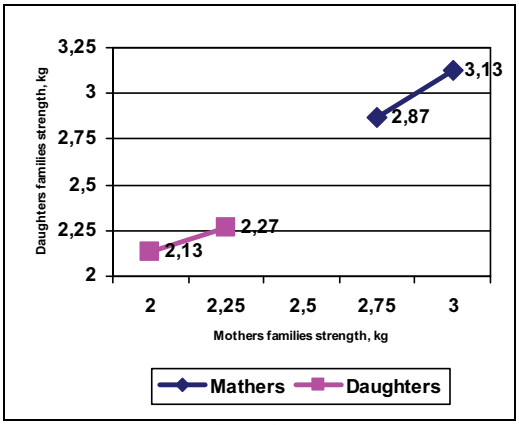

Figure 3. Genotypical correlation between mothers families strength and daughters families strength

Based on the obtained data, we can affirm, that, the bee family strength is a biological morpho-productive quantitatively determined character, mostly (61%), of the family genotype, and influenced, although less (39%), but quite significant, by environmental factors.

Heritability research by another groups of bee families morpho-productive biological characters with a larger phenotypic variability, such as brood's viability and honey quantity gathered in the nest, has shown, that, the genotypical correlation of these characters between mothers families and daughters families has the same tendency, except that the genotypical correlation coefficient value **(r_{mf})** is of average level, and of heritability coefficients **(h^2)** - of bellow average level.

Thus, the genotypical correlation of brood's viability, between mothers families and daughters families, represents, on average, **r_{mf}** = 0.60 ± 0.07, and heritability coefficient value - **h^2** = 0.36 with a quite definite significance (P<0.01).

From obtained data, it was found, that, increasing the brood's viability of mothers families-mothers, leads, moderately, to the brood's viability increasing in daughters families (Table 5, Figure 4).

Once with brood's viability increasing in mothers families from 88.1 ± 0.0% up to 90.0 ± 0.3%, or with 2.2%, will increase also the brood's viability from daughters families, from 87.5 ± 0.0% up to 90.2 ± 0.3%, or with 3.1%. With the continued increase of brood's viability in mothers families from 90.0 ± 0.3%, up to 95.5 ± 0.0%, or with 6.1%, will increase also the brood's viability in daughters families, from 90.2±0.3% up to 95.0±0.0%, or with 5.3%.

Table 5. Broods viability at mothers families and daughters families

Broods viability classes, %	**Broods viability, %**			
	Mothers families		**Daughters families**	
	N	**M ± m**	**N**	**M ± m**
95 – 96	1	95.5 ± 0.0	1	95.0 ± 0.0
93 – 94	-	-	3	93.5 ± 0.1
91 – 92	13	91.7 ± 0.2	11	91.7 ± 0.2
89 – 90	5	90.0 ± 0.3	4	90.2 ± 0.3
87 - 88	1	88.1 ± 0.0	1	87.5 ± 0.0
Average	**20**	**91.3** ± 0.3	**20**	**91.6** ± 0.4

Despite the fact, that, the character's variability of brood's viability is not so big (but only 8.4%), the size of the heritability coefficient is bellow average (0.36). It denotes, that the variability rate, determined by the genotype in the general phenotypic variability, is only 36%, whereas, the paratypical variability share, determined by environment is predominant (64%). The decisive environmental factors, which influence the variability of this important biological character can be some diseases, which are affecting the brood. Hence, it results the conclusion that the selection effectiveness performed according to brood's viability will always be lower than that of characters with a higher heritability coefficient.

Last, and most important biological morpho-productive character of bee families, examined by us, in order to establish the heritability, is the honey quantity gathered in the nest (Table 6, Figure 5). The research has shown, that, between the honey quantity gathered in the nest of mothers families and the honey quantity gathered in the nest of daughters families, is a genotypical correlation of middle level (r_{mf} = 0.56 ± 0.09) and a heritability coeficient of bellow average level (h^2 = 0.31).

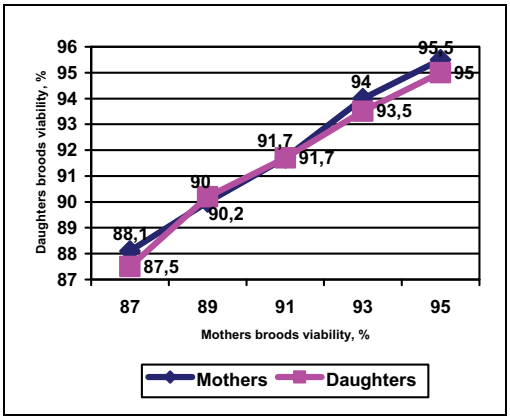

Figure 4. Genotypical correlation of brood's viability between mothers families and daughters families

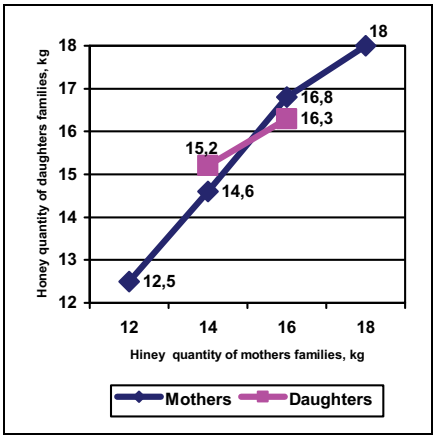

Figure 5. Genotypical correlation of honey quantity gathered in the nest by mothers families and daughters families

Table 6. Honey production gathered in the nest by mothers families and daughters families

Honey quantity classes, kg	Honey quantity, kg			
	Mothers families		Daughters families	
	N	M ± m	N	M ± m
18 – 19	4	18.0 ± 0.0	-	-
16 – 17	7	16.8 ± 0.2	4	16.3 ± 0.1
14 – 15	5	14.6 ± 0.2	16	15.2 ± 0.1
12 – 13	4	12.5 ± 0.3	-	-
Average	**20**	**15.6** ± 0.5	**20**	**15.4** ± 0.2

In order to compare, we mention that, in researches of various authors, the heritability coefficient of honey production of honey is, also, low and varies within limits: 0.23 (Rinderer, 1986); 0.22-0.23 (Билаш, 1991); 0.22-0.25 (Siceanu, 2005).

The Table 6 shows, that once the character value of honey quantity gathered in the nest by mothers families increases, from 14.6 ± 0.2 kg up to 16.8 ± 0.2 kg, or with 15.1%, will be noted an increase of the honey quantity gathered in the nest by daughters families, from 15.2 ± 0.1 kg up to 16.3 ± 0.1 kg, or with 7.2%. The honey quantity gathered in the nest, being a biological morpho-productive character, with the highest phenotypical variability (22.1%), compared to the other biological morpho-productive characters of bee families, has the weakest genotypical correlation between mothers families and daughters families and the lowest heritability coefficient.

This fact denotes, that the genotypical variability rate, determined by heredity in general phenotypical variability of this character, is lower, compared to the other characters, and it is only 31%. Otherwise, the paratypical variability rate in total phenotypical variability of the honey quantity gathered in the nest, prevails, and is determined, mostly preponderant (69%), by the environment factors, such as: harvest melliferous resources, weather conditions, bee families care and maintenance technology, prevention and control of diseases etc.

The results of heritability researches, of the main biological morph-productive characters, obtained by us, are used in the selection process, for bee families genetic improvement plan development and prediction of selection effect, calculated by the formula:

$$E_s = h^2 \cdot d$$

where:

E_s – selection effect of selected character in a generation;

h^2 – heritability coefficient of selected character;

d – selection diferential, calculated by the formula:

$d = M_{lp} - M_{st}$,

where:

M_{lp} – selected characters average value per a bee family from the breeding batch;

M_{st} - selected character's average value per a bee family from whole apiary.

Based on the above mentioned formula, it is clear that the selection efficiency carried out at the apiary of IZ ASM, was bigger at the characters with a higher heritability coefficient such as: resistance to diseases (hygienic instinct), which reached, recent years, the average level at the apiary high enough - up to 90-95%, the broods viability up to 90-94%, quuens prolificacy - 1700 -1800 eggs/day and family strength – 3.0-3.2 kg, while the honey production of bee families, reached in recent years, the level of only 35-40 kg.

The selection effect value varies, depending on the year, selection intensity and, widely, on weather conditions of the respective year.

CONCLUSIONS

According to the heritability coefficient ($\mathbf{h^2}$), the main biological morph-productive characters of *Apis mellifera carpatica* bee families of researched population, can be divided into two groups: the first group - qualitative-quantitative characters with a high genetic determinism and narrow phenotypical variability, whose heritability coefficient $\mathbf{h^2}$ is comprised within the limits of over average of 0.6-0.75; the second group - quantitative characters with reduced genetic determinism subsided and large phenotypical variability, whose heritability coefficient $\mathbf{h^2}$, is contained within the limits of bellow average 0.3-0.4.

The bee families selection effect by biological morpho-productive characters of Ist group, with heritability coefficient ($\mathbf{h^2}$) over average, is much bigger, than the effect achieved in the bee families selection by characters from the second group, with the heritability coefficient ($\mathbf{h^2}$) bellow average.

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ANALYSIS CONCERNING THE INFLUENCE OF COLOUR GENES IN KARAKUL SHEEP ON MILK PRODUCTION

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Abstract

The purpose of this paper is to verify if the genes that determine the colour of lamb pelts of Karakul type influences the milk production. In other words, we have seen if the lamb pelt colours are associated with characters related to milk production. The biological material was composed of sheep population belonging to the Botosani Karakul breed. Sheep of the six colour varieties of this breed were taken in the experiment. The experimental conditions were assured equally for all sheep varieties. The first mathematical processing is a statistical description of the data depending on colour groups. The statistical description refers to the calculation of statistical parameters (average, standard deviation etc.). The average variation of colour groups was graphically represented. The representation was made also as a histogram depicting the standard deviation, too. The variance analysis was performed. Two homogeneity tests of variance are used to see the legitimacy of the analysis of variance: Leaven and Brown- Forsthe; the relation between average and standard deviation was graphically represented to see if it is linear. Comparative analysis of averages was done with different test: LSD, Sheff, Newman-Keuls, Duncan, Tukey. Data processing was performed with a statistical program. Analysis of variance shows that, in general, the colour of hair fibre does not significantly influence the milk production.

Keywords*: sheep, Karakul, lamb pelts, colour variety.*

INTRODUCTION

Currently, in the world, the sheep breeding is generally made trough the specialization on a certain production type: meat, wool, milk and lamb pelts. Rarely, the focus is on mixed production types even if between the segments of production metabolism were not found antagonistic relations (Taftă et al., 1997, Buzu, 2012).

The Botosani Karakul breed is specialized for lamb pelt production, which, besides the physical and morphological of their curling (shape and size of curls, quality and lustre of hair fibres) it is appreciated for the colours of the hairy coating of lamb pelts, too (black, greyish, brown, gray, pink and white) (Filote et al., 1966; Niga et.al., 1989). But this breed is exploited also for the milk production. Originating in Asia, Karakul sheep is considered a breed with a lower milk production (especially in black variety) compared to other sheep breeds. At the Research and Development Station for Sheep and Goat Breeding Popauti-Botosani several experiments were carried out on creating a mixed production line (milk – lamb pelts), in particular in coloured varieties showing promising results. The specialty literature presents an association between milk production and colour (milk production of greyish sheep is superior to the other colour varieties). (Filote and Filote, 2002)

In the context of these statements, the purpose of this paper is to verify if the genes that determine the colour of lamb pelt in Karakul lambs can influence the milk production. The hypothesis is to be verified. In other words, we want to see if the hair fibre colours of lamb pelts are associated statistically with milk production in order to use this association in making decisions regarding the sheep selection for increasing the milk production in this breed.

MATERIALS AND METHODS

The biologic material is composed of Botosani Karakul sheep belonging to the population from Research and Development Station for

Sheep and Goat Breeding Popauti. Sheep of all colour varieties of this breed were taken in experiment. Experimental conditions were ensured equally to all varieties within this investigational approach.
The statistical parameters used in experiments were taken from common mathematical statistics (average, standard deviation, variance, standard error etc.) (van Vleck, 1987; Sandu, 1995).
Statistical processing was performed according to the calculation methodology of the software *STATISTICA 8.0* (developed by the manufacturer StatSoft, 1984-2002).

Database description. The data were systematized in a file: File *MilkProd.*
The file contains a database with the following fields:
• Colour;
• Lactation period (days);
• Milk production (liters).

Data: MilkProd* (3v by 183c)

	1 Colour	2 LactPer	3 MilkProd
1	KN	121	73.290
2	KN	125	53.787
3	KN	169	71.844
4	KN	181	94.066
5	KN	181	63.085
6	KN	123	20.006
7	KN	160	21.278
8	KN	110	58.497
9	KN	108	25.902
10	KN	117	19.001
11	KN	143	57.820
12	KN	108	39.885
13	KN	142	111.468
14	KN	81	7.490
15	KN	112	12.151
16	KN	136	41.747
17	KN	177	79.619
18	KN	186	24.574

Figure 1. File *MilkProd*

Data processing
The first statistical processing is statistical description of the data on colour groups. The statistical description refers to the calculation of statistical parameters: average (mean), standard deviation, variance, standard error and so on.
The variation of averages in colour varieties is plotted. In addition the representation is made in histogram form depicting the standard deviation, too.
The variance analysis is carried out. To see the legitimacy of variance analysis two tests of variance homogeneity are performed: Leavene and Brown-Forsthe. The relationship between average and standard deviation is represented graphically to see if this relationship is linear. The comparative analysis of averages is made by the tests:
• LSD;
• Sheff;
• Newman-Keuls;
• Duncan;
• Tukey.
Data processing was performed with STATISTICA software.

Abbreviations used in the Data Base File:
• Colour – colour variety;
• LactPer – lactation period;
• Milkprod – milk production;
• KN - Black Karakul;
• KA - White Karakul;
• KM - Brown Karakul;
• KR - Pink Karakul;
• KS - Gray Karakul;
• KB - Greyish Karakul.

RESULTS AND DISCUSSIONS

The statistic parameters for the character *Milk production* in colour varieties of Karakul sheep are shown in Table 1. This file contains the following fields: colour variety, milk production average, individual number, standard error. Last article includes statistical indicators on the entire population.

Table 1. Descriptive statistics

Breakdown Table of Descriptive Statistic N=183 (No missing data in dep. var. list)			
Colour	MilkProd Means	MilkProd	MilkProd Std.Dev.
KN	53.96561	41	29.68602
KB	63.58850	32	33.44977
KM	43.86815	26	30.93618
KS	50.29254	28	29.08239
KR	46.99253	15	25.03952
KA	56.35441	41	29.51363
All Grps	53.61532	183	30.31437

The table 2 presents the variance analysis for the character *Milk production* in colour varieties of Karakul sheep breed. ANOVA shows no significant differences in milk production among the colour varieties

To validate the ANOVA test it is necessary that the populations to be homogeneous. This validation was done by homogeneity tests (Leaven, Brown-Forsythe).

The table 3 illustrates the homogeneity of dispersions by the Leaven test. They are homogeneous, so that the variance analysis is legitimate.

The table 4 illustrates the homogeneity of dispersions by the Brown-Forsythe test. They are homogeneous, so that the variance analysis is legitimate.

Table 2. Variance analysis

Variable	Analysis of Variance (MilkProd) Marked effects are significant at p < .05000 SS Effect	df Effect	MS Effect	SS Error	df Error	MS Error	F	p
MilkProd	6932.750	5	1386.550	160318.2	177	905.7523	1.530827	0.182442

Table 3 The Levene test for variance homogeneity

Variable	Levene Test of Homogeneity of Variances MilkProd) Marked effects are significant at p < ,05000 SS Effect	df Effect	MS Effect	SS Error	df Error	MS Error	F	p
MilkProd	258.0010	5	51.60020	55736.81	177	314.8972	0.163864	0.975456

Table 4. The Brown-Forsythe test for variance homogeneity

Variable	Brown-Forsythe Test of Homog. of Variances (MilkProd) Marked effects are significant at p < ,05000 SS Effect	df Effect	MS Effect	SS Error	df Error	MS Error	F	p
MilkProd	190.2227	5	38.04454	62246.59	177	351.6756	0.108181	0.990397

The figure 2 is a graphical representation of the statistical parameters of milk production in the colour varieties of Karakul breed. The chart shows that the highest milk production is obtained from sheep of greyish variety (KB) and the lowest from sheep of brown variety.

The chart below shows that the average and standard deviation are not correlated and therefore the variance analysis is legitimate (fig. 3). Because the points on the chart mean-dispersion do not align to regression line, it results that the variance analysis carried out is valid.

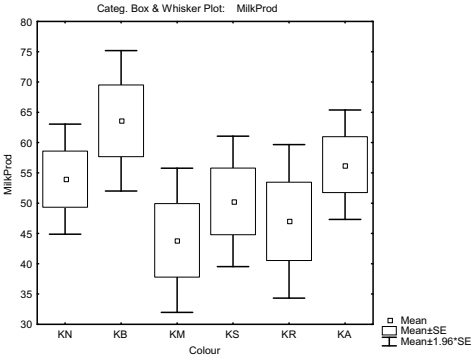

Figure 2. Histogram regarding variation of average depending on colour varieties

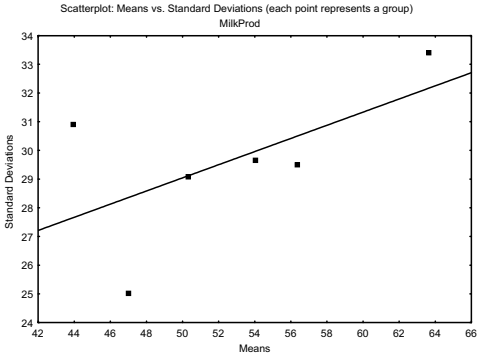

Figure 3. The relationship between average and standard deviation

The figure 4 configures averages and standard deviations for milk production in colour varieties of Karakul sheep.

The following analysis of the paper compares the milk productions among all colour varieties.

The table 5 presents the analysis of differences between averages concerning lactation period in colour varieties of Karakul sheep (according to LSD test).

The table 6 presents the analysis of differences between averages concerning lactation period in colour varieties of Karakul sheep (according to Scheffe test).

The table 7 presents the analysis of differences between averages concerning lactation period in colour varieties of Karakul sheep (according to Newman Keuls test).

The table 8 presents the analysis of differences between averages concerning lactation period in colour varieties of Karakul sheep (according to Duncan test).

The table 9 presents the analysis of differences between averages concerning lactation period in colour varieties of Karakul sheep, according to Tukey test for unequal groups.

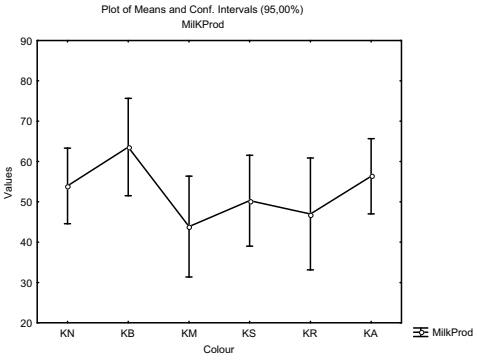

Figure 4. Average variation of milk production in colour varieties

Table 5. The LSD test for significance of difference between averages

Colour			LSD Test; Variable:MilkProd (MilkProd) Marked differences are significant at p < ,05000 {1} M=53,966	{2} M=63,589	{3} M=43,868	{4} M=50,293	{5} M=46,993	{6} M=56,354
	KN	**{1}**		0.176979	0.182520	0.619230	0.443613	0.719740
	KB	{2}	0.176979		**0.014007**	0.089532	0.079752	0.309582
	KM	{3}	0.182520	**0.014007**		0.434215	0.749207	0.099719
	KS	{4}	0.619230	0.089532	0.434215		0.732239	0.412422
	KR	{5}	0.443613	0.079752	0.749207	0.732239		0.304011
	KA	{6}	0.719740	0.309582	0.099719	0.412422	0.304011	

Table 6. The Scheffe test for significance of difference between averages

Colour		Scheffe Test; Variable: MilkProd (MilkProd) Marked differences are significant at p < ,05000 {1} M=53,966	{2} M=63,589	{3} M=43,868	{4} M=50,293	{5} M=46,993	{6} M=56,354
KN	{1}		0.870362	0.876454	0.998483	0.988297	0.999689
KB	{2}	0.870362		0.296105	0.713042	0.683865	0.958930
KM	{3}	0.876454	0.296105		0.987144	0.999824	0.739900
KS	{4}	0.998483	0.713042	0.987144		0.999754	0.984080
KR	{5}	0.988297	0.683865	0.999824	0.999754		0.956858
KA	{6}	0.999689	0.958930	0.739900	0.984080	0.956858	

Table 7. The Scheffe test for significance of difference between averages

Colour		Newman-Keuls test; Variable: MilkProd(MilkProd) Marked differences are significant at p < ,05000					
		{1} M=53,966	{2} M=63,589	{3} M=43,868	{4} M=50,293	{5} M=46,993	{6} M=56,354
KN	**{1}**		0.466096	0.603551	0.652861	0.669396	0.769893
KB	{2}	0.466096		0.150962	0.362651	0.250453	0.375701
KM	{3}	0.603551	0.150962		0.711218	0.702028	0.543470
KS	{4}	0.652861	0.362651	0.711218		0.686135	0.738269
KR	{5}	0.669396	0.250453	0.702028	0.686135		0.660677
KA	{6}	0.769893	0.375701	0.543470	0.738269	0.660677	

Table 8. The Duncan test for significance of difference between averages

Colour		Duncan test; Variable: MilkProd (MilkProd) Marked differences are significant at p < ,05000					
		{1} M=53,966	{2} M=63,589	{3} M=43,868	{4} M=50,293	{5} M=46,993	{6} M=56,354
KN	**{1}**		0.269313	0.265380	0.652861	0.425018	0.769893
KB	{2}	0.269313		**0.032201**	0.139418	0.069536	0.375701
KM	{3}	0.265380	**0.032201**		0.462615	0.702028	0.178008
KS	{4}	0.652861	0.139418	0.462615		0.686135	0.488403
KR	{5}	0.425018	0.069536	0.702028	0.686135		0.302510
KA	{6}	0.769893	0.375701	0.178008	0.488403	0.302510	

Table 9. The Tukey test for significance of difference between averages

Colour		Unequal N HSD; Variable:MilkProd (MilkProd) Marked differences are significant at p < ,05000					
		{1} M=53,966	{2} M=63,589	{3} M=43,868	{4} M=50,293	{5} M=46,993	{6} M=56,354
KN	{1}		0.796686	0.832351	0.997522	0.988449	0.999219
KB	{2}	0.796686		0.169575	0.563130	0.657711	0.930018
KM	{3}	0.832351	0.169575		0.972605	0.999751	0.666955
KS	{4}	0.997522	0.563130	0.972605		0.999675	0.975016
KR	{5}	0.988449	0.657711	0.999751	0.999675		0.957594
KA	{6}	0.999219	0.930018	0.666955	0.975016	0.957594	

The test results show that, in general, the milk production differences among the sheep groups composed by their affiliation to the colour varieties are not significant. However, some tests (LSD and Duncan) shows that the differences between greyish (KB) and brown (KM) varieties on milk production present statistical assurance.

CONCLUSIONS

The analysis of variance in Karakul sheep breed shows that the determinant genes of wool fibre colour does not significantly influence the milk production.

Statistically, the difference relevance among the milk productions in Karakul sheep classified by colour varieties is certified by significance tests.

Generally, the milk production differences among the colour varieties are not significant. Only significant difference is found between greyish and brown varieties.

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MOLDOVAN TYPE OF BLACK-MOTLEY CATTLE

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Abstract

The object of research was the cattle of dairy production. In the Republic of Moldova to the early 80-ies of the last century were used Red Steppe and Simmental cattle breeds that have a low potential for milk production, poor adaptability to machine milking. The material is animals, which was converted to the type of high-yielding dairy cattle. The transformation was carried out by cross-breeding cows the Red Steppe and Simmental bulls with black-and-White and Holstein breeds. The purpose was to study the effectiveness of selection for a new breed of dairy cattle. As a result of breeding hybrids of different genotypes it was found that they had an average milk production advantage the cows local breeds. On the efficiency of the selection process in the dairy farming is evidenced by the fact that by the end of the third stage of launch a new type in the country for 550 farms and complexes average milk yield per cow per year was 3500 kg of milk and in 51 - more than 5000 kg. Yield of milk from one cow in all categories of farms reached 4016 kg of milk.By 2000, the program for launch a new type has been completed, and in 2008 was approved Moldavian type of black-and-white cattle. In the alelophond of a new type of bulls in locus AEB prevails genetical markers $G_2Y_2E'_1Q'$, G_2Y_1D', G_1I_1, I_2, O_1, B_2G_2, $B_2O_1Y_2D'$, B_2O_1, $B_1O_3Y_2A'_2E'_1P'Q'G''$, $Y_2E'_2G'O'$, Q', E'_3, E'_3Q', I' .

Keywords**:** *alleles, body measurements, cattle, hybrids, new type of cattle.*

INTRODUCTION

In the province of Bessarabia, in pieces, which is now the territory of Moldova, primordial breeds of cattle were Simmental and Red Steppe, and in more remote times bred gray Bessarabian steppe cattle.

Red Steppe breed was formed in the late 19th and early 20th century, in the south of Ukraine and Moldova of the German population of red cattle imported by colonists. Red steppe cattle for a body type are typical of dairy breeds. It is well adapted to local, tough enough climatic and fodder conditions. Animals of Red Steppe breed, bred in the country, distinguished by low milk production. Thus, according to appraisal of cows (1975-1979 years) at 62661 cows milk yield per I lactation amounted 2816 kg of milk with fat content 3.68%.

Simmental breeds. Animals of this breed were imported into Bessarabia from Switzerland - the birthplace of the breed. Under the influence of specific local conditions of feeding and management, and as a result of cross-breeding with local Bessarabian gray cattle he purchased some of the features distinguishing it from Simmental cattle in other countries. Cattle of this breed have a great diversity of types (coarse, medium and soft) and well adapted to local conditions. The area of its distribution is the farms of the northern zone of the country.

Signs of milk yield of Simmental cows are expressed medium. Productivity of young cows in the farms of the republic for 1970-1975, reached 2692-2711 kg of milk with fat content of 3.61-3.66%. Under high level of feeding into force of the combined productive direction at cows of this breed often comes obesity that entails a reduction of milk yield.

Addition to the above species in the country are widely used other breeds of cattle, which at the beginning of 80th years of the last century had a low potential for milk production, poor adaptability to machine milking, making them unsuitable for the industrial management of dairy cattle breeding industry. Therefore, the Government of the Republic decided on the industrialization of dairy farming industry in

connection with which any number of problems, one of which was the creation of a population corresponding the conditions of industrial milk production technology, which is a new type of breeding animals.

MATERIALS AND METHODS

The conversion of low productivity of local Red Steppe and Simmental breeds in the type of high-yield dairy cows were conducted by cross-breeding with the best global gene pool of specialized breeds of dairy productivity - Black-and-White and Holstein. Employees of laboratory breeding technology and exploitation of cattle was developed program breeding animal populations of the "northern" and "southern" zone subtypes of Moldavian-type black-and-white cattle (The program launch Moldavian black and white breed of cattle, 1985) and the materialization of this process - the importation of seed material and producers of black-and-White and Holstein breeds.

The purpose was to study the effectiveness of selection for a new breed of dairy cattle.

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

RESULTS AND DISCUSSION

The experience of industrial complexes showed that their performance is dependent on a number of factors, among which one of the most important is the presence of animals that are highly productive, ability of well-paying food, long maintain high productivity.

In terms of industrial technology from an economic point of view the breeding of cows with the productivity less than 4.0 thousand kilograms of milk per year is not justified.

The breeding program of Moldavian-type black-and-white cattle with two subtypes of zonal "northern" and "southern" was of incremental and for each of which pose a particular challenge. Already in the early stages of the breeding program was found on milk yield advantage in F1 hybrids of different genotypes in comparison with the original (parent) species (Table 1).

Table1. Approbation of the productivity of cows of different genotypes

Breeds and breed	Number of cows	Productivity		
		Milk yield, kg	Fat	
			%	kg
Red Steppe, purebred	2105	2991	3.74	111.8
Red Steppe × Black-and-White, F_1	1839	3192	3.63	117.5
The difference (±) in favor F_1	-	201	-0.06	5.7
Simmental purebred	467	2529	3.63	91.8
Simmental × Black-and-White, F_1	1087	3136	3.63	113.8
The difference (±) in favor F1	-	607	0	22.0
Simmental × Holstein, F_1	1116	3385	3.60	121.8
The difference (±) in favor F_1	-	856	-0.03	30.0
For all the initial breeds	2572	2907	3.72	108.2
For all F_1	4042	3230	3.64	117.4
The difference (±) in favor F_1	-	323	-0.08	9.2

The analysis showed that hybrids F_1, as the Bulls of Black-and-White as well as Holstein breeds, on average, a dairy cow productivity advantage over native species. The fat content in the milk of several hybrids below without significant difference, and the output fat of milk in all variants the higher have hybrids.

As a result, hybrids breeding of different genotypes in 1985 the average milk yield per cow was 3420 kg milk in a number of breeding farms have been bred herds with a yield of 4.0-

4.5 thousand kg of milk, and in the breeding groups - 5,0-5,5 thousand kg of milk per cow with fat content 3.7-3.9% with 3-3.3% protein.

About the effectiveness of the selection process in the dairy farming is evidenced by the fact that by the end of the third stage of breeding a new type in the country in 550 farms and complexes average milk yield per cow per year was 3.5 thousand kg of milk and in 51 - more than 5.0 thousand kg. The dynamics of the milk production of a new type of animals at different stages of its elimination is presented in Figure 1.

As can be seen from the material presented at the maximum efficiency of a new type of animal was in the third stage.

During this period in the country produced 1.548 thousand tons of milk, and the milk yield per cow, as seen in the figure, in all categories of farms reached 4016 kg of milk, milk production per inhabitant of the republic amounted to 353 kg.

By 2000, the launch program of a new type has been basically completed, and in 2008 was approved by the Moldavian type of Black-and-White cattle with the contents of Holstein genes in the "northern" zonal subtype 75-87.5% and the "southern" - 62.5-75% respectively (Table 2).

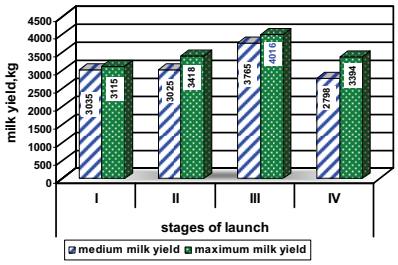

Figure 1. Milk production of the cows in different stages of launch of the Moldavian type of black-and-white cattle

Table 2. The main parameters of cows' zonal subtypes of the Moldavian type of Black-and-White cattle

Indicators		Zonal subtypes	
		"northern"	"southern"
The genetic potential for milk production		7000-8000	6000-7000
The actual milk yield and fat content per lactation:	1st lactation	5070 – 3.63%	4420 – 3.72%
	Third lactation and older	6340 – 3.67%	6200 – 3.79%
Live weight:	heifers 18 months	400 kg	380 kg
Cows:	1st lactation	550 kg	500 kg
	Third lactation and older	600 kg	550 kg
Form of udder		Bath-shaped, rounded	Bath-shaped, rounded
Speed of milk let		1.8-2.0 kg/min	1.8-2.0 kg/min
Height at withers, cm		133-135	132-134

The animals of the new type - dairy production. The cows head is clearly defined, proportional to the body, a broad nasal mirror, moderately concave forehead, limbs correctly placed, the hoof has an optimal angle setting 43.7˚.

The high-altitude measurements of body of cows "southern" zone subtype 132-134 cm, the "northern" subtype - 133-135 cm, (Smirnov et al., 2007). Chest width the shoulder blades – 42.7-42.9 cm, the depth of the chest – 68.4-68.0 cm, width at the hip joints – 46.8-46.7 cm, respectively, the "northern" and "southern" subtypes. The length of the body 150.4-151.1 cm and girth of the metacarpus - 18.1 cm. The udder with a large margin tightly attached and balanced development of the teats of medium length and thickness. Animals of the new type are well suited for use as industrial complexes, and in the farms.

One of the most important elements in creating Moldavian type Black and White cattle was to study its genetic structure.As a result of a detailed analysis of the antigen spectrum of blood group found that crossbred animals on the basis of Red Steppe (Table 3) in the dynamics of generations the concentration of antigens Y_2 , P ', G "(B - system), R_2 (C - system) gradually decreased from 56.6 to 54.0 %, from 28.5 to 5.3 %, from 35.1 to 12.7 %, from 57.4 to 25.5 % (agrofirm "Friendship") and from 47.8 to 44.5 %, from 36.0 to 18.5 %, from 21.7 to 15.4 %, from 52.2 to 30,8% (STE

"Maximovca") respectively. Conversely, the frequency of antigen G_2, G_3, E'_2, O', D' (B - system), X_2 (C - system), M (M - system) with increasing of blood on black-motley breed grew. Thus, the number of carriers antigen G_2 increased from 13.7 to 38.1 and 42.0 %; O_1 antigen from 1.9 to 20.0 and 26.4, M antigen from 0.0 to 8.5 and 9.2 % respectively in the standard of herds agricultural firm "Friendship" and experienced department STE „Maximovca".

Table 3. Genetic features of hybrids on the basis of red steppe dynamics for some generations antignam,%

System	Antigens	Red Steppe breed	Agricultural firm "Friendship"				STE „Maximovca"			
			F_1	F_2	F_3	F_4	F_1	F_2	F_3	F_4
B	G_2	13.7	12.1	31.7	37.7	38.1	47.8	36.2	45.9	42.1
	G_3	0.0	0.0	31.2	40.5	44.0	43.5	36.2	58.3	46.5
	Y_2	62.7	56.6	55.3	61.5	54.0	47.8	60.9	57.3	44.5
	D′	23.5	25.4	14.6	22.9	36.2	15.2	20.3	8.3	31.3
	E_2'	11.7	17.9	32.4	33.1	38.3	30.4	42.0	46.8	34.6
	O′	1.9	2.3	4.2	8.1	20.0	19.5	20.3	19.8	26.4
	P′	31.4	28.5	19.2	8.1	5.3	26.0	23.2	11.5	18.6
	G″	31.4	35.1	24.1	21.9	12.7	21.7	31.9	12.5	15.4
C	R_2	66.7	57.4	41.9	36.3	25.5	52.2	50.7	42.7	30.8
	X_2	56.8	40.6	64.6	70.3	59.7	73.9	78.2	73.9	78.3
M	M	0.0	1.2	4.6	13.5	8.5	10.8	11.6	7.9	9.2

Significant changes have occurred in crossbred on the basis of Simmental breed animals (Table 4). Thus, with increasing relationship on black and white breed increased incidence antigens G2, Y2, E'_2 (B - system), X_2 (C - system) from 36.0 to 43.5% and from 26.2 to 56.1 %, 32.0 to 38.2% and from 18.3 to 51.2%, from 56.0 to 61.7% and from 57.6 to 63.4% in the herds of agricultural association "Tetskan" and STE "Selectsia"

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Table 4. Genetic features of hybrids on the basis of the dynamics of Simmental generations for some antigens,%

System	Antigens	Simmental breed	Agricultural association "Tetskan"			STE "Selectsia"			
			F_1	F_2	F_3	F_1	F_2	F_3	F_4
B	G_2	38.2	36.0	41.3	43.5	26.2	24.7	46.9	56.1
	Q	29.4	12.0	8.6	12.3	16.6	12.8	6.1	2.4
	Y_2	41.2	68.0	49.8	50.0	56.3	54.4	75.5	73.2
	D′	32.3	32.0	23.2	18.2	21.8	20.85	26.5	29.3
	E_2'	20.6	32.0	36.5	38.2	18.3	16.8	51.0	51.2
	O′	20.6	8.0	9.2	10.6	17.0	21.8	34.7	29.3
	Y′	8.8	20.0	7.0	8.8	12.2	9.9	2.0	9.7
C	R_2	73.5	64.0	64.4	62.9	47.1	39.6	40.8	46.3
	X_2	50.0	56.0	51.4	61.7	57.6	64.3	53.1	63.4
M	M	2.9	8.0	3.2	1.7	8.3	1.9	2.0	0.0

If the hybrids on the basis of Red Steppe in the dynamics generations, an increase in frequency of antigen M, then the crossbred based on Simmental breed the contrary, with the increase of relationship on black-motley breed is a gradual decrease in the frequency of its occurrence from 8.0 to 1.7% (agricultural association "Tetskan") and from 8.3 to 2.0% (STE "Selectsia").

In the alelophond of a new type of bulls in locus AEB prevails genetical markers $G_2Y_2E'_1Q'$, G_2Y_1D', G_1I_1, I_2, O_1, B_2G_2, $B_2O_1Y_2D'$, B_2O_1, $B_1O_3Y_2A'_2E'_1P'Q'G''$, $Y_2E'_2G'O'$, Q', E'_3, E'_3Q', I' .

The population of the nev type has a high genetic potential, due to the homozigotic level (Cα) which varies in limits 2.6 till 7.9% that allows the maintaining of varied genetics of

structural units in the process of improvement of the bulls of new type.

CONCLUSIONS

By the end of the third stage launch of a new type in the country for 550 farms and complexes average milk yield per cow per year was 3.5 thousand kg of milk and in 51 - more than 5.0 million kg.

Milk yield per forage cow in farms of all types have reached 4016 kg of milk, milk production per inhabitant of the republic amounted to 353 kg.

In the alelophond of a nev type of bulls in locus AEB prevails genetical markers $G_2Y_2E'_1Q'$, G_2Y_1D', G_1I_1, I_2, O_1, B_2G_2, $B_2O_1Y_2D'$, B_2O_1, $B_1O_3Y_2A'_2E'_1P'Q'G''$, $Y_2E'_2G'O'$, Q', E'_3, E'_3Q', I' .

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EFFECT OF SELECTION PRESSURE ON FIXING THE QUALITATIVE FEATURES OF LAMB PELTS OF KARAKUL TYPE

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Abstract

*This paper describes comparative aspects of the qualitative features of lamb pelts in two populations of lambs belonging to two farm types of Karakul sheep: elite farm within the R-DSSGB and private farm within the SGBA "MOLDOOVIS" Botosani. The qualitative analysis of lamb pelts by the complex (multifactorial) estimation method revealed the morphological and production superiority of individuals from elite farm compared to those from private farm concerning their distribution in zootechnical (breeding) classes. In the elite farm three quarters of lambs are in Record class and a quarter of them belong to Elite class; the presence of individuals in Ist class is sporadic. In contrast, in the private farm, half of individuals fall within the Elite class and only a third of them are in the Record class; the lambs belonging to Ist class have a relatively high frequency. Differences between the two farms regarding the lamb pelt quality have statistical assurance validated by high value of the test χ^2 (253.39***). The differences between the two types of farms, in this respect, would be caused by the accuracy of selection system and of reproduction process. In the elite farm the selection pressure is more intense and the activities of selection and mating matching are made with a higher accuracy than in the private farm. Also, in private farm the selection was focused on immediate commercial characters (shape and size of curls) whereas in the elite farm the selection works took into account the setting of some finer characteristics (such as lustre and quality of hair fibre). However, due to the fact that in both populations the zootechnical classes IInd and IIIrd are missing shows that in both kinds of farms the animal selection is made accurately, with rigor distinctions between them.*

Keywords: Botosani Karakul sheep, lamb pelts.

INTRODUCTION

The lamb pelt of Karakul type, through its characteristics, is the only qualitative production trait met in ovine species. In the other sheep breeds, all characters of production have continuous (quantitative) distributions (meat, wool, milk) (Taftă et al., 1997; Pascal, 2007). Currently, there are two major trends concerning sheep breeding for the lamb pelt production, both equally important: selection for improvement of morphological features of lamb pelt curling and selection for strengthening the colours of hairy coating in the colour varieties and diversifying their shades (Marin and Niga, 1975; Niga et al., 1989; Filote et al., 1994). In the greatest extent, these qualitative characteristics have a profound genetic determinism and are less influenced by the environment or technological factors, therefore non-genetic influences (Taftă et al., 1997).
Exteriorization of the qualitative features of Karakul lamb pelt is determined by the selection criteria applied by the sheep breeder (improver) according to his preferences to achieve a certain type of lamb pelt (Taftă et al., 1997; Pascal, 2007).
In this context, this paper aims to analyze comparatively how the selection was reflected on the morphological traits of lamb pelt curling in two types of farms in which the selection criteria used were differentiated to obtain various characteristics of lamb pelts.

MATERIALS AND METHODS

For the aim pursued, we made experiments in two populations of Botosani Karakul sheep belonging to two types of farm, in each of them the selection pressure and accuracy of reproduction process have had differentiated valences: 194 lambs of the farm elite within the *Research and Development Station for Sheep and Goat Breeding Popauti* (RDSSGP Popauti) and 161 lambs into a private farm

within the *Association of Sheep and Goat Breeders "MOLDOOVIS"* Botosani (SGBS "MOLDOOVIS" Botosani).
The main qualitative physical and morphological features of their pelts were revealed by multifactorial estimation method, as follows (Filote et al., 1994; Hrinca et al., 1994).

- curl shape, with characteristics: cylindrical tube, tube+grain, grain, flat tube, varia (heterogeneous shapes of curls);
- curl size, with characteristics: middle, middle-small, small, big;
- hair fibre quality, with characteristics: silky, normal, rough, soft;
- hair fibre lustre, with characteristics: intense, good, satisfactory, weak-metallic-mat;
- framing in zootechnical classes. The two populations of lambs were analyzed depending on their productive performances, too, by distribution of the animals in zootechnical classes; in the Botosani Karakul breed this operation is performed at the time of estimating the qualitative characteristics of lambs and represents a summation of the scores obtained by all morphological traits of lamb pelt.

To see the significance of differences between the two types of farm as regards the qualitative features of lamb pelts we used the Hi^2 test (χ^2).

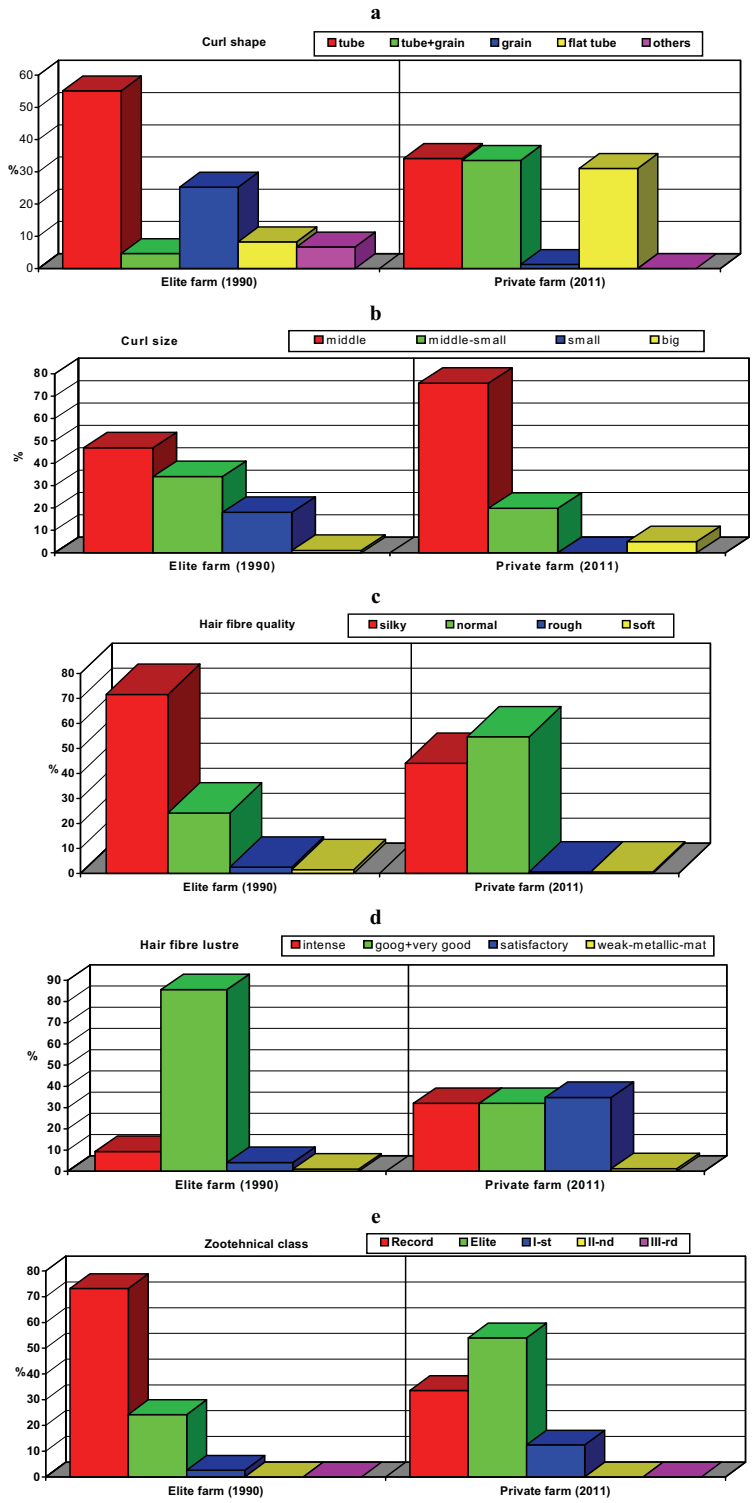
RESULTS AND DISCUSSIONS

The comparative analysis of general panel, relating to the qualitative features of lamb pelts, the elite farm of RDSSGB Popăuţi and the private farm of SGBS "MOLDOOVIS" Botosani, records some differences, as fallows (fig 1).

Curl shape (Figure 1a)
For a long time the classical shapes of "tube" and "grain" were considered the most valuable, from the economic point of view, in particular the "tube" shape presenting a special curling of hair fibres. The combined type of the first two shapes, “tube+grain”, is also an appreciated zoo-economic feature. The diversified practice of improvement systems lead to the occurrence of a "flat tube" type of curl, which (though it seems that presents some histological and chemical deficiencies regarding the curling of hair fibres) has a very nice design. Therefore, all four curl types, with their specific histo-chemical and morphological peculiarities, are valuable economically, their qualitative appreciation being a fashion matter. The last feature, "varia", shows a very heterogeneous design, with Tsurcana aspect, and therefore has a low zoo-economic value.
In the elite farm, the "tube" and "grain" shapes are more frequent than in the private farm. In contrast, the incidence of "flat tube” and of combined shape "tube+grain" in lambs of private farm are higher than those of elite farm. It is however surprisingly the presence of lambs with inhomogeneous curls (right in low rate) in elite farm, such samples missing in private farm. In elite farm, the large presence of lambs having curls with "tube" and "grain" shapes may be due to breeding of black variety in specialized zootechnical lines, too: the line 5 is composed of individuals whose hairy coating is curled in “tube” shape and line 1557 includes individuals with a considerable presence of the "grain".

Curl size (Figure 1b)
The first two sizes of curls present high economic value, while the extreme sizes are undesirable traits in the livestock practice.
The "middle" size of curl is the essential characteristic found in the private farm, while in the elite farm the size characteristics of curls have a more balanced distribution. In the elite farm, as in the private one, the "middle" size is more frequent than the other sizes, but as well the curls with “middle-small” size represent an important share. Among the less valuable characteristics, the lambs with "small" curls are missing in the private farm, and in the elite farm registering a moderate incidence, while the lambs with “big” curls have low frequencies in both farms, but in the private farm their frequency (5%) is five times higher than in the elite farm (1%).

Quality of hair fibre (Figure 1c)
Only the first feature corresponds, to the highest degree, to the economic exigencies and partially the second feature, while the last two characteristics are attributes that must be eliminated from population.
In the elite farm, most lamb pelts have "silky" hair and to a lesser extent the hair fibre is "normal", while in the private farm the percentages of the two qualities of hair fibre are in a sensible balance. The fibres with "soft" and "rough" hair are rarely or sporadically found in lambs of both types of farm.



χ^2 =253.39*** p<0.01

Figure 1. Incidences of qualitative features of lamb pelts in Botosani Karakul sheep from two farm types

Lustre of hair fibre (Figure 1d)
The "intense" lustre is one of the most important properties of lamb pelts, but it is obtained hard enough, its incidence being relatively low, so that most valuable lamb pelts have a "good+very good" lustre (very accepted trait in the selection respect). The "satisfactory" lustre is increasingly harder accepted in selection works, and the "weak-metallic-mat" one confers to lamb pelts a very poor quality.
In elite farm the hair lustre is mostly "good+very good" (approximately 86%); a certain part of lamb pelts have hair fibres with "intense" lustre (approximately 10%). In the private farm, the three categories of lustre record almost equal frequencies (approximately 1/3 for each lustre characteristic). Noteworthy that in the private farm the "intense" lustre is three times more frequently than in the elite farm, this fact being correlated with a high incidence of "flat tube" curls found at lamb pelts from the first farm type, but the "good+very good" lustre is very incident in lambs of elite farm towards the one of private farm. On the other hand, if in the private farm the "satisfactory" lustre is present to a third of lamb population, then in the elite farm this feature is very little common (4%). In both types of farm the lambs with "weak-metallic-mat" lustre are quite rarely met (1%).

Framing in zootechnical classes (Figure 1e)
Depending on the production performance of animals, the zootechnical classes in the Karakul sheep breed are ranked thus: Record, Elite, I^{st}, II^{nd} and III^{rd}.
Cumulating all qualitative features of lamb pelts which determine the framing of individuals into zootechnical classes, it comes out that in the elite farm three quarters of the lambs are in Record class, and a quarter of them belongs to Elite class; the presence of individuals in I^{st} class is sporadic. In contrast, in the private farm, half of individuals fall into Elite class and only a third of them are in Class Record; the lambs belonging to I^{st} class register a relatively high frequency (approximately 13%). The classes II^{nd} and III^{rd} are missing in the two populations, sign that in both types of farm the animal selection is made accurately, with certain differences between them concerning the application rigors of selection process.
This configuration of qualitative features of lamb pelts (especially their distribution in zootechnical classes) confers a certain economic and production advantage to elite farm in comparison with the private one. The differences between the two farm types, in that regard, would be caused by the accuracy of selective system and reproductive process. In the elite farm the selection pressure is stronger than in the private farm. Also, the selection and matching mating activities are more elaborated and are made with higher accuracy in the elite farm towards the private farm. This issue is determined by the herd size of the two types of farms. In the elite farm the selection area is wider, thanks to bigger herds which it holds. Also, the sheep breeding on the basis of zootechnical lines in elite farm confers on it a more elevated selective advantage compared to private farm. Related to this, a specification would be required. The analysis moments were 1990 for farm elite and 2011 for private farm. In 1990, there were more than 15,000 animals in the elite farm, so that the selection range was very wide. It is possible that during the last years, these features have undergone a certain dynamics because of drastic decline in the animal number in this farm, thus the selection area being narrowed too, and therefore it is possible to decrease the incidence of valuable traits of lamb pelts. That is a hypothesis which should be taken into account, but it must be tested by further observations and experiments.
In the private farm there were preponderantly aimed the shape of curls and their size, while in the elite farm the selection was focused on all qualitative features of lamb pelts. In elite farm the selection works aimed fixing of traditional shapes of curls ("tube" and "grain"), while in private farm the main target of breeder was to obtain "flattened" curling. However, it should not be overlooked from the improvement equation that in experimentation period in elite farm the Line 2000 (with "flattened" curling) was at the beginning of its creation or have had a very short history, so that the fixing of its characteristics was at a low level. It seems that only in the respect of curl size, the private farm presents a slight productive advantage on the elite farm. As regards the features of hair fibres (quality and lustre), the superiority of the elite farm on the private farm is more than obvious.

At the same time, the morpho-production superiority of elite farm towards the private one might also be due to the fact that in the private farm the selection was focused on immediate commercial characters (shape and size of curls), while in the elite farm the selection works took into consideration fixing certain features of fineness (especially those concerning quality and lustre of hair fibres).
From the economic point of view, to respond quickly to the changing market demands, it is necessary that the improvement of Karakul sheep to be carried out on all the qualitative features of their individuals to obtain various assortments of valuable lamb pelts; doing so the unilateral selection can be avoided.

CONCLUSIONS

Between the elite farm and the private farm there are some production differences as regards the qualitative features of lamb pelts as a result of the selection criteria applied in a differentiated manner in the two farm types.
In the private farm the selective process aimed preponderantly shape and size of curls, whereas in the elite farm the selection was focused on all the qualitative features of lamb pelts (shape and size of curls, quality and lustre of hair fibres).
The individual distribution in zootechnical classes, depending on the qualitative features of lamb pelts, confers a certain economic and production advantage to the elite farm on the private one.

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USE OF POTASSIUM SORBATE AND SODIUM ASCORBATE FOR EXTINDING THE SHILFLIFE OF REFRIGERATED GROUND BUFFALO MEAT

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Abstract

Ground buffalo meat was preblended with either 0.3% potassium sorbate, 0.05% sodium ascorbate, and 0.3% potassium sorbate. 0.05% sodium ascorbate stored in refrigerator at 4ºC ±1ºC. Color (L, a, b), pH value, water holding capacity (WHC), cooking loss 2-Thiobarbituric acid (TBA) number, and total volatile bases, (TVB) were determined. Aerobic plate counts (APC), anaerobic bacterial count, psychrophilic bacterial count, total coliform and sensory properties were also determined. The results revealed that ground buffalo meat treated with 0.3% potassium sorbate and 0.05% sodium ascorbate had the highest Hunter "a" value (redness). pH values, TBA number, and TVB increased along with storage period. Ground buffalo treated with potassium sorbate alone or potassium sorbate mixed with sodium ascorbate had lower anaerobic and psychrophilic bacterial counts than samples treated with sodium ascorbate alone and still accepted for panelist after 10 days of refrigerated storage.

Keywords: *buffalo meat, potassium sorbate, sodium ascorbate.*

INTRODUCTION

The ground meat is produced mainly from very old unproductive animals which results in it being coarse and tough in texture, and dark in color. Such meat is profitably utilized by comminuting and using in a variety of meat products (Sahoo and Anjaneyulu, 1997a). Spent male and female buffalo meat is more suitable for processing in chunks (Kandeepan et al., 2009). Ground meat tends to become brown and rancid more rapidly than whole muscle retail cut since grinding exposes more of the muscle surface to air and microbial contamination (Mitsumoto et al., 2005). Such changes are attribute to rapid formation of metmyoglobin, the undesirable brown color and oxidative rancidity. Lipid oxidation in meats leads to the development of off-flavour, loss of color and nutritive value (Pearson et al., 1983). Microbial growth in fresh meat is the primary factors associated with meat quality reduction, and spoilage. The off-odour compounds that characterize spoilage meat originate largely from the nonprotein nitrogen compounds. Spoilage flora attacks the nonprotein nitrogen components and produces amines and ammonia from these simple components (Jay and Shelef, 1978).

Extended shelf life and meat product safety require maintaining low microbial numbers during fabrication, packaging, and storage of meat at refrigeration temperature. A variety of additives which have the potential for inhibiting microorganisms associated with fresh meat products have been investigated. A concentration of 0.1% potassium sorbate delayed the growth of the spoilage microflora, retarded growth of *Salmonellae*, and *Staphylococcus aureus*, and growth and toxin production by *C. botulinum* (Sofos and Busta, 1981; Robach and Sofos, 1982; Sofos, 1989). The sorbate has also inhibited bacteria (i.e.,total *Psychrotrophs, Pseudomonas spp., B. thermosphacta, Lactobacillus spp., Enterobacteriaceae, Sallmonella* and *Staphylococcus aureus, Cl. botulinum* yeast and molds) and extended the shelf life of raw beef (Robach and Ivey, 1978; Zamora and Zaritzky, 1987b; Zamora and Zaritzky, 1987a; Sofos, 1989).

The use of antioxidant like ascorbic acid had a significant effect in reducing oxidation of pigments and lipids of ground and beef steaks

(Greene et al., 1971; Shivas et al., 1984; Okayama et al., 1987; Mitsumoto et al., 2005). Sodium ascorbate (SA) at 500 ppm retarded pigments and lipids oxidation and extended the shelf life of ground buffalo meat from 4 to 8 days under refrigerated storage at 4°C+1°C (Sahoo and Anjaneyulu, 1997a). Extending the shelf life of fresh meat is very important consideration for both consumers and meat packers. The storage life of fresh meat can prolong by limiting the extent of discoloration, lipid oxidation and microbiological contamination.

The objectives of this study were to evaluate effect of adding potassium sorbate and sodium ascorbate used alone or in combination on the quality of ground buffalo meat during refrigerated storage.

MATERIALS AND METHODS

12 kg meat chunks of about 2 kg size from top round of spent female buffalo, about 10 years age was obtained within 4 hours of slaughter from local market in Minia city. Meat chunks were packed in polyethylene bags, transported to Food Science Department, Faculty of Agriculture, Minia University and kept for conditioning in a refrigerator at 4°C±1°C for about 24 hr. The meat chunks were trimmed of separable fat and loose connective tissue, cut into small cubes and ground by using a meat grinder (Moulinex, HV2, Model A14, France) with a 8-mm hole plate with adding 20% fat. Ground buffalo meat 20% fat was divided into 4 portions and mixed with either 0.3% potassium sorbate (Sofos, 1989), 0.05% sodium ascorbate was bought from Sigma Chemical Company (St. Louis, MO, USA), (Sahoo and Anjaneyulu, 1997b), 0.3% potassium sorbate/0.05% sodium ascorbate, and minced again with 4 mm hole plate for uniform dispersion of additives. Both control and treated ground buffalo meat were divided into 200 g, placed in Styrofoam tray and overwrapped with stretch film (saran). All trays were stored in refrigerator at 4ºC ±1ºC for 12 days. The samples were examined for quality parameters at 4 days intervals during storage.

Microbiological analysis

Aerobic plate counts (APC), Anaerobic count, Psychrophilic count, and coliform count of the treated and the untreated ground buffalo meat were determined as CFU/g according to the methods described in the standard methods (APHA, 1985; Vaderzant and Splittstoesser, 1992). BBLGasPak^R anaerobic chamber with BBL GasPak CO_2 gas packs (Becton Dickinsin Microbiology System, Boston, MA) was used to create an anaerobic environment for incubation.

Color values (lightness L*, redness a*, and yellowness b*) were measured for treated and untreated ground buffalo meat at zero time and during storage period with a colorimeter (Color Tec PCM Color Meter Tec. NJ, USA). Four random measurement spots on each sample were made and the average data were recorded according to Holownia et al. (2003). pH was determined by homogenizing 10 g of ground meat in 90 ml distilled water using a homogenizer (VIRTIS Model 6-105 AF, The VIRTIS Company, NY, USA) for 5 min and measuring the pH of the resulting slurry with a digital pH meter (Model 41250, ICM, OR, USA), standardized at pH 4 and 7 (Lee and Yoon, 2001). The average of three reading was recorded.

Expressible water was determined according to Alvarez et al. (1992), while water-holding capacity (WHC) was calculated. Thiobarbituric acid reacting substances (TBARS) number, TBARS was determined following the distillation method described by Tarladgis et al. (1960). Total volatile basic nitrogen was measured according to Pearson (1975). Cooking loss, Meat samples (25 g each) were tightly wrapped in polyethylene bags and cooked, totally immersed, in water bath at 80^{o}C for 20 min. After cooking they were cooled, dried with paper towels and cooking losses were determined from the weights before and after cooking (Anjaneyulu et al., 1989).

Sensory evaluation. Samples from each group were randomly assigned for sensory evaluation according to Sahoo and Anjaneyulu (1997a). Twelve panel members with previous panel experience were chosen to evaluate ground meat buffalo odor and color discoloration during storage. Sensory score for odor was obtained by following a 5-point scale where 1 =

very unpleasant, 2 = moderately unpleasant, 3 = moderately pleasant, 4 = pleasant and 5= very pleasant. The score for color discoloration was 1 = pale pink, 2 = pink, 3 = pinkish red, 4 = bright red and 5 = reddish-brown.

Statistical analysis. Data was analyzed with GLM (General Linear Model) program using statistical analysis system (SAS, 1987). Mean values were compared by Duncan's Multiple Test.

RESULTS AND DISCUSSION

Data in Table 1 revealed that the L values (lightness) of all samples increased during storage time. The value (redness) of all sample decreased after 4 days of storage except samples treated with sodium ascorbate (SA) only, SA and potassium sorbate. No changes were found in the redness of samples treated with sodium ascorbate and potassium sorbate after 8 days of storage. Sahoo and Anjaneyulu (1997a) found that 500 ppm sodium ascorbate treatment increased the lovibond tintometer red color units of ground buffalo during storage at 4°C. Redness of control and sample treated with potassium sorbate only was sharply decreased after 4 days of storage.

Table 1. Effect of potassium sorbate and sodium ascorbate on the color (L*, a* and b*) of ground buffalo meat during refrigerated storage at 4°C

Treatments	Storage time days				
	Color	0	4	8	12
Control	L	42.12b	42.90a	44.96 a	
	A	19.63a	b	13.43 b	
	B	10.27a	12.59b	8.76b	
			10.43a		
Potassium sorbate	L	45.44b	46.16b	47.09 b	45.72 b
	A	20.13a	13.64b	13.59 b	11.44 c
	B	8.69ab	10.20a	9.66a	7.65b
Sodium ascorbate	L	45.49a	45.49a	45.21 a	45.50 a
	A	23.56a	23.06a	16.08 b	12.79 c
	b	12.77a	10.44a	10.52 a	11.09 a
Potassium sorbate + Sodium ascorbate	L	43.69b	42.33c	45.37 b	45.22 b
	A	c	23.82a	23.21 a	22.80 a
	b	23.56a	11.26a	11.56 a	11.19 a
		11.63a			

a,b,c Mean values in the same row not followed by the same letter are significantly different ($P \leq 0.05$)

Generally, the pH was increased gradually with increased storage time (Table 2). The highest values of pH were found in sodium ascorbate (5.24) and control (5.20) at the fourth day and at the eighth day of storage (Table 1). Shelef and Jay (1970) reported that the difference between freshness and incipient spoilage ground beef usually dose not exceed 0.3-0.5 of a pH unit during the first 4 days of storage. The increase of pH may have been owing to bacterial metabolic by-products, such as amino sugar during storage (Jay, 1992).

Table 2. Effect of potassium sorbate and sodium ascorbate on the pH and water holding capacity (WHC) % of ground buffalo meat during refrigerated storage at 4°C±1°C

Treatments	Storage (Time Days)				
		0	4	8	12
Control	pH	4.9c	5.20b	6.17a	
	WHC*	31.36 c	38.81 a	37.83 b	
Potassium sorbate	pH	5.33b c	5.08c	5.22b	5.53a
	WHC	34.77 c	37.06 a	36.02 b	36.03 b
Sodium ascorbate	pH	4.85c	5.24b	5.81a	5.95a
	WHC	32.49 c	39.69 a	38.53 b	38.20 b
Potassium sorbate +Sodium ascorbate	pH	4.97b	5.06a	5.23a	4.98b
	WHC	31.94 b	32.81 b	35.75 a	35.22 a

a,b,c Mean values in the same row not followed by the same letter are significantly different ($P \leq 0.05$)
*WHC =Water Holding Capacity

Water holding capacity (WHC) for all samples increased during storage time and the lowest values of WHC was found in the sample treated with potassium sorbate and sodium ascorbate at 4, 8, and 12 days of storage (Table 2).

It is well known that lower WHC is associated with lower pH. Both increasing a pH as a result of ammonia production and amino sugar complex formation has the effect of increasing the WHC of meats during refrigerated storage (Jay and Shelef, 1978).

Cooking loss gradually decreased along with storage time. The treated samples with potassium sorbate had higher values of cooking loss than that of sodium ascorbate treated samples at 4 and 8 days of storage (Figure 1).

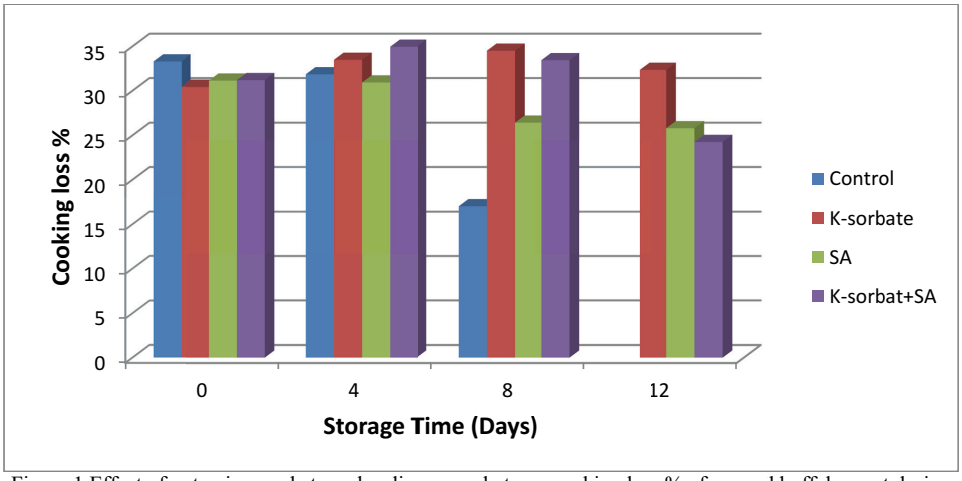

Figure 1 Effect of potassium sorbate and sodium ascorbate on cooking loss % of ground buffalo meat during refrigerated storage at 4°C±1ºC

Jay (1992) reported that free amino acids and related simple nitrogenous compounds utilized by bacteria during the first days of refrigerated storage and the primary proteins are not attacked until the supply of simpler constituents has been exhausted. The total volatile basic nitrogen (TVBN) could be used as a quality indicator for fish products and is associated with the amino acid decarboxylase activity of microorganisms during storage (Jay, 1992). Changes in TVBN values during storage are shown in Figure 2.

TVBN values of all treatments increased with increasing storage time and potassium sorbate / potassium sorbate and sodium ascorbate had lower TVBN values than other treatments. Control, and SA treatments remained at higher TVBN values suggesting greater bacterial populations and activity, which in agreement with microbial counts (Figures 4, 5, 6, and 7). Only control sample had 34.56 mg/100 g TVBN and became unacceptable after 8 days of storage.

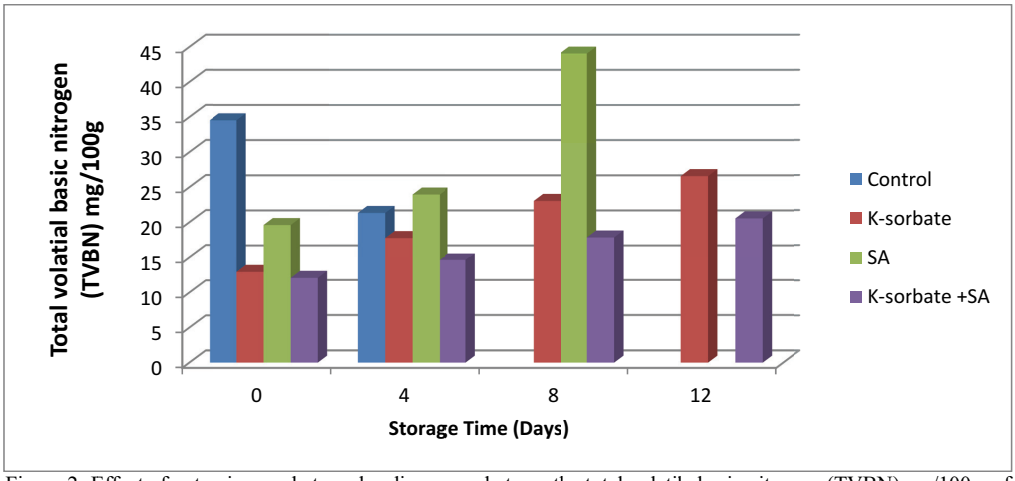

Figure 2- Effect of potassium sorbate and sodium ascorbate on the total volatile basic nitrogen (TVBN) mg/100 g of ground buffalo meat during refrigerated storage at 4ºC±1°C

TBARS values increased over time for all samples. The increment was rapid for the control samples and the greatest changes occurring between the 8 and 12 days of storage.

Samples treated with SA had lower TBARS than other samples (Figure 3). On the other hand, Rhee et al. (1997) reported that TBARS were higher in antimicrobial treated samples, which suggested that microorganisms in the untreated meat may have removed malonaldehyde and other TBARS. Sahoo and Anjaneyulu (1997a) reported that sodium ascorbate at 500 ppm contributed to the lowest TBARS value (0.26 mg malonaldehyde/kg) in refrigerated ground buffalo meat indicating that it inhibited lipid oxidation.

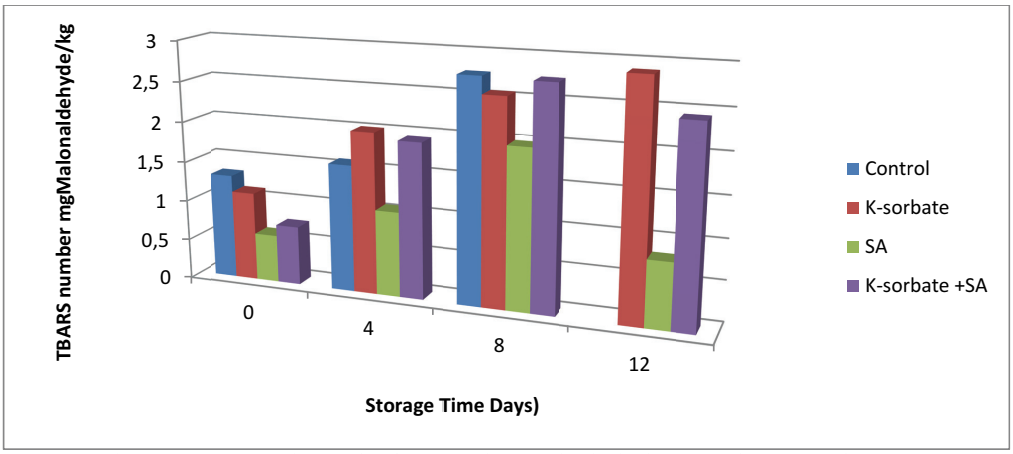

Figure 3. Effect of potassium sorbate and sodium ascorbate on the thiobarbituric acid reacting substances (TBARS) number of ground buffalo meat during refrigerated storage at 4°C±1°C

The growth of microbes in meat is one of the main factors that cause discoloration and spoilage. Aerobic plate counts (APC) for samples treated with SA increased with increasing storage time and reached 6 Log CFU/g after 8 days (Figure 4). However, APC for K-sorbate, K-sorbate and SA, treated samples decreased after 4 and 8 days and then increased after 12 of storage. According to the guidelines from the Meat Hygiene Manual (Canadian Food Inspection Agency), these maximum values are 7 and 3 log CFU/g for total aerobic mesophilic and coliform count (Saucier et al., 2000). Aerobic plate count of control sample was over the accepted limit on the day 12 but exhibited off-odor on the day 8. APC of samples treated with K-sorbate alone or K-sorbate mixed with SA were less than 5 log CFU/g after 12 days of storage. Aerobic plate count remained under the maximum value (7 log CFU/g) after 12 day of storage for all samples except the control. Zamora and Zaritzky (1987a,b) reported that potassium sorbate treatment inhibited the bacterial growth and extended the shelf life of refrigerated beef slices. Sorbic acid is a lipophilic acid preservative with a short chain length and this kind of substances inhibits both gram positive and gram negative bacteria (Sofos and Busta, 1981).

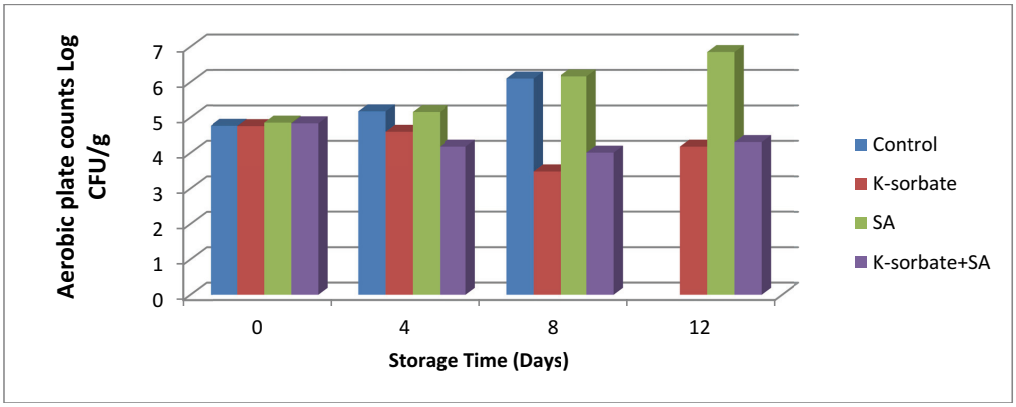

Figure 4. Effect of potassium sorbate and sodium ascorbate on the aerobic plate counts in ground buffalo meat during refrigerated storage at 4°C±1°C

Psychrophilic counts and anaerobic count of all samples increased with storage time (Figure 5 and 6). In case of refrigerated meat under aerobic conditions, the spoilage flora is dominated by *Pseudomonas spp.* and under anaerobic condition by *Lactobacillus spp.* (Marth, 1998). Coliform counts had the same trend of APC. The control sample had the highest number of coliform during storage time. The sample treated with K-sorbate alone or mixed with SA had lower coliform count than other treatments and its coliform counts less than 3 Loge after 12 days of storage (Figure 7).

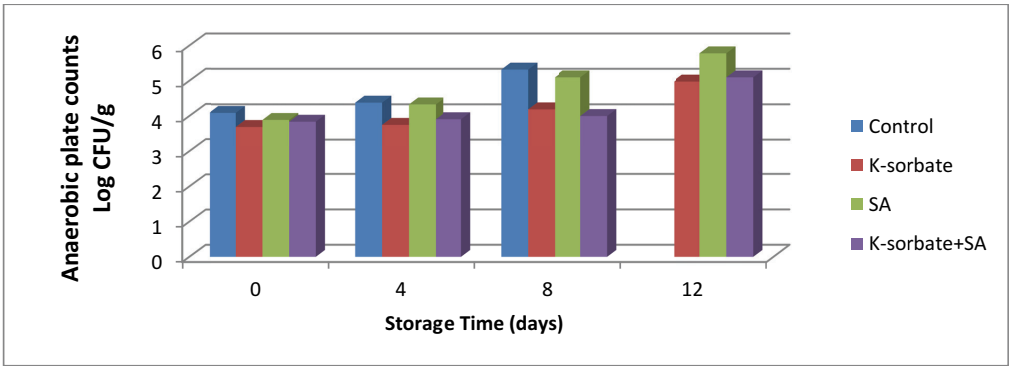

Figure 5. Effect of potassium sorbate and sodium ascorbate on the anaerobic plate counts in ground buffalo meat during refrigerated storage at 4°C±1°C

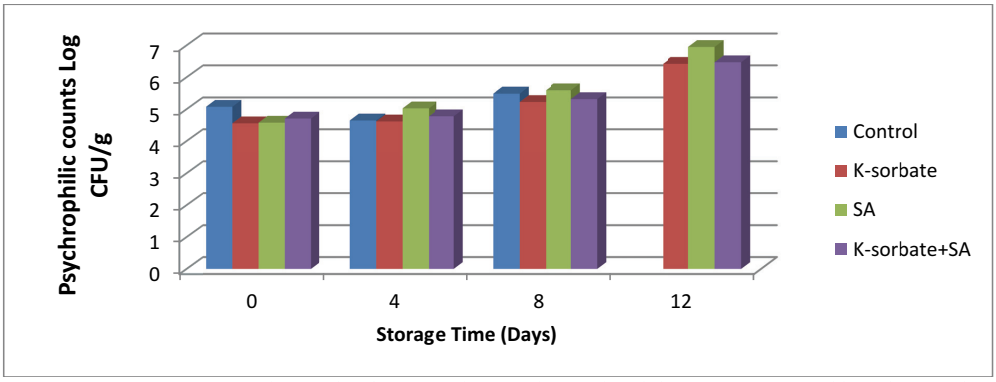

Figure 6. Effect of potassium sorbate and sodium ascorbate on the psychrophilic counts in ground buffalo meat during refrigerated storage at 4°C±1°C

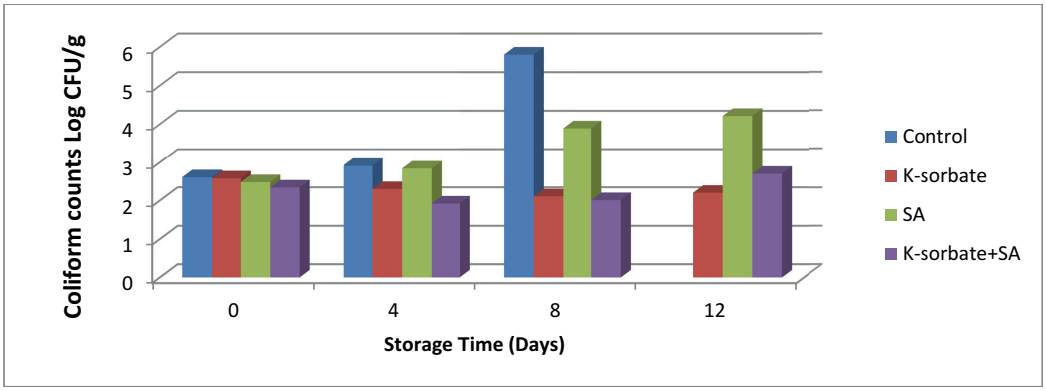

Figure 7. Effect of potassium sorbate and sodium ascorbate on the coliform counts in ground buffalo meat during refrigerated storage at 4°C±1°C

Color score of all samples slightly decreased after 4 days of storage (Figure 8).

The control, K-sorbate, and SA treated samples have lower color score than treated sample with K-sorbate mixed with SA on the day 8. The samples treated with K-sorbate mixed with SA had the highest color score during 12 day of storage time. Discoloration may be attributed to alteration or destruction meat pigments. Myoglobin may be oxidized to brown metmyoglobin. It may combined with H_2S, produced by bacteria, to form sulphmyoglobin (Lawrie, 1998). Rancid flavor and odors arise from oxidative changes occurring in the meat during refrigerated storage.

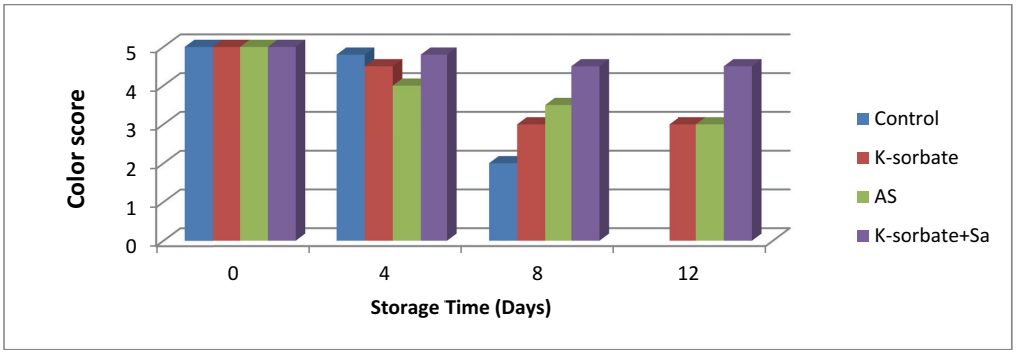

Figure 8. Effect of potassium sorbate and sodium ascorbate on the color score of ground buffalo meat during refrigerated storage at 4°C±1°C.

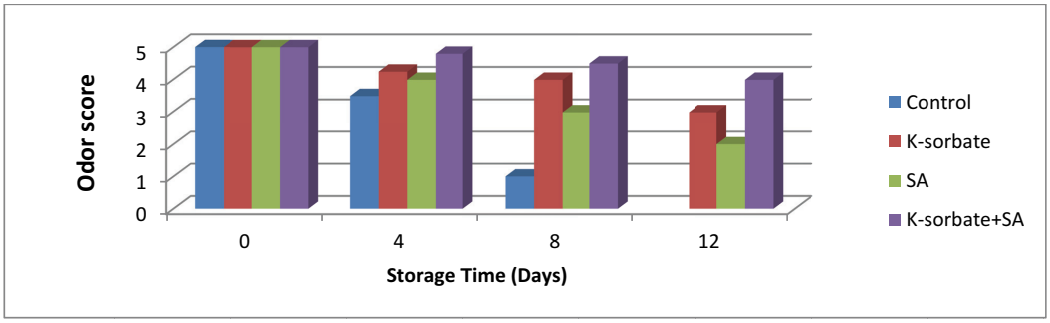

Figure 9. Effect of potassium sorbate and sodium ascorbate on the odor score of ground buffalo meat during refrigerated storage at 4°C±1°C

Odor score of control sharply decreased after 8 days of storage (Figure 9). The samples treated with SA have lower odor score than other treatments during storage time. The mixture and K-sorbate and SA treatments have higher odor scores than other treatment after 12 days of storage. The off-odor of meat may be due to the organisms attacked glucose initially and amino acids subsequently, producing hydrogen, carbon dioxide and ammonia (Jay, 1992). Van Laak (1994) reported that off-odors become noticeable in chilled meat and poultry, when bacterial numbers are between 7.0 and 7.5 log CFU/cm^2. Sahoo and Anjaneyulu (1997a) found that 500 ppm sodium ascorbate extended the shelf life of ground buffalo meat from 4 to 8 days stored at 4°C.

CONCLUSIONS

From these results it could be concluded that the shelf life of ground buffalo meat treated with potassium sorbate alone or mixed with sodium ascorbate (populations of microorganisms chemical and sensory quality) could be extended from 8 to 12 days. The sodium ascrobate treatment extended the shelf life of ground buffalo meat from 4 to 8 days under refrigerated storage.

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DECREASING CHOLESTEROL AND TRIGLYCERIDE LEVEL ON BLOOD BY ADDING ORANGE (*Citrus sinensis*) WASTE ON PADJAJARAN I SHEEP

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Abstract

Sweet orange (Citrus sinensis) waste content of flavonoids, namely quercetin and kaempferol, which is expected to inhibit the growth of bacteria. Kaempferol have antibacterial activity, denaturate bacterial cell proteins and cell membranes without damage irreparable. While quercetin has increased permeability porin protein activity on other bacteria. Research about Decreasing Cholesterol and Triglyceride Level on Blood by Adding Sweet orange (Citrus sinensis) waste (SOW) on Sheep Padjajaran 1. This research was done by using Completely Randomized Design with four treatments level i.e. 0, 4, 6 and 8% SOW in ration and repeated five times. The observed variables were blood's lipid profile, consists of cholesterol, HDL, LDL, and triglyceride. The observation showed that level of blood's lipid profile were not significantly changed, but the trend of cholesterol, HDL, LDL, and triglyceride was decreased. The conclusion is using sweet orange waste (SOW) until 8% has not showed statistically significant differences but have positive effect on lowering blood lipids, i.e. cholesterol level decreased 8.05%, LDL 10%, triglyceride 23.4 % on R4 (8%) while using 4% SOW, triglycerides level decreased 24.8% and decreased 33.53% on using 6%.

Keywords*: sweet orange waste, blood lipid, cholesterol, triglyceride, Padjadjaran 1 sheep.*

INTRODUCTION

The sheep is the fourth meat producer after poultry, cattle and swine. Currently, the sheep contributes 16.12% for national meat production while the population almost 59.52% in West Java (Agriculture Ministry/Deptan, 2013). The excellence mutton have full-flavored high typical, but their meat has high fat and cholesterol levels which in turn has impact on health. Hence some people assumed that consume mutton can trigger vascular disease and atherosclerosis.

The production of a citrus fruit in Indonesia are 2.355.550 tons per year (Agriculture Ministry/Deptan, 2010), and the orange waste almost 60%, which is composed of skin, the membrane and seeds. Considering the waste potential sweet orange can be used as feed supplements that play a role in improving the profile of blood fat.

MATERIALS AND METHODS

According to Bandiati (2012), *Padjadjaran 1* sheep is a local genetic material that has been cross bred and still in Garut sheep family that came from Wanaraja Garut regency, still in breeding process for meat sheep with white hair and wide years as their identity.

This research used 20 *Padjadjaran 1* sheep with body weight averages 30.42 $\pm$ 4.50 kg. Sheep breeding cages are obtained from Padjadjaran Station Farm, University Padjadjaran. The research conducted during 8 weeks. The rations consist of 40% concentrates and 60% is *Brachiaria brizantha grass lawn* and *Pennisetum purpureum* mixed grass. The content of the feed materials of food substances of rations of dried materials based on the research are presented in Table 1.

Table 1. Nutrient Composition (%)

Ingredient	Nutrient Content						
	BK	Ash	CP	CF	CF	BETN*	TDN*
	---------- % ----------						
Rice Brand	87.70	13.60	11.00	14.00	8.60	40.50	67.90
Palm oil Cake	86.00	4.10	15.00	22.00	11.90	33.00	79.00
Tofu Waste	14.60	5.10	30.30	22.20	9.90	32.50	77.90
Coconut Cake	86.00	8.20	19.00	14.00	10.90	33.90	78.00
Citrus sinensis	90.01	7.70	6.50	12.76	3.40	59.65	79.00
CGF	90.40	4.33	17.71	20.11	5.40	42.85	73.68
Skin Bean	90.75	24.30	9.18	25.80	4.49	26.98	50.70
Molases	82.40	11.00	3.90	0.40	0.30	66.80	70.70
Pollard	88.50	5.90	18.50	9.80	3.90	50.40	69.20
Cassava cake	79.80	2.40	1.80	8.90	0.30	66.40	78.30

Source: Proximat analysis Laboratorium Ternak Ruminansia and Kimia Makanan Ternak, Animal Husbandry University Padjadjaran (2010)

Data Analysis

The data were analysis to know the differences between the treatments with Duncan test. The parameters are: Blood cholesterol, HDL (High Density Lipoprotein), LDL (Low Density Lipoprotein) and triglyceride

RESULTS AND DISCUSSIONS

The effect of using sweet orange (*Citrus sinensis*) waste meal in the ration on cholesterol levels LDL, HDL and triglycerides blood sheep Padjadjaran 1, in Tables 2 and Figure 1.

Table 2. The Cholesterol , HDL, LDL, and Triglyceride Blood on Padjadjaran 1 Sheep

Treatment	Cholesterol	HDL	LDL	Triglyceride
	mg/dL			
R1	69.6	44.8	23.4	32.8
R2	73.4	44.8	25.4	24.8
R3	71	46.4	22.8	21.8
R4	64	40.2	21	23.4

Notes:
R1 = Control diet (Placebo)
R2 = Contol died + sweet orange waste 4%
R3 = Control diet + sweet orange waste 6%
R4 = Control diet + sweet orange waste 8%

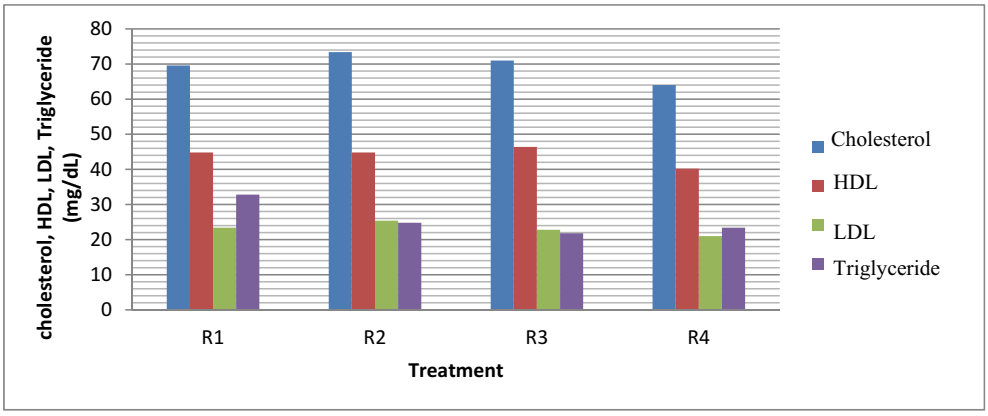

Figure 1. The effect of Sweet Orange Waste meal on Padjadjaran 1 Sheep on blood cholesterol, HDL, LDL, Triglyceride

From Figure 1, it shows that the highest cholesterol level is 73.40 mg/dL (P2=4% SOW), while the lowest cholesterol is 64.00 mg/dL (P4=8% SOW). And for HDL, the highest is 46.40 mg/dL (P3 = 6% SOW) and the lowest is 40.20 mg/dL (P4 = 8% SOW). For the highest LDL is 25,40 mg/dL (P2 = 4% SOW) while the lowest LDL is 21.00 mg/dL (P4 = 8% SOW). The highest triglycerides are 34.80 mg/dL (P1 = placebo), while the lowest triglyceride is 21.80 mg/dL (P3 = 6%). The results of the analysis range shows that giving SOW has no significant ($P < 0.05$) in decreasing blood cholesterol levels, LDL, HDL, and triglycerides Padjadjaran sheep, but the trend is decrease.

Based on the results of Duncan multiple range test, that the granting of SOW in all cases, indicating that the results did not differ significantly compared with R1 (placebo).

Table 3. Duncan Test Effects treatment on Cholesterol, HDL, LDL, and triglyceride Blood Level on Padjadjaran 1 Sheep

	Significancy 0,05			
Treatment	Cholesterol	HDL	LDL	Triglyceride
R1	a	a	a	a
R2	a	a	a	a
R3	a	a	a	a
R4	a	a	a	a

Note: same superscripts indicate non significantly different effect ($P > 0.05$).

Although the results are not significantly different (Table 3), but there is a tendency to decrease the cholesterol in R4, LDL in R3 and R4, and the triglycerides in R2, R3, and R4. This indicates that the active substances contained in SOW can work to decrease cholesterol, LDL, and triglycerides level. This

is consistent with Chaudry et al., (2004), which states that the granting of orange waste powder with 5% decreased blood level sheep lipoproteins. The reduction in blood cholesterol may result from the fusion of active substances and crude fiber contained on orange waste powder. The crude fiber foods tend to accelerate the rate of passage of food in the digestive tract, so the absorption of cholesterol and other substances will also decrease. According to Lovita et al., 2013, such circumstances are strengthened, that cholesterol levels dropped in line with rising crude fiber content because of the orange waste in rations. A sweet orange waste is rich of pectin that helps in the process of lowering blood cholesterol due to molecular interaction between lipid and pectin (Jenkins et al., 1976; Selvendran, 1978). Pectin can increase the fat excretion due to anaerobic fermentation in the digestive tract (Dutta and Hlasko, 1985). Pectin may reduce the absorption of fat, so the absorption of triglycerides decreased (Sutardi, 1992). Flavonoid is vital in oranges, the herperidin has been proven to lower high blood pressure and cholesterol in animal experiments, and has anti-inflammatory properties (Lovita et al., 2013). The flavonoid is vital in oranges, the herperidin has been proven to lower high blood pressure and the cholesterol in animal experiments, and has anti-inflammatory properties. Most of these phytonutrients found in the white part and possibly beyond the meat of the citrus, not at the center of the orange liquid, and these compounds will be damaged by the processing. When the animals with high cholesterol are given feed that contains 1% PMFs (especially tangeretin), the levels of total cholesterol, VLDL and LDL is reduced until 19-27, 32 and 40%, and when the animals were given feed containing a mixture of 3% of two different flavonones (naringin and hesperidin) grapefruit, cholesterol decreased, caused by tannins and saponins in sweet orange that is preventing an increase in the secretion of bile salt so can inhibited the formation of cholesterol. Cholesterol decreasing also caused by the role of flavonoids and essential oil in inhibiting the early stages of the reaction by freeing 1 H atoms of the hydroxil cluster, and attached with 1 free radicals. This bond will stabilize the radical peroxide who made the activation energy is reduced, and will further inhibit the oxidation of LDL cholesterol (Nurwahyunani, 2006). The oxidation reaction inhibits the enzyme work i.e. 3-Hydroxy-3-metilglutaril-CoA (HMG-CoA reductase), acts as a catalyst for the biosynthesis of cholesterol (Martin et al., 1981). The sheep blood triglyceride levels decrease due to the content of the active substance contained in the waste of sweet orange. The active substances such as antioxidant flavonoids reduce inflammation and can capture free radicals or oxygen compounds (Reynertson, 2007) and ultimately inhibit the synthesis of triglycerides. The initial formation of the triglycerides compound of glycerol-3-phosfat from glycerol, di-hydroxide acetone phosphate that are experiencing reduction in the presence of NADH, to synthesize Glycerol-3-Phosphate of disease (GPDH) for the synthesis of triglycerides.

He et al. (2009) reported that the essential oil was decrease the activity of Glycerol-3-Phosphate of disease (GPDH) enzyme, which is involved in the biosynthesis of triglycerides. Free radicals oxidize the cholesterol, so it works after oxidized cholesterol does not stick to the walls of the arteries that build the plaque, can eventually grow and large enough to hinder or completely blocked the blood flow and vitamin C contained in flour sweet citrus waste can neutralize free radicals.

The saponins have a lipophilic molecule that is able to dissolve fats and emulsions that can lower serum cholesterol (Harborne, 1987) and can lower blood cholesterol levels of animals (Francis et al., 2002). Then saponins may decrease the animal hypercholesterolemia. Saponin in feed containing triglycerides formed a bond that difficult to absorbe by the intestines, so the absorption of triglycerides is hampered. Tannins in the body will bind the

protein and will coat the intestinal wall, so the mucus layer compaction in the digestive tract and inhibit the absorption of food substances, including cholesterol. In accordance with the research, the tannins may increase the excretion of cholesterol. Tannins in the body shall be bound to a protein that will coat the intestinal wall.

The reduction in blood cholesterol levels can be due to a combination of active substances and crude fibers contained in flour sweet citrus waste. Giving a rough fibers tend to accelerate the rate of passage.

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CONCLUSIONS

1. The blood cholesterol level decreased when the sheep has been given 8% orange waste meal, from 69.6 mg/dL to 64 mg/dL, and also the LDL decreased with 10%, while the HDL has not increased. The LDL : HDL ratio are better, because the ratio are more wider than the ratio that has not been added with sweet orange waste meal.
2. The triglyceride level in sheep blood has been decreased although according statistical analysis were non-significant; the sheep that given SOW meal 4% has 24.8% , 33.53% in 6% SOW and 23.4 % in 8% SOW compared with the placebo
3. The sweet orange waste meal can be used as cholesterol and triglyceride decreasing in sheep blood. The optimum is 8% orange waste meal.

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EFFECT OF DIFFERENT LEVELS OF FLAXSEED POWDER AS A SOURCE OF OMEGA-3 ON THE WEIGHT MUSCLES AND FAT DISTRIBUTION FOR CARCASSES OF KARADI LAMBS

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Abstract

This experiment was conducted at College of Agriculture, University of Sulaimany to study the effect of different levels of Flaxseed powder (FP) as a source of omega-3 on the on the weight muscles and Fat partitioning and distribution for carcasses of Karadi lambs.It used 20 male Karadi lambs, with an average live-weight of 28 ± 0.398 kg and 4-5 months. They were randomly distributed to 4 treatments. FP was supplemented at the levels of 3%, 6% and 9% compared with the control group for 88 days. At the end of the experiment 12 lambs were slaughtered after overnight fasting of feed. Carcasses were chilled for 24 h at 4°C. Then, several measurements of carcass characteristics were taken.
The results showed: higher ($p<0.05$) weights of the individual weight muscles located in different carcass region (pelvic limb, dorsal region and thoracic limb) were observed in Flax seed powder supplementation treatments. There were significant differences ($p<0.05$) in the carcass fat, offal and total fat in half animal body partitioning and distribution among treatments. Also it is noticed that the results of these traits were various. It can be concluded that using Flax seed powder (FP) as a source of omega-3 supplementation was increased muscles weight and reduced of carcass fat . These results were led to improvement of efficiency of meat production in Karadi lambs.

Keywords: *Flax seed powder, muscles weight, fat carcass, Karadi lambs*

INTRODUCTION

Today, one of the principal concerns of animal scientists is to determine the possibility of improving the health quality of animal food products. Among the most effective ways to do this is to use vegetable oils in nutrition (Bas and Morand-Fehr, 2000). In light of many studies conducted mainly with beef cattle, its essential to the efficient use of vegetable oils by ruminants is prevention of polyunsaturated fatty acids (PUF) in feeds from biodegradation in the rumen. The simplest and cheapest way of achieving this is to feed whole oilseeds (Oprządek and Oprządek, 2003). Many scholars think this requires supplementing the feeds with antioxidants, vitamin E being the most efficient and natural one (Barowicz, 2000). The previous studies have shown that Omega-3 source has affected some carcass characteristics, such as carcass weight, rib eye area, 12th rib fat thickness, and muscle weight fat distribution and carcass cuts. As well as, some studies have shown that carcass traits, marbling scores, and quality grade have been affected by flaxseed supplementation for instant (Ponnampalam et al., 2001b;Wistuba et al., 2006; Marinova et al., 2007.)

Flaxseed can be effectively used in feedlot rations. Several studies have demonstrated the use of up to 20% flaxseed in the diet without negatively affecting performance (Newkirk, 2008). Flaxseed has high levels of energy and protein and promotes feed intake and weight gain. Flaxseed has also been shown to offer additional benefits over its nutritional value alone, however flax is a highly palatable feed ingredient and contains high levels of nutrients (Drouillard et al., 2002). Ground flaxseed increased marbling and grade scores when the finishing diet was supplemented with ground flaxseed (Newkirk, 2008). Flaxseed is the richest land-based source of the omega-3 fatty acid α-linolenic acid, or ALA (Connor, 1999). However flaxseed is unique among oilseeds because of its exceptionally high content of ALA (18:3, n-3), contains 35 to 45% oil, of

which 45 to 52% is ALA (Bhatty, 1995). The deposition and distribution of body fat observed in the study of Marinova et al. (2007) suggested that the polyunsaturated fatty acids from the fish oil could be a repartitioning factor for carcass fats in lambs and could have a favorable effect on the carcass fatness and the quality of lamb meat. Therefore, the objective of this study is to investigate the effect of dietary supplementation of Flaxseed powder supplementation as a source of Omega-3 on the weight muscles and Fat partitioning and distribution for carcasses of Karadi lambs.

MATERIALS AND METHODS

Housing and Feeding Trail Experiment

Twenty male Karadi lambs purchased from unknown local contractor were individually housed in pens (1 × 1.5 m^2) at the animal production farm, faculty of agriculture, University of Sulaimany. The ration was gradually introduced to the lambs over a period of 2 weeks as adaptation period. Four treatments of FP (Fat partitioning) supplementation on voluntary feed intake were conducted with 20 male Karadi lambs (live body weight 28 ± 0.398 kg and 4-5 months old) at the start of the experiment. The lambs were randomly allocated into four treatments to receive either control ration no FP, T1 or ration containing 3% FP, T2 or diet containing 6% FP, T3 or ration containing 9% FP. All the lambs were received an equal daily allowance of concentrate ration (3% of the body weight). The formulation and approximate chemical composition of concentrate diet are presented in Table 1. The lambs were randomly penned individually indoors on dry earth bedding and the concentrate was supplied once daily (9:00 am). The straw was given *ad libitum*. Each ration treatment was tested for 2 weeks adaptation and 13 weeks of feeding periods respectively. Daily feed intake and refused were measured and sampled for 13 weeks. The lambs were weighed once a week from the beginning till the end of the experiment.

Table 1. Formulation and chemical composition of concentrate diets

Ingredients (%)	Control	T1	T2	T3
Barley	40	40	40	40
Wheat bran	27	27	27	27
Corn	15	15	15	15
Soybean meal	15	12	9	6
Flaxseed powder	0	3	6	9
Mineral &Vitamin mixture	2	2	2	2
Salt	1	1	1	1
Urea		0.2	0.4	0.6
Chemical composition				
CP %	15.38	15.31	15.23	15.14
ME (MJ/KG)*	12.77	11.63	11.82	12.01

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

Slaughtering and Carcass Characteristics

At the end of feeding trial (13 weeks), from each treatment three lambs were randomly slaughtered after feed was withdrawn overnight**.** The lambs were weighed immediately before slaughter to provide slaughter body weight (SBW). The slaughtering was performed according to Islamic law by severing the jugular vessels, esophagus and trachea without stunning. The lambs were slaughtered in an experimental abattoir. The carcasses were longitudinally split into two equal sides, right and left, after removing the tail fat from the carcasses.

Selected groups of muscle were dissected from three main regions of the right half of carcass which represent pelvic limb, dorsal region and thoracic limbs, using special dissection method according to (Butterfield et al., 1983). Then each muscle was weighed separately by electronic balance. After that the surfaces of muscles are cleaned from all fats and connective tissues. Fat deposition was conducted from left half carcass which included (subcutaneous fat, intermuscular fat, kidney and pelvic fat, tail fat) also offal fat which included (omental fat, mesenteric fat, and heart fat). After that all the mentioned fat was weighed by balance. But also percentages of these mentioned fats were measured on the base of total fat from left half carcass.

Statistical Analysis

Data were analyzed using XL Stat, version 7.5, 2005. The significant differences between means of traits included in this study were determined using Duncan's multiple range tests under the probability (P<0.05) (Duncan, 1955).

RESULTS AND DISCUSSIONS

Major muscles in pelvic limbs

The effect of flaxseed powder supplementation on the muscles growth in pelvic limbs in lambs is presented in Figure 1. The results in figure 1 revealed that significant differences (P<0.05) in muscles weight pelvic limbs among treatments as affected by the supplementation of FP. It is noticed that adding FP led to increase (P<0.05) muscles weight of *Semitendinosus* (ST), *Semimembranosus* (SM), *Biceps femoris* (BF), *Rectus femoris* (RF), *Adductor* (AD), *Gracilis* (G), *Vastus medialis* (VM) and *Vastus intermedialis* (VI) in T1. While the mean weight in C group were recorded, decrease in muscles weight. It can be observed that T2 achieved the highest weight in both RF and VL muscles (172.667 and 142.667 g) respectively. Also, the weight of muscles in other treatments (T2 and T3) were recorded significant differences (P<0.05).

Major muscles in dorsal region

The effect of flaxseed powder supplementation on the muscles growth in dorsal region in lambs is shown in Figure 2. The results revealed that there was significant differences (P<0.05) in main muscles weight in dorsal region. T2 recorded the highest weight (545.000, 72.500 and 35.000 g) in *Longissimus dorsi* (LD), *Psoas major* (Pj) and *Psoas minor* (PM), respectively. The C group recorded the lowest weight (462.500, 61.653 and 25.167 g) in LD, Pj and Pm respectively. As well as, the weight of muscles in other treatments (T2 and T3) recorded significant differences (P<0.05).

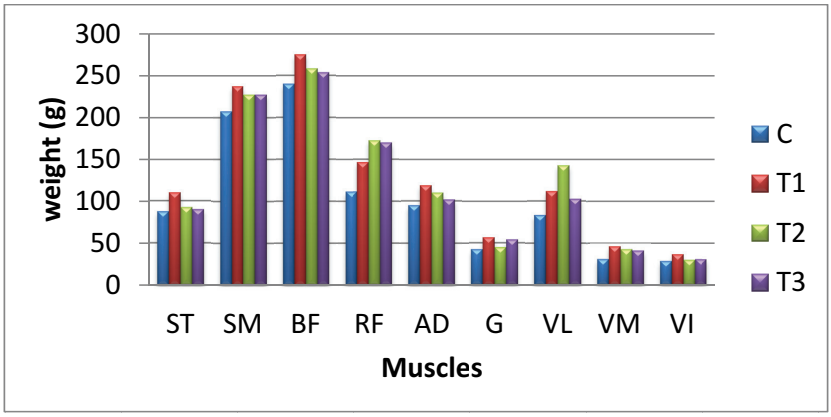

Figure 1. Effect of flaxseed powder supplementation on major muscles in pelvic limbs

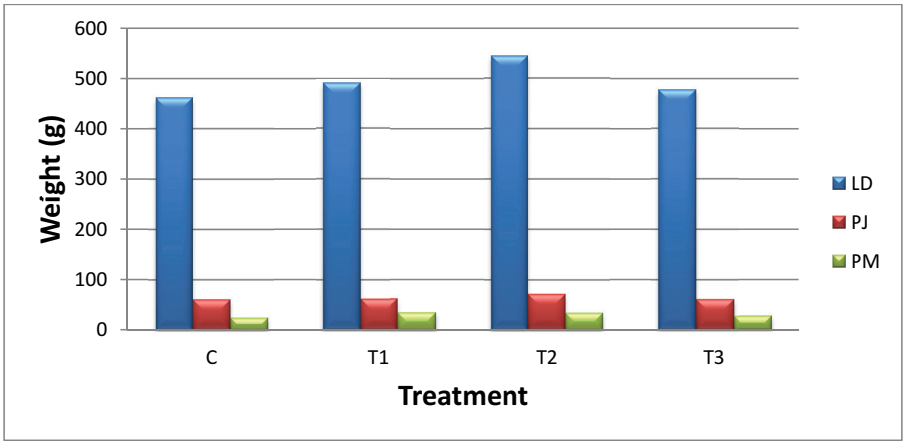

Figure 2. Effect of flaxseed powder supplementation on major muscles in dorsal region

Major muscles in thoracic limbs

Figure 3 showed the effect of FP on the muscles growth in thoracic limbs. The data revealed significant differences ($P<0.05$) among treatments in main muscles weight in thoracic limbs, except for SC muscle which is not different. The highest weight of *Infraspinatus* (IS), *Supraspinatus* (SP) and *Brachialis* (B) muscles respectively, were found in T2, while the highest weight *Subscapularis* (SC) and *Triceps brachii* (TB) muscles respectively, were found in T1, finally the highest weight of *Biceps brachii* (BP) muscle was found in T3. On the other hand, the lowest weights were recorded in C group.

It can be concluded that there is a difference in the rate of growth of muscles in lambs affected by supplementation of the FP that was reflected in the differences of muscles weight. This response may contribute to improve mass of lean and point to the importance of the FP supplementation in increasing meat efficiency through increasing muscle production and decreasing fat deposition in the carcasses. These results confirm data referred to previously about increasing the percentages of meat in the main cuts and whole half carcass and the full effect of the positive effect of the FP to add to the diets of lambs (Zahir, 2012).

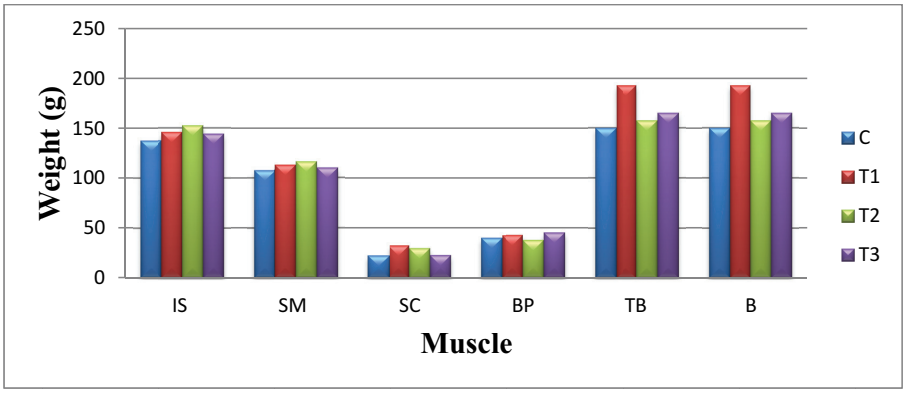

Figure 3. Effect of flaxseed powder supplementation on major muscles in thoracic limbs

Table 2. Effect of flaxseed powder supplementation on weight precipitating* and percentages of (subcutaneous fat, intermuscular fat, kidney and pelvic fat, tail fat and total carcass fat in half carcass (Mean ± standard error)

Treatment	Subcutaneous fat		Intermuscular fat		Kidney &Pelvic fat		Tail fat		Carcass fat	
	(g)	%	(g)	%	(g)	%	(g)	%	(g)	%
C	1565.833^{a} ± 16.729	33.389^{a} ± 0.702	305.500^{ab} ± 0.289	6.515^{a} ± 0.140	37.500^{b} ± 15.877	0.786^{b} ± 0.321	2621.250^{a} ± 82.994	55.828^{ab} ± 0.663	4530.083^{a} ± 101.405	96.518^{a} ± 0.155
T1	1100.000^{b} ± 46.188	25.519^{b} ± 0.669	305.000^{ab} ± 37.528	7.058^{a} ± 0.760	86.250^{ab} ± 7.939	2.009^{ab} ± 0.216	2655.000^{a} ± 24.537	61.695^{a} ± 1.547	4146.250^{a} ± 51.240	96.281^{a} ± 0.335
T2	1459.167^{a} ± 23.467	30.688^{a} ± 0.694	365.833^{a} ± 5.833	7.713^{a} ± 0.418	137.500^{a} ± 30.311	2.945^{a} ± 0.752	2541.250^{a} ± 235.992	53.128^{b} ± 2.920	4503.750^{a} ± 223.058	94.473^{a} ± 1.057
T3	1465.000^{a} ± 50.083	31.867^{a} ± 1.331	292.500^{b} ± 12.990	6.387^{a} ± 0.502	85.000^{ab} ± 8.660	1.863a^{b} ± 0.251	2520.000^{a} ± 213.620	54.540^{ab} ± 2.802	4362.500^{a} ± 201.168	94.658^{a} ± 0.891

Means having different letters at the same column are significantly different (P<0.05).
*It was measured on the total fat of half animal body

Fat partitioning and distribution

Fat partitioning in half carcass

The effect of FP supplementation on weight and percentage of fat (calculated by the total fat content in the half of animal body), Fat deposition in half carcass including (subcutaneous fat, intermuscular fat, kidney and pelvic fat and tail fat), are summarized in Table 2. The supplementation of FP decreased ($P<0.05$) fat deposition, the lowest weight and percentage of subcutaneous fat were found in T1 as compared with other treatments, while oppositely were recorded in C group the highest weight and percentage of subcutaneous fat. However, intermuscular fat in T2 has given the highest weight and percentages as compared with other treatments, T3 has given opposite results in intermuscular fat; it recorded lowest weight and percentages. The highest weight and percentages in kidney and pelvic fat were found in T2, while the lowest weight and percentages fat recorded in C group.

Offal fat deposition

The effect of flaxseed powder supplementation on weight deposition and percentages of (Omental fat, Mesenteric fat, Heart fat, Offal fat in half carcass) are presented in Table 3. The results showed that significant differences ($P<0.05$) were generally found among all treatments in relation to omental fat and offal fat while, no significant differences ($P>0.05$) were found in mesenteric and heart fat as response to FP supplementation (Table 3). The highest weight and percentages of omental fat were found in T3 and the lowest weights with the lowest percentages were found in C group. However, the highest weight and percentages of mesenteric fat were found in T2, while the lowest weight and percentages were found in T1. It is also observed from the results that the highest weight and percentages of heart fat were found in T1 and the lowest weight with the lowest percent was found in T2. The results also denoted that highest weight of fat deposition on offal slaughtering and percentages were found in T2. Then the weight and percentages gradually decreased in other treatments.

Generally, in the current study there was observed from the results decreasing in percentages and amounts of deposition fats in the carcasses from lambs were fed on FP and increasing in percentages and amounts on offal fats. This is expressed as a positive tendency in pattern of fat distribution and muscle production which reflected to efficiency of meat production and this is what the scientists want to achieve now in decreasing fat percent in the carcasses and increasing in the offal fat because of being so easy to separate and get rid of it. As shown from the results of the positive role of flaxseed powder added to diets to improve utilization of nutrients present in the diets and to increase the formation of protein and deposited at the expense of lower deposition of fat in the carcasses.

Total fat deposition

The effect of flaxseed powder supplementation on weight deposition and percentages of total fat in half empty body weight are presented in Table 4. The results reveal that there were no significant differences in weight and percentages of total fat in half empty body weight. But, results indicate that there is a mathematical decrease in weight and percentages of total fat in half empty body weight due to FP supplementation. It is noticed that the lowest weight (4306.875) and lowest percentage (19.775%) of total fat were found in T1, while the highest weight (4763.125) and highest percentages (21.098%) were in T2. It can be concluded that T1 was the best one in meat production efficiency and also it is the best one in relation to produced carcass quality as compared to other treatments.

CONCLUSIONS

These results indicate that the low level (3%) of flaxseed powder is the best to improve and increase the efficiency of meat production and reduce the deposition of fat in the animal's body.

Table 3. Effect of flaxseed powder supplementation on weight deposition* and percentages of (Omental fat, Mesenteric fat, Heart fat, Offal fat in half carcass (Mean ± standard error)

Treatment	Omental fat		Mesenteric fat		Heart fat		Offal fat	
	(g)	%	(g)	%	(g)	%	(g)	%
C	62.500^{b} ± 15.877	1.319^{b} ± 0.311	81.875^{a} ± 17.681	1.761^{a} ± 0.411	18.750^{a} ± 2.165	0.402^{a} ± 0.054	163.125^{b} ± 3.969	3.482^{a} ± 0.155
T1	70.625^{b} ± 10.464	1.633^{ab} ± 0.217	70.625^{a} ± 9.743	1.633^{a} ± 0.200	19.375^{a} ± 3.248	0.452^{a} ± 0.083	160.625^{b} ± 16.960	3.719^{a} ± 0.335
T2	131.250^{a} ± 10.825	2.781^{ab} ± 0.335	113.125^{a} ± 29.950	2.431^{a} ± 0.725	15.000^{a} ± 0.722	0.315^{a} ± 0.003	259.375^{a} ± 40.054	5.527^{a} ± 1.057
T3	135.000^{a} ± 28.868	2.982^{a} ± 0.724	90.625^{a} ± 4.691	1.980^{a} ± 0.170	17.500^{a} ± 0.722	0.380^{a} ± 0.005	243.125^{ab} ± 32.837	5.342^{a} ± 0.891

*Was measured on the total fat of half animal body
Means having different letters at the same column are significantly different ($P<0.05$).

Table 4. Effect of flaxseed powder supplementation on weight deposition and percentages of total fat in half empty body weight (Mean ± standard error)

Treatments	Total fat	
	(gm.)	%
C	4693.208±97.492[a]	21.384±0.508[a]
T1	4306.875±68.200[a]	19.775±0.530[a]
T2	4763.125±183.016[a]	21.098±0.573[a]
T3	4605.625±169.559[a]	21.725±1.008[a]

Means having different letters at the same column are significantly different (P<0.05).

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RESEARCH CONCERNING THE INFLUENCE OF USING DIFFERENT DOSES OF NON-PROTEIN NITROGEN IN COWS FEED OVER THE MILK QUANTITY AND QUALITY

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Abstract

The experiments followed the increase of nitrogen from a source inorganic (urea) used in feeding cows and its effect on milk production and milk quality. The OPTIGEN product is used in proportions between 1 and 3% of fodder for cows in lactation, which means quantities of urea between 60 and 180 g/head/day , the inorganic total crude protein weight source intake of cows varied between 10 and 20%. The quantitative determination of milk production and its qualitative analysis have revealed very little differences both between batches and between determinations from the same batch, which after processing the statistical differences proved insignificant. These results demonstrate that inorganic nitrogen in the OPTIGEN product can be harnessed properly by the lactating cows, also causing nutrition specialists to find solutions allowing both increasing the weight ratio of the inorganic source, and improving the milk production and quality parameters.

Keywords*: crude protein, lactating cows, milk production, non-proteic nitrogen, protein and fat from milk.*

INTRODUCTION

Achieving and maintaining a nutritional standard for a human being assumes a constant supply in the market with products that provide a nutritionally balanced calorie intake and at an affordable price allowing food preferences and satisfaction for all categories of population (Raducuta, 2008).

One of the foods that have taken part in very old times to balance the power equilibrium of the human being, thus contributing to maintaining health and improving the quality of life is the milk and, in particular, the one belonging to cows that provide about 90% of the market of this product (Akay et al., 2004).

If in terms of nutrient content is almost universally recognized that milk is a complete food, the question of solving the shortcomings related to the inconsistent production and price fluctuations is put (Inostroza et al., 2010; Bach et al., 2005), both affected mainly by the expenses in animal feed.

Growing specialized breeds for milk production and feeding them with feed ingredients to turn them up to maximum physiological peculiarities (Bourg et al., 2012) is the main route by which these shortcomings can be corrected.

The objective of this experiment is to track how different levels of substitution of a classic protein ingredient with non-protein nitrogen sources influence the milk production and the quality parameters of cow milk.

MATERIALS AND METHODS

The experiments were conducted in the Biobase ICDCB-Baloteşti over three months (December-March), the biological material was represented by 20 cows from the Romanian Black Spotted breed (BNR), divided into 4 groups, meaning 5 heads/batch .

Feeding of the animals was done with the entered feed rations specific to the winter season and different amounts of non-protein nitrogen have been used (Table 1).

Measuring the amount of milk which has been milked was done volumetric individually at each milking, and the determination of the physico-chemical parameters was performed using the Ekomilk Ultra apparatus (Banu et al., 2010).

The statistical data processing was done using Microsoft Excel spreadsheet application. The

database has been developed with the sequences of corresponding variations, each sequence being encoded in accordance with the specific studied information. To test the statistical significance of the differences between the average environment characters studied the Single Factor ANOVA algorithm was used.

Table 1. Experimental scheme

Batch	n	Treatment	Exp. period / determinations	Objectives
Control batch (C)	5	Vegetable protein soy meal 18%	Dec.-march (winter) The begining of the experience = R1 45 days of experience = R2 The end of experience (90 days) = R3	The evolution of: -milk production; - milk content in dry matter and its components; - values of milk pH
I Experimental Batch (E I)	5	Soy meal 12% +OPTIGEN 1%		
II Experimental Batch (E II)	5	Soy meal 6% +OPTIGEN 2%		
III Experimental Batch (E III)	5	OPTIGEN 8%		

RESULTS AND DISCUSSIONS

The situation of the production and composition of the produced milk during the progress of the experience is presented in Table 2. It can be noticed that, in terms of milk production, there were differences in both batches (maximum 7.73%) as well as between the determinations in the batches (7.36%), but these differences were not statistically covered (Table 3).

Table 3. The calculated values for Fisher test (F)

Calculated values F	Milk production	Fat	Protein	Glucides	Mineral subst.	pH
R1	0.590	1.649	1.334	0.742	1.909	2.538
R2	0.559	0.267	0.300	0.319	0.342	1.361
R3	1.352	0.816	0.520	0.567	0.768	0.927
C batch	0.324	0.392	0.537	1.224	0.504	0.863
E I batch	1.638	2.061	0.684	1.392	2.649	0.234
E II batch	0.286	0.964	0.576	0.371	2.519	0.357
E III batch	2.011	0.827	0.811	0.279	0.301	1.295

F tabular =3.24 for sampling (R1, R2, R3) between batches
F tabular =3.88 for differences in the experimental batches
F tabular > F calculated – insignificant differences

The milk fat content does not show significant differences in any of the determinations made both between batches and in those performed in the same batch, but it has been found that there is a single batch, the experimental batch II, where there is a constant increase over the experience of this parameter, the values being of 4.022 g/100 ml milk at first determination to 4.370 g/100 ml at the second determination and 4.690 g/100 ml at the third determination.

The determination of the nitrogenous substances highlighted a small fluctuation intensity values over the experience, the only batch where there was a continuous growth, but of low intensity (from 2.960 g/100 ml to 3.028 g/100 ml of milk), being the batch witness, all the other batches having an increasing trend between the first and the second measurement (maximum 7.82%) followed by a decline of about the same intensity (maximum 4.82%) between the second and third measurement.

The increasing trend between the first two measurements are recorded even for glucides in all the 4 batches of experience, the most obvious difference being recorded at the experimental batch E I (0.332 g%), followed by the E II batch (0.32 g%), the control batch with 0.18 g% and the batch E III with 0.07 g%. And glucides values recorded an increasing trend between the first two measurements in all the experimental batches (0.052 g% in the batch I E, 0.050 g% in the batch E II and 0.006 g% in the experimental batch E III) and descending to determine the third, except the control batch (0.05 g%), the decrease being of 0.22 g% for the experimental batch E II, 0.14 g% for the batch E I and 0.04 g% for the experimental batch E III. For all the experimental batches are recorded positive differences, but statistically uninsured between the first and last measurement.

The brute ash (minerals) has the same slight increase tendency between the first and the second measurement in the experimental batches, remaining the same for the control batch, after which the control batch encounters a slight increase (0.69 g% compared to 0.68 g %) and the experimental batches suffer a decrease between 0.002 g% for the experimental batch E III and 0.032 g% for the experimental batch E II. And in the case of the brute ash it can be observed that for all the batches the values are consistently higher between the first and last measurement.

The milk pH recorded very close values, between 6.322 and 6.590; the differences were recorded both between the batches and between measurements in the same batch by not being assured statistically.

Table 2. The evolution of physico-chemical parametrs of milk

Batch	Sampling	Milk production (l)		TCDM (%)		Fat (%)		Protein (%)		Glucides (%)		Mineral subst. (%)		pH	
		$x \pm s_x$	CV%	$x \pm s_x$	CV%	$x \pm s_x$	CV%	$x \pm s_x$	CV%	$x \pm s_x$	CV%	$x \pm s_x$	CV%	$x \pm s_x$	CV%
C	R1	20.83± 1.151	16.81	12.332± 0.67	5.46	4.372± 0.40	9.16	2.958± 0.11	3.85	4.314± 0.31	7.25	0.688± 0.05	7.36	6.544± 0.03	0.57
	R2	20.10± 1.66	18.46	12.55± 0.67	5.34	4.380± 0.48	11.14	3.000± 0.08	2.70	4.490± 0.12	2.81	0.680± 0.01	2.75	6.544± 0.05	0.90
	R3	20.06± 1.45	16.20	12.468± 1.02	1.16	4.212± 0.63	15.04	3.028± 0.16	5.57	4.538± 0.25	5.57	0.690± 0.03	5.70	6.322± 0.45	7.19
E I	R1	20.22± 0.75	8.31	11.866± 1.13	9.76	4.062± 0.71	18.39	2.864± 0.18	6.29	4.290± 0.26	6.26	0.650± 0.03	5.65	6.544± 0.03	0.49
	R2	21.32± 0.68	7.15	12.75± 1.87	14.27	4.400± 1.20	25.71	3.088± 0.25	8.36	4.622± 0.38	8.29	0.702± 0.05	8.26	6.590± 0.02	0.33
	R3	21.10± 0.38	4.03	12.508± 1.09	8.87	4.050± 1.03	26.91	2.994± 0.04	1.36	4.486± 0.06	1.37	0.684± 0.01	1.30	6.540± 0.04	0.66
E II	R1	19.74± 0.29	3.24	11.848± 0.71	5.99	4.022± 0.61	15.21	2.874± 0.05	2.00	4.302± 0.08	2.06	0.650± 0.01	2.17	6.514± 0.03	0.61
	R2	19.82± 0.51	5.75	12.782± 1.11	8.73	4.370± 0.67	15.55	3.086± 0.17	5.71	4.626± 0.26	5.76	0.700± 0.04	5.80	6.576± 0.02	0.41
	R3	20.05± 0.31	3.49	12.71± 0.56	4.43	4.690± 0,54	11.64	2.944± 0.11	3.75	4.408± 0.16	3.76	0.668± 0.02	3.57	6.520± 0.01	0.21
E III	R1	20.62± 0.88	9.60	12.684± 0.45	3.62	4.546± 0.39	8.60	2.986± 0.07	2.67	4.470± 0.12	2.68	0.682± 0.01	2.17	6.490± 0.03	0.56
	R2	20.12± 0.28	3.13	12.918± 0.75	5.86	4.658± 0.40	8.64	3.032± 0.13	4.49	4.540± 0.20	4.41	0.688± 0.03	4.40	6.578± 0.03	0.46
	R3	21.60± 0.20	2.08	12.31± 1.25	10.17	4.116± 1.11	27.01	3.004± 0.07	2.47	4.504± 0.10	2.36	0.686± 0.01	2.21	6.470± 0.01	0.24

CONCLUSIONS

The lactating cows use better the nitrogen from a non-protein source (urea) included in rations between 60 and 180 g/head/day.

The amount of milk obtained from cows during the experience was not significantly influenced by the use of doses between 10 and 30 g/100 kg body weight.

The physic-chemical features of the milk collected during the experience recorded very similar values both between batches and between the determinations from the same batch, the differences recorded were not assured statistically.

It is necessary to find solutions allowing both the increase in the proportion of non-protein nitrogen in the ration, as well as to improve the production and the milk quality.

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DRY MATTER PRODUCTION AND NUTRITIVE VALUE OF CEREAL SPECIES HARVESTED AT BOOT OR DOUGH STAGE OF MATURITY

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Abstract

*The comparative advantages of winter cereals have not been thoroughly evaluated for their forage production potentials in the semi-arid conditions of the Central Anatolian Region of Turkey. The effect of maturity on dry matter (DM) production (t/ha) and nutritive value of the whole-crop cereal forage of barley (*Hordeum vulgare L.*), wheat (Triticum aestivum* L.*), rye (*Secale cereale L.*), triticale (X* Triticosecale *wittmack) and oat (*Avena sativa L.*) were investigated at booting and dough stages of the forage cereals. Barley had higher (P<0.001) DM production at booting stage, whereas DM production of triticale, rye and oat was the highest (P<0.001) at the dough stage. Overall, the increase in DM production and digestible DM production with advancing maturity was 34, 42, 60, 49, 51 % and 23, 29, 51, 43, 38 % for barley, wheat, triticale, oat and rye, respectively at dough stage. The metabolizable energy values (ME MJ/kg DM) of cereal forages were higher (P<0.05) at booting stage. Neutral detergent fibre did not differ (P>0.05) with maturity or forage species, while non-fiber carbohydrates increased (P<0.001) with advancing maturity.*
In conclusion, all cereal crops should be harvested for forage production at their boot stage of maturity to obtain higher nutritive value forage. Barley provided more promising cereal forage at booting stage with its higher DM and digestible DM production. Triticale, rye and oat produced more DM at dough stage with triticale having higher digestible nutrients indicating its superior value compared to other cereal forages at this stage. However, the choice of cereal for forage production should include consideration of class and breed of livestock to be fed, agronomic characteristics and soil type requirements under the semi-arid conditions of Central Anatolia.

Keywords: *Cereal forage, digestibility, dry matter production, nutritive value.*

INTRODUCTION

The cereal crops are mainly grown for grain production in the Central Anatolia region where crop–livestock farming is a common practice. Despite the fact that high quality forage is in shortage in the region, using cereal crops for forage production is not a widespread practice. However, in a double cropping system, small grain forage cereals are sown as winter crops in rotation with maize planted in summer. In this system, the cost of the maize silage is often lower because early seeding allows maize to benefit from the late spring rains more efficiently and provides increased dry matter (DM) production. However, the low dry matter content of cereal crops in early stage of maturity requires longer wilting time which may pose a challenge for hay or silage productions, particularly in rainy weather conditions. On the other hand, as maturity advanced from boot to dough stage, dry matter content of cereal crop increases proportionally an average of 0.42 (Khorasani et al., 1997). At this DM level (over 350 g/kg), cereal crops do not require pre-wilting before ensiling to get sufficient silage fermentation (McDonald et al., 1991) and the time necessary to get well dried hay production is shorter. Moreover, when cereal crops reach the dough stage of maturity it is easier to produce well fermented silages or well dried hay with less soil contaminations due to more favourable and drier weather conditions in late spring. Furthermore, because high drying rate in late spring compared to early spring, less mechanization is needed to produce silage or hay production from cereal crop harvested at dough stage.

It is well documented that the DM production of cereal crops is low but the nutritive value of cereal crop is high when they are harvested at early stage of maturity (Helsel and Thomas, 1987; Crovetto et al., 1998; Beck et al., 2009). Because DM production are related to

increased DM concentration and biomass accumulation with maturity, delaying the harvest time from boot to dough stages increases DM production. However, this may also reduce the nutritive value of the forages (Khorasani et al., 1997; Nadeau, 2007). There were also differences between cereal crops for their feeding value (Helsel and Thomas, 1987; Khorasani et al., 1993; Emile et al., 2007).

The studies investigating the effect of maturity on the DM production and nutritive value of different cereal species (barley, wheat, triticale, rye and oat) are not consistent and present significant variations. Moreover, few studies have assessed the responses to maturity of a wide range of cereal species which could be grown for forage production. The main aim of this study was to assess the DM production potential and nutritive value of barley, wheat, rye, triticale and oat to get the information necessary on which cereal species should be most advantageous when they are harvested at booting or dough stage of maturity.

MATERIALS AND METHODS

Establishment and experimental design

This study was carried out at Bahri Dagdas International Agricultural Research Institute (37° 51' N, 32° 33' E, 1008 m a.s.l.), Konya, Turkey from October 2010 to July 2011. The site was on a clay–loam soil with slightly alkaline characteristics. The cereal grains of barley (cv. "beysehir"), wheat (cv. "goksu"), rye (*cv* "aslim"), triticale ("tatlicak") and oat ("faikbey") were sown in 16 m x 78 m plots using a commercial grain drill with 0.2 m row spacing on 9 November. Treatments were arranged with three replicates. Based on soil test results, a total of 100 kg ha^{-1} fertilizer (18% N and 46% P_2O_5) was applied at sowing. Cereal grains were seeded at rates typical for the region, which were 210 kg/ha for wheat, 200 kg/ha for triticale, 172 kg/ha for rye 166 kg/ha for barley and 146 kg/ha for oat. 2.4–D was applied by small sprayers for weed control in each plot on 12 April. The dry matter production (kg/ha) of cereal crop was measured by three quadrat cutting selected to be representative of each plot in cereal crop's boot and dough stage of maturity. All herbage from the quadrat cuts was weighed and DM content was determined.

Meteorological data

Average mean temperature and monthly rainfall at the trial site during the growing season are presented in Table 1. Total rainfall was 396 mm between October 2010 and July 2011 which was 102 mm higher than the long–term average. Of note was that the higher than usual rainfall in the spring and in the early summer was evenly distributed. The mean temperature was higher during November through February, while it was lower for the rest of the growing season.

Table 1. Monthly rainfall and mean daily air temperatures at Bahri Dagdas International Agricultural Research Institute, Konya, Turkey during the 2010–2011 growing season

	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	June
Temperature (^oC)	12.0	8.8	4.6	1.1	1.7	4.6	8.7	13.2	18.4
LTM	12.6	5.9	1.3	-0.3	1.2	5.8	11.0	15.8	20.3
Rainfall (mm)	71.8	2.4	71.2	37.8	40.4	23.0	44.6	62.6	42.6
LTM	33.3	35.3	41.8	32.9	24.5	25.6	37.4	40.5	22.9

Analytical procedures

The forage samples were assayed for DM by oven drying at 60 ^oC for 48 h. Crude protein (CP) was determined by Kjeldahl method (AOAC, 2003). The ash and the crude fat (CF) was also determined by AOAC (2003). Neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL) was assayed according to Van Soest et al., 1991. The NDF was expressed with the inclusion of a heat stable amylase and sodium sulfite, but both NDF and ADF expressed inclusive of residual ash. Neutral (NDICP) and acid (ADICP) detergent insoluble CP necessary for calculating metabolizable energy (ME; NRC, 2001) was determined on the samples obtained from NDF and ADF residues and discussed in previous paper (Coskun et al., 2013) together with protein quality. Non-fiber carbohydrates were: 100-(NDF+ash+CP+CF). *In vitro* true DM digestibility (DMD) was determined with the DAISY[11] incubator. Ruminal fluid used for

DMD was collected from a non-pregnant, dry cow fed an alfalfa pellet and concentrate (60:40).

Statistical analysis

The experimental data were analysed via analysis of variance for a split-split plot design. Cereal crop was treated as main plot and stage of maturity was sub-plot in SPSS 10. Where ANOVA was significant, comparisons between treatments were made using the least significant difference procedure.

RESULT AND DISCUSSIONS

The mean DM content and DM production of cereal crops are summarized in Table 2. Among the cereal crops, rye had the lowest (P<0.001) DM content at booting stage which is in line with the work reported by Helsel and Thomas (1987). This could be a challenge when ensiling rye as it would require more drying time at booting stage especially in time when drying conditions are not favourable in early spring. The higher (P<0.001) DM content at dough stage of wheat silage was also reported by the (Beck et al., 2009). Cereal species used in this experiment may be partly due to the fact that these crops were originally developed for grain production. However, higher DM of cereal crops also poses a challenge when making high DM baled silage where there is no precision chopping (Keles and Demirci, 2011).

Table 2. Agronomic data of the cereal forages

Stage of maturity	Cereal crop	Dry matter, g/kg DM	DM production, t/ha	Digestible DM production, t/ha
Boot	barley	211	9.9	7.2
Boot	wheat	220	8.9	6.7
Boot	rye	140	7.2	5.3
Boot	triticale	184	9.1	6.6
Boot	oat	183	8.6	6.1
Dough	barley	370	14.9	9.3
Dough	wheat	509	15.5	9.5
Dough	rye	438	18.0	10.9
Dough	triticale	439	17.7	11.5
Dough	oat	438	17.6	9.9
	s.e.m[1]	4.6	0.20	0.15
P				
Stage of maturity		***	***	***
Cereal crop		***	***	***
Maturity x crop		***	***	***

[1]: for the two-way interactions. *= P < 0.05; **= P < 0.01; ***= P < 0.001.

The DM production ranged between 7.2 (rye) and 9.9 t/ha (barley) at boot and varied from 14.9 (barley) to 18.0 t/ha (rye) at dough stage. The rye had lower (P<0.001) DM content than other cereal forages which also resulted in rye having the lowest (P<0.001) DM production. The DM production of rye, triticale and oat were higher (P<0.001) than DMP of wheat and barley at the dough stage. Digestible DM production increased (P<0.001) with maturity with the value being the highest (P<0.001) in triticale at dough stage. The highest digestible DM production of barley at boot stage was contrasted with the lowest DM production (P<0.001) at dough stage compared to other cereal crop. Overall DM productions of barley, wheat, oat and rye at boot and dough stage of maturity were higher than the values reported by Helsel and Thomas (1987), who also reported that DM production could vary among different barley cultivars. But, DM production of wheat at dough stage was similar to the value reported by Filya (2003) who measured 15.2 t/ha DM production for “Gonen” wheat.

The rainfall in Konya during spring 2011 was higher than the long term means and evenly distributed through the spring period providing favourable growing conditions for plant growth. Due to unusually wet spring, it is hard to identify which cereal crop was superior to another for their dry matter production potentials. In dryer conditions, the advantage of cereal species may change. Barley having had more (P<0.001) DM production as well as more digestible DM production was evaluated the most promising cereal forage at boot stage, while triticale was superior over the other species at dough stage. However, the same samples were also used for protein quality (Coskun et al., 2013) and, in terms of digestible CP production, oat was evaluated the more promising species at dough stage. The DM production of cereal crops increased (P<0.001) with advancing maturity but at different rates (P<0.001). The increase in DM production with advancing maturity were higher than the mean values reported by Helsel and Thomas (1987), but lower than the value reported by Filya (2003) with two wheat cultivar harvested at flowering and dough stage or wheat reported by Crovetto et al. (1998). Different rate in DM production with maturity among cereal crops could a key factor when choosing which crop could be harvested different stage of maturity for DM production together with the nutrient composition.

Table 3. Chemical composition of cereal crop

Factors		Chemical composition[1]								
Maturity	Crop	CP	Ash	CF	NDF	ADF	ADL	NFC	DMD	ME[2]
Boot	barley	169	97	26	521	297	45	187	729	8.5
Boot	wheat	142	87	21	503	292	51	247	755	8.5
Boot	rye	162	80	31	541	308	44	186	735	9.0
Boot	triticale	161	94	30	516	308	50	199	726	8.7
Boot	oat	123	82	32	524	317	51	240	706	8.7
Dough	barley	100	63	20	509	288	67	309	622	8.2
Dough	wheat	91	59	23	497	314	67	330	612	8.4
Dough	rye	74	49	28	516	316	64	334	605	8.4
Dough	triticale	85	54	25	504	278	64	332	650	8.4
Dough	oat	93	67	42	511	316	68	287	562	8.4
s.e.m[3]		2.7	2.2	1.9	15.9	6.4	2.4	16.7	10.2	0.11
p										
Stage of maturity		***	***	NS	NS	NS	***	***	***	*
Cereal crop		***	***	***	NS	**	NS	NS	**	*
Maturity x crop		***	**	**	NS	NS	NS	NS	*	***

[1]: CP: crude protein, g kg^{-1} DM, CF: crude fat, g kg^{-1} DM; NDF: Neutral detergent fibre, g kg^{-1} DM; ADF: Acid detergent fibre, g kg^{-1} DM; ADL: Acid detergent lignin, g kg^{-1} DM; NFC: non fibre carbohydrates, g kg^{-1} DM; DMD: *in vitro* dry matter digestibility, g kg^{-1} DM.
[2]: ME: Metabolizable energy (MJ/kg DM). Calculated according to tabular value of NRC (2001)
[3]: for the two-way interactions. *= P < 0.05; **= P < 0.01; ***= P < 0.001.

The chemical composition of cereal crops are presented in Table 3.The main effect of the stage of maturity was significant for CP, ash, ADL, NFC, DMD and ME of cereal crops with the values being higher (P<0.05) with maturity for ADL and NFC. Nutritive values of cereal crops were higher (P<0.05) when they were harvested at their booting stages as evidenced by higher CP, ash, ME and DMD values. The highest (P<0.001) decrease in nutritive value occurred in CP content for all cereal crops and this was the sharpest (P<0.001) in rye with an overall 54 % decrease. This is in line with the results, reported by Helsel and Thomas (1987) for the barley, wheat, rye and oat silages and by Beck et al. (2009) for the wheat silage. The reduction in DMD was not as sharp as CP as similarly reported by Crovetto et al (2009) and

NDF content of forages were similar at both maturity. This was due to accumulation of NFC with maturity (Nadeau, 2007).

CONCLUSIONS

The main aim of the study was to assess the DM potential and nutritive value of cereal species to get the information necessary which cereal species should be most adventanegeous when they harvested their suggested stage of maturity. Barley at boot stage and triticale at dough stage are more promising cereal species for forage production in terms higher digestible values under the semi-arid conditions of Central Anatolia rather than their nutritive value or DM production. However, because requirement of climate and soil factors are different, the choice of cereal species for forage production should include consideration of animal type to be fed, requirement for protein quality and agronomic characteristics under the semi-arid conditions of Central Anatolia.

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USAGE OF CONTINUOUS PRESSURE READER IN *IN VITRO* GAS PRODUCTION TECHNIQUE FOR EVALUATION OF FEEDSTUFFS FOR RUMINANTS

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Abstract

This experiment was conducted to determine possibility of usage of automatic system that continuously record pressure and sample gas at certain intervals in in vitro gas production technique. Rumen fluid was collected from 2 ruminally cannulated Holstein heifers weighing an average of 400 kg. Medium was prepared by mixing macromineral (200 ml), micromineral (0.1 ml), buffer (200 ml), reduction (40 ml) and resazurin (1 ml) solutions as well as distilled water (400 ml). The mixtures contained alfalfa hay, maize silage, wheat, maize, cottonseed meal, and soybean meal. Mixtures differing in the roughage:concentrate ratio (20:80, 40:60, 60:40, and 80:20) were formulated to contain rapidly fermentable fraction (B1+B2), insoluble but slowly fermentable fraction (B3,) and 50% of these fractions at three different fermentation characteristics. Gas productions at 6, 12, 24, and 48 h relative to incubation determined using system recording continuously. In automatic system, "dual-pool logistic equation" representing factions either soluble in NDF solution and rapidly fermentable fraction (NDF-F) and fractions or insoluble in NDF solution, but slowly fermentable (NDF-S) were used. The fermentation was completed within 12 h in fully automatic system. While cumulative gas production from NDF-F was positively correlated with gas production within 3 h ($r=0.77$, $P<0.001$), cumulative gas production from NDF-S was positively correlated with gas production within 3-20 h ($r=0.88$, $P<0.001$). Differences in time to reach maximal fermentation rate from NDF-F and NDF-S increased with increasing NDF ($r=0.76$, $P<0.001$) and ADF ($r=0.85$, $P<0.001$) levels. These data suggest that fermentation kinetics parameters, such as lag time and time to reach maximal fermentation rate from fractions soluble and insoluble in NDF solution, should be considered in formulation and evaluation of rations.

Keywords*: automatic gas system, in vitro gas production technique, feedstuff evaluation.*

INTRODUCTION

In vitro gas production technique is considered a unique method for determining nutritive value of feedstuffs and compound feeds (Getachew et al., 2005). This technique is employed ration evaluation when excess carbohydrates are fed to minimize metabolic disturbances. Projection of fermentation characteristics and kinetics can help elucidate intake depression especially in early lactation (Johnston and Tricarico, 2007; Pell and Schofield, 1993). Using systems that record continuously allow determination of kinetics of gas, which enables to reduce production of CH_4 and (Ramin and Huntanen, 2012). This system provides information that is invaluable to project the fate of nutrients and establish alternative feeding management. Automated *in vitro* gas system time-dependent changes are modeled to determiner fast and slow degradable fractions (Johnston and Tricarico, 2007; Pell and Schofield, 1993; Schofield et al., 1994) or degradable and undegradable fractions as well as gas production (Dijkstra et al., 2005; France et al., 2005). Degradation of fractions (B_1- rapidly degradable fraction, mainly starch; B_2- slowly degradable soluble fraction; and B_3- insoluble but slowly degradable fraction, mainly cellulose and hemicellulose) is described in detail. Imbalance between rapidly and slowly degradable fractions leads to low production and dry matter intake, acidosis, laminitis, milk fat depression, displaced abomasum, and liver abscess (Johnston and Tricarico, 2007).

MATERIALS AND METHODS

Rumen fluid was obtained from two ruminally cannulated Holstein heifers. They were fed twice daily. Ration consisted of the roughage:concentrate ratio of 60:40 to meet maintenance plus 0.5 kg weight gain and contained 5 kg alfalfa (13.43% CP and 43.50% NDF) and 3.5 kg compound feed (17.32% CP and 37.82% NDF).

The mixtures included alfalfa hay, corn silage, wheat grain, corn grain, cottonseed meal, and soybean meal. Corn silage, wheat grain, and soybean meal predominantly contain rapidly fermentable fractions (F), others predominantly contain insoluble, but slowly fermentable fractions (S) (B_3) (Mahanna, 2010). The mixtures were prepared to exist in different roughage:concentrate ratios (20:80, 40:60, 60:40, and 80:20). Final mixtures were predominant in F and S as well as equal amount of them (50F). They were isonitrogenous.

To determine gas production and gas kinetics NDF residues of these two pools (NDF-F and NDF-S) were obtained (Van Soest et al., 1991; Pell and Schofield, 1993; Schofield and Pell, 1995). Ground mixtures and their NDF residues (460 mg) were put in 100-ml Pyrex tubes containing ruminal fluid medium [(macromineral (200 ml), micromineral (0.1 ml), buffer (200 ml), reduction (40 ml) and resazurin (1 ml) solutions as well as distilled water (400 ml)] and incubated at 39°C (Menke and Steingass, 1988). Pressure due to gas production was monitored and recorded every minute using data-logger (RHT50, Extech Instruments, USA). Gas was released 6 times within 12 h, 3 times within 12-24, and 3 times within 24-48 h. Cumulative gas production was calculated using pressure recorded by digital manometer (Lopez et al., 2007).

"dual-pool logistic equation" (soluble in NDF solution and rapidly fermentable; insoluble in NDF soluble and slowly fermentable) was used to calculate gas kinetics in curve subtraction technique (Schofield et al., 1994; Schofield and Pell, 1995). Formulas were:

$$\text{Gas, ml} = V_{1F}\ \{1+\exp(2+4S_1(\lambda_1-t))\}^{-1} + V_{2F}\ \{1+\exp(2+4S_1(\lambda_2-t))\}^{-1}$$

V_{1F} and V_{2F}: maximal gas in both pools.

S_1 and S_2: specific fermentation constant for both pools.

t: incubation time.

λ: lag time (λ_1 and λ_2 represent pools)

Data were subjected to 2-way ANOVA in a completely randomized design in which groups were arranged in 4 x 3 factorial fashion (SPSS, 2006). The linear model in data analyses was as follows:

$$Y_{ijk} = \mu + (R:C)_i + (FC)_j + (R:C \times FC)_{ij} + e_{ijl}$$

Y_{ijk} = response variable
μ = population mean
$R:C_i = i^{th}$ roughage:concentrate ratio
$FC_j = j^{th}$ fermentation characteristics
e_{ijk}= experimental error

RESULTS AND DISCUSSIONS

Fermentation was almost completed within 12 h and was at a very low level between 24 and 48 h. As the R:C ratio decreased, pH decreased linearly (P<0.023). The substrate rich in F decreased pH more dramatically than the substrate rich in S (P<0.002). As the incubation advanced NH_3-N concentration increased (P< 0.002). Lag time for fractions soluble in NDF solution there was no effect of the roughage proportion and fermentation characteristics (F and S). However, lag time for fractions insoluble in NDF solution increased with increasing the roughage proportion (P<0.025) and providing S (P<0.001). The gas production decreased from NDF-F (P<0.053) and increased from NDF-S (P<0.005) as the roughage proportion increased. Expectedly, gas production from NDF-S was low at earlier phase of incubation. Gas production from NDF-F and NDF-S was similar 24 h after incubation.

There was a positive correlation between cumulative gas production and gas production within 3 h of incubation from NDF-F (r = 0.77; P<0.001). For NDF-S, this relationship was evident cumulative gas production and gas production between 3 and 20 h of incubation (r = 0.88; P<0.001).

Difference in times to reach maximal fermentation of substrates soluble and insoluble in NFD got longer as the NDF level increased (r =0.76; P<0.001). Similar observation was noted for ADF, especially for fractions of S (r = 0.85; P<0.001) and difference in times to

reach maximal fermentation for F and S (r = 0.85; P< 0.001). These indicated importance of focusing on times to reach maximal fermentation of F and S substrates in ration evaluation. The specific ratio of NDF-S for concentrates was greater than that for roughages (Table 1).

Table 1. Gas production parameters of substrates differing in roughage:concentrate (R:C) ratio and fermentation characteristics (FC; F- fast; S- Slow; 50F- mixture of F and S) in fully automated *in vitro* gas production system

		Gas Measurements[1]									
R:C	FC	CGP (ml)	S-NDF (ml)	Is-NDF (ml)	cS-NDF (h^{-1})	cIs-NDF (h^{-1})	tS-NDF (h)	tIs-NDF (h)	Δt (h)	S-Lag (h)	Is-Lag (h)
	F	163	135	27	0.087	0.105	7.5	6.3	-1.2	1.68	1.48
20:80	50F	152	112	40	0.078	0.095	7.4	7.2	-0.2	1.12	2.03
	S	142	107	35	0.082	0.090	7.3	9.6	2.3	1.00	3.94
	F	165	129	37	0.087	0.098	6.8	7.0	0.2	1.03	1.93
40:60	50F	151	112	40	0.081	0.091	6.3	8.4	2.1	0.28	2.97
	S	145	109	36	0.087	0.085	6.9	10.9	4.0	1.15	4.95
	F	141	96	45	0.087	0.080	7.3	7.1	-0.1	1.41	0.85
60:40	50F	141	97	44	0.114	0.071	6.2	9.3	3.2	1.73	2.35
	S	143	111	32	0.103	0.078	5.6	12.2	6.5	0.83	5.74
	F	155	99	56	0.072	0.072	7.5	8.5	1.0	0.60	1.06
80:20	50F	152	105	47	0.093	0.066	4.8	11.5	6.8	0.70	4.08
	S	142	108	33	0.110	0.080	5.5	13.6	8.2	0.98	7.21
SEM		2.36	2.66	1.28	0.03	0.02	0.15	0.30	0.39	0.14	0.28
Effect		---------- $P <$ ----------									
R:C		0.287	0.053	0.005	0.051	0.001	0.001	0.001	0.001	0.437	0.025
FC		0.107	0.385	0.005	0.123	0.118	0.001	0.001	0.001	0.812	0.001
R:CxFC		0.214	0.006	0.843	0.085	0.369	0.022	0.273	0.001	0.639	0.098

[1]CGP = cumulative gas production. S-NDF = gas produced from fraction soluble in NDF solution. Is-NDF = gas produced from fraction insoluble in NDF solution. cS-NDF = maximal gas production constant for fraction soluble in NDF solution. cIs-NDF = maximal gas production constant for fraction insoluble in NDF solution. tS-NDF = time occurring maximal gas production from fraction soluble in NDF solution. tS-NDF = time occurring maximal gas production from fraction insoluble in NDF solution. Δt = difference in times to reach maximal fermentation from fractions soluble and insoluble in NDF solution. S-Lag = lag time for fractions soluble in NDF solution. Is-Lag = lag time for fractions insoluble in NDF solution.

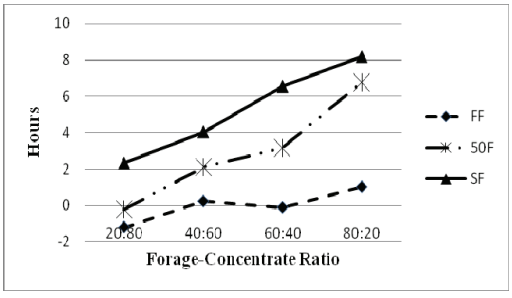

Figure 1. Times to reach maximal gas production from fractions soluble (NDF-F) and insoluble (NDF-S) in NDF solution using fully automatic *in vitro* gas system (SEM = 0.39). FF = fractions rapidly fermented; SF = fractions slowly fermented; 50F = mixture of FF and SF.

Microbial mass started to differ 12 h after incubation depending upon fermentation characteristics of substrates. It was greater for FF than for SF. Microbial mass production efficiency was 43.3, 44.0, 45.4, and 46.0% for 20, 40, 60, and 80% the roughage proportions, respectively at the end of 24-h incubation (P<0.001).

CONCLUSIONS

Lag time and time to reach maximal fermentation are important fermentation kinetics parameters and differ by solubility of fractions in NDF solution. Considering these parameters could improve nutritional efficiency and well-being of the ruminant animal.

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THE EFFECT OF SUPPLEMENTAL DIFFERENT LEVEL OF ROSELLE FLOWER IN DIET ON JAPANESE QUAIL PERFORMANCE

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Abstract

This experiment was conducted at the Poultry Farm, Poultry Research Station, State Board of Agriculture Research, Ministry of Agriculture, to study the effect of supplemental Roselle (Hibiscus sabdariffa) to Japanese quail diets on productive performance. A total of 360 Japanese quail (Coturnix coturnix japonica) female 60 days old were used in this study. They were randomly distributed to 3 treatments. Roselle flowers were supplemented at the levels of 0.2, 0.4% and compared with the control group for 140 days. The result showed a significant increase (P<0.05) in egg production percentage , accumulative egg number, egg weight and egg mass, while feed consumption increased significantly and feed conversion ratio improved significantly by supplementing 0.4% Roselle flower compared with supplementing 0.2% Roselle flower and control groups. Significant decrease in mortality percentage in Roselle treatments compared with control group. There were significant increase (P<0.05) in some egg interior quality by supplementing Roselle flower at two levels 0.2, 0.4% compared with control group.

Keywords*: Roselle flower, Japanese quail, Productive performance, egg quality.*

INTRODUCTION

In recent years the science has started paying attention to the properties of spice (Chaudhry and Tariq, 2006). Due to the side effects of medicine, the use of natural products as an alternative to conventional medicine and antibiotics has been rise in the last few decades (Ansari et al., 2006).

Many medicinal plants and their extracts are used widely in poultry diets because the herbs have biological activities and served as antioxidants (Chithra and Leelamma, 1999), stimulate the digestive system (Jamroz and Kamel, 2002), increase the production of digestive enzymes and improve utilization of digestive products through enhanced liver functions (Hernandez et al., 2004).

Roselle plant (*Hibiscus sabdariffa*) is one of medicinal plants related to Malvaceae family (Norman, 1992). Flowers are part from the plant that used, the active ingredient concentrated in sepal leaves of flowers such as phenolic compounds, glycosides and hydrochloride hibisine (Resendiz et al., 1998).

Roselle is a herb which was used to lower blood pressure to the normal levels (Faraji and Hagi Tarkhani, 1999), also AL-Obeidy (2008) reported that Roselle flower contain vitamin C about 45-50 mg/100 ml solution, in addition it contain citric acid, tartaric acid from 3 to 4% which play an important role on gut flora and then enhance nutrient absorption (Mazza and Miniti, 1993), on the other hand there were colour pigments in sepal leaves of flower like anthocyanine pigments (Kowalczgk et al., 2003; Marco et al., 2005). However, no reports to my knowledge is available on the effect of supplementing Roselle flower on Japanese Quail, wherefore the objective of this study is to investigate the effect of Roselle flower on Japanese Quail performance and egg quality during summer month in Iraq.

MATERIALS AND METHODS

An experiment was conducted at Poultry Research Station, State Board of Agricultural Research, Ministry of Agriculture. A total of 360 Japanese Quail female 60 days old were used during summer season.

The quail birds were randomly distributed to three treatments by supplemented Roselle flower (*Hibiscus sabdariffa*) to diet at levels 0.2 and 0.4% and compared with control group for 140 days, each treatment content four replicates 30 birds per replicate.

Experimental diet and calculated chemical composition is presented in Table 1. The diets

were isocaloric and isonitrogenous. Diets and water given *ad libitum*.

Table 1. Composition of experimental diet and chemical composition

Ingredient	%
Yellow corn	56
Wheat	3
Soybean meal (44%cp)	29
Protein concentrate *	5
Corn oil	2
Limestone	4.7
Salt	0.3
Total	100
** Calculated Chemical Composition	
Crude protein %	20
ME, kcal/kg feed	2902
Calcium %	2.4
Phosphorus %	0.4
Lysine %	1.12
Methionine %	0.4
Methionine + cystine %	0.75

* Protein concentrate type COLOM contains 40% CP and 2100 kcal ME

** Calculated composition according to NRC, 1994

Birds were raised in environmentally controlled poultry house. The performance and the egg quality were measured, which included Hen Day egg production percentage, accumulative egg number (egg/bird), egg weight (g) egg mass (g), feed consumption (g), feed conversion ratio (g feed/g egg) and mortality rate (%). While egg quality parameters include yolk weight, shell weight and thickness also albumin and yolk height were measured.

The data were subjected to analysis of variance Utilizing Complete Randomizes Design (CRD) according to SAS (2001) and significant means were separated by Duncan's multiple range test (1955).

RESULTS AND DISCUSSIONS

Results indicated that Hen–Day egg production was significantly ($P<0.05$), higher by increasing Roselle flower supplementation in the diet. The egg production was 74 and 79% for treatments 0.2 and 0.4%, respectively compared with 70% for control group (Table 2).

Table 2. Effect of supplementing different levels of Roselle flower in Quail diet egg production %, accumulative egg production (egg/hen), egg weight (g), egg mass (g) during 140 days

Treatment	Egg production %	Accumulative egg number (egg/bird)	Egg weight (g)	Egg mass (g)
Control	*70±1.10 b	98.0±0.52b	10.5±0.20b	1029.0±21.3c
Roselle flower 0.2%	74±0.32 a	103.6±1.08a	10.8±0.10a	1118.8±15.2b
Roselle flower 0.4%	79±0.64a	110.6±0.34a	11.2±0.08a	1238.7±20.6a

* Means in the same column with different super scripts are significantly different ($P<0.05$)

The accumulative egg number (egg/bird) and the egg weight(g) follow similar trends as egg production during the experimental period (140 days), they were recorded 103.6, 110.6 (egg/bird) and 10.8, 11.2 g for 0.2 and 0.4% Roselle flower respectively compared with 98.0 (egg/bird) and 10.5 g for control treatment. The egg mass (g) for treatment 0.4% Roselle flower had significant ($P<0.05$) higher 1238.7 g followed by treatment 0.2% Roselle flower 1118.8 g, finally control group 1029.0 g. The improvement in egg production, the accumulative egg number and the egg weight as supplementing two levels of Roselle flower was increased as compared to control group could be due to containment ascorbic acid levels in Roselle (45-50 mg/100 ml juice) (Al-Obeidy, 2008), and the important role of ascorbic acid in Lowering body temperature and stimulate thyroid gland especially during high ambient temperature, synthesis of vitamin for physiological action was enough during optimum temperature, but when ambient temperature raised from 21°C to 31°C, it leads to decrease the level of vitamin C in blood because of their endogenous partial attrition then decrease vitamin synthesis (Pardue and Thaxton, 1989) and reflected on productivity of birds. Also vitamin C play an important role in increasing estrogen which support the synthesis of yolk precursor and Lipoproteins and their transport to the liver then to the ovary, which resulted in improvement in egg production, accumulative egg numbers and egg weight (Whitehead et. al., 1990; Keshavarzr, 1996).

The significant increase in egg mass due to the increase in average eggs weight and accumulative egg production for quails supplemented with 0.2 and 0.4% Roselle flower.

Table 3. Effect of supplementing different levels of Roselle flower in Quail diet of feed consumption (g/day), feed conversion ratio (g feed/g egg) , feed conversion ratio (g feed/g eggs) and mortality (%) during 140 days

Treatment	Feed consumption (g/day)	Feed conversion (g feed/g eggs)	Feed conversion (g feed/g egg)	Mortality (%)
Control	*20.2±0.68b	2.7±0.04a	28.9±0.11a	4.2±0.30a
Roselle flower 0.2%	20.8±0.01b	2.6±0.10a	28.1±0.15a	1.6±0.02b
Roselle flower 0.4%	21.4±0.20a	2.4±0.01b	27.0±0.08b	0.0±0.00c

* Means in the same column with different super scripts are significantly different (P<0.05)

There was a significant increase (P<0.05) in feed consumption (g/day) in Roselle flower 0.4% treatment (21.4 g) compared with Roselle flower 0.2% (20.8 g) and control (20.2 g) treatments.

The feed conversion ratio (g feed/g egg) and (g feed/g egg) are presented in Table 3 for quail birds that were supplemented with different levels from Roselle flower.

The quails that were supplemented with 0.4% Roselle flower improved significantly (P<0.05) recorded (2.4 g feed/g eggs and 27.0 g feed/g egg), respectively compared with other treatments.

The mortality rate was significantly (P<0.05) decrease by increase Roselle flowers in quail diets. The supplementation of 0.4% Roselle flower determined lowest mortality (0.0%), while 0.2% Roselle and control treatments recorded 1.6 and 4.2% respectively.

The improvement in Roselle flower treatments could be attributed to the active material that have stimulating effect on animal digestive systems (Langhout, 2000; Williams and Losa, 2001) they explained that these effects could be due to the increased production of digestive enzymes and the improved utilization of digestives products through enhanced liver functions, also the content of Roselle flower of vitamin C which may improve the utilization of feed nutrient despite the increase in ambient temperature. This finding were supported by Hai et al. (2003), who defined that supplementation of vitamin C for layer diets improve feed conversion, also vitamin C is important to cellular metabolism and digestion and utilization of nutrients (Lohakare et al., 2005).

Whitehead and Keller (2003) confirm our result and stated that supplementation of vitamin C resulted in improvement in egg production, feed conversion ratio through lower the negative effect of heat stress and protect the liver and other vital organs from oxidative damage.

Table 4. Effect of supplementing different levels of Roselle flowers in Quail diet on Yolk diameter (mm),Yolk height (mm), Yolk weight (gm), Albumen height (mm), shell weight (gm) and shell thickness (mm) during 140 days

Treatments	yolk diameter (mm)	yolk height (mm)	yolk weight (g)	Albumen height (mm)	Shell weight (g)	Shell thickness (mm)
Control	*22.5±0.16	9.1±0.02b	3.1±0.01b	2.9±0.12b	0.84±0.06b	0.15±0.04b
Roselle flower 0.2%	22.2±0.20	9.8±0.01a	3.2±0.03b	3.4±0.33a	0.90±0.01a	0.18±0.02a
Roselle flower0.4%	23.8±0.72	10.4±0.80a	3.5±0.10a	3.8±0.11a	0.93±0.02a	0.20±0.01a

* Means in the same column with different super scripts are significantly different (P<0.05)

About the effect of supplemental Roselle flower on some egg quality parameters Table 4 explained that no significant difference between treatments in yolk diameter, but there were significant (P<0.05) higher in 0.2 and 0.4% Roselle flower treatments in yolk and albumen height and in weight and thickness shell compared with control group, while yolk weight increase significantly just in 0.4% Roselle flower treatment compared with other two treatments.

It could be noted from the results of Table 4 that most egg quality parameters were improved in supplemented groups as compared to the control. The improvement in most egg quality parameters could be due to the presence of vitamin C in Roselle which play an important role in Ca absorption and reabsorption from the bone and improve shell thickness and interior egg quality (Tollba et al., 2006). These results confirm the role of vitamin C in Roselle flower which lowest heat stress

effect during summer months, through lowering body temperature of the birds and the increase in PCo_2 and bicarbonate in the blood. The increase bicarbonate which is considered the major constituent of egg shell (Cheng et al., 1990) and the role of vitamin C in the estrogen synthesis increase Ca^{++} in the blood through increased its absorption from the intestine (Mahmoud et al., 1996), also Whitehead and Keller (2003) explained that vitamin C increase the metabolism and increase total protein which resulted in providing more protein from albumin formation.

It could be concluded that the inclusion 0.2 and 0.4% Roselle flower in quail diets could be used as growth promoter and reduce the effect of heat stress especially on mortality and shell thickness that composed the most problem in quail bird.

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EFFECT OF LACTIC ACID ON *ENTODINIUM CAUDATUM* MONOCULTURE

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Abstract

This experiment was carried out to evaluate the effect of lactic acid on Entodinium caudatum monoculture in vitro. After thawing, E. caudatum was grown at 39°C under anaerobic condition to yield 10^5. Four groups were established by inclusion of 0, 0.5, 1, and 2 mM DL-lactic acid (Fluka Chemica, 69775). E. caudatum started to selectively use lactate to maintain 1.2 mM concentration at the highest lactic acid concentration. Increasing lactic acid concentration in medium was associated with reduction in pH (P<0.0001) and increase in total volatile fatty acids (P<0.0001), but no change in ammonia concentration. There was a reduction in acetate (P<0.04) and increases in propionate (P<0.02) and butyrate (P<0.0001) proportions as lactic acid concentration in medium increased. Stoichiometrically calculated gas production and CH4 amount increased accordingly with total volatile fatty acid production. In conclusion, E. caudatum grows to utilize lactate in case of acidosis.

Keywords: *Entodinium caudatum, lactic acid, in vitro rumen.*

INTRODUCTION

In order to meet energy demand, feeding excessive amount of readily fermentable carbohydrate sources can disturb rumen flora and microbial fermentation, which may result in acute and/or subacute acidosis (Umucalılar and Gülşen, 2005; Umucalılar et al., 2012).

Protozoa, especially ciliatas play a significant role in lactic acid metabolism in rumen when excess grains are fed. They engulf starch and soluble carbohydrates, which limits their utilization by amylolytic bacteria. This reduces lactic acid production (Nagaraja et al., 1992). Moreover, protozoa increases lactate fermentation, which reduces lactic acid accumulation in rumen (Nagaraja et al., 1992; Russell and Hespell, 1981). Entodiniomorphid ciliates help maintain ruminal pH (Dehority, 2005), by storing starch to minimize its utilization by starch-utilizing bacteria (Schwartzkopf-Genswein et al., 2003). This in vitro experiment was conducted to evaluate *E. caudatum* cultures in response to increased lactic acid concentration in medium.

MATERIALS AND METHODS

After thawing frozen *E. caudatum* cultures at 39ºC, they were allowed to grow in Medium M at 39ºC under anaerobic conditions to enumerate 10^5 (Dehority, 1998). Media were enriched 1.5% wheat flour and 1% ground alfalfa daily.

Cultures were then added with 0, 0.5, 1, and 2 m*M DL*-lactic acid (Fluka Chemica, 69775). Upon condensation, 1 of 10^{th} of the sediment were added with 96.6 ml Medium M, to achieve 10^3-10^4/ml. After incubation at 39ºC, 0.2 ml medium and 1.2 ml substrate solution were refreshed everyday at the same time. Every 3 d, half of the media was added with fresh Medium M (Dehority 1998). Media pH were measured before adding and 5 h after adding the substrate solution. On d 5 and refreshment of the media 1 ml sample was taken for determination of lactic acid and volatile fatty acid (VFA) concentrations and enumeration of protozoon.

Stoichiometrical Calculations (Blümmel et al., 1999):

CO_2 production (CO_2fer), mmol = acetate/2 + propionate/4 + 1.5 x butyrate

CH_4 production (CH_4fer), mmol = acetate + 2 x butyrate - CO_2
CO_2 released from buffer (CO_2buff), mmol = total VFA
Gas production, ml = (CO_2fer + CH_4fer + CO_2Isobutyrate + CO_2buff) x 0.0821 x 312
Methane level (Wolin, 1960; Ramin and Huntanen, 2012) was calculated using formula as follows:
Methane, ml = 22.4 x [(0.5 x acetate) – 0.25 x propionate) - (0.5 x butyrate) – (0.25 x valerate)]
In a completely randomized design experiment data were analyzed using 2-way ANOVA (SPSS, 2006).

RESULTS AND DISCUSSIONS

Increasing lactic acid addition up to 2 m*M* decreased pH (Table 1; Figure 1). Excess lactate appeared to be used by *Entodinium caudatum* to maintain its level by 1.2 m*M* (Figure 2).

Table 1. Effects of lactic acid addition on medium pH, ammonia, lactate concentrations and *Entodinium caudatum* numbers

Trt[1]	pH	Ammonia m*M*	Lactate m*M*	Protozoon
0	6.60	2.98	---	$4.7x10^3$
0.5	6.61	2.91	0.83	$6.6x10^3$
1	6.56	3.02	1.17	$8.1x10^3$
2	6.50	3.04	1.27	$9.4x10^3$
Effect	---------- *P* > F ----------			
Trt	0.0001	0.31	0.0001	0.0001
T	0.0001	0.0001	0.0001	0.0001
Trt x T	0.001	0.08	0.0001	0.0001

[1]Trt = treatments, lactic acid, mM. T = time, day.

Table 2 Effects of lactic acid addition to medium containing *Entodinium caudatum* monocultures on VFA profile and fermentation parameters

	Lactic Acid (m*M*)					
Parameters	0	0.5	1.0	2	SEM	*P* > F
Acetate (%)	56.4	57.8	54.9	52.8	0.84	0.040
Propionate (%)	22.2	21.5	23.6	24.3	0.44	0.017
Isobutyrate (%)	6.0	5.5	5.4	5.0	0.13	0.009
Butyrate (%)	9.1	9.1	10.1	12.5	0.32	0.000
Isovalerate (%)	4.9	4.8	4.5	4.0	0.27	0.572
Valerate (%)	1.4	1.2	1.5	1.4	0.07	0.373
Σ VFA (m*M*)	0.42	0.46	0.45	0.52	0.01	0.001
CO_2fer (ml)	0.20	0.22	0.22	0.27	0.01	0.000
CH_4fer (ml)	0.11	0.13	0.12	0.13	0.001	0.028
CO_2buff (ml)	0.40	0.43	0.42	0.49	0.01	0.000
Gas (ml)	18.8	20.4	20.0	23.6	0.45	0.000
NGR[1]	3.60	3.82	3.36	3.36	0.09	0.070
e-CH_4[2] (ml)	2.52	2.80	2.61	2.97	0.06	0.027

[1]NGR = nonglucogenic VFA:glucogenic VFA.
[2]e-CH_4 = estimated methane production.

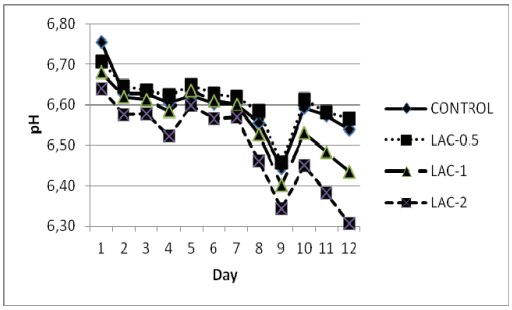

Figure 1. Alterations in pH in media containing *E. caudatum* upon addition of different concentrations of lactic acid (SEM = 0.06).

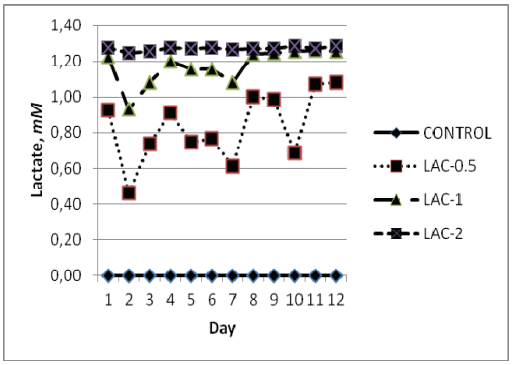

Figure 2. Alterations in lactate concentrations in media containing *E. caudatum* upon addition of different concentrations of lactic acid (SEM = 0.01).

While total VFA increased, proportion of acetate decreased and proportions of propionate and butyrate increased as concentration of lactic acid increased in media containing *Entodinium caudatum* (Table 2).

Increased lactic acid concentration caused increases in total VFA production and CO_2 as well as CH_4. Increased CO_2 release from buffer is a way to neutralize pH. These increases led to increases in stoichiometrically calculated gas production and CH_4 (Table 2).

CONCLUSIONS

Lactic acid inclusion up to 2 m*M* decreased pH in media containing *E. caudatum*. Reduction in pH associated with stimulation of *E. caudatum* to maintain pH, through modifying rumen fermentation.

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EFFECT OF BANANA PEEL APPLICATION IN RATION ON HEMATOLOGICAL LEVEL, NITROGEN RETENTION AND BODY WEIGHT GAIN OF HEAT EXPOSED BROILER CHICKEN

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Abstract

Banana peel application on heat exposed chicken was conducted by using 80 broiler chickens aged 21 days in complete randomize design method. Four treatment that was given based on the level of banana peel ration are R_0=0%, R_1=10%, R_2=20% and R_3=30%. Each of the treatment was repeated 5 times to 4 chickens for each repetition. The study was held for 21 days. The observed variables are haematocrite value, haemoglobin level, erythrocyte, MCHC level, ration consumption, nitrogen retention and body weight gain. The result of this study showed that haematological parameters between the treatment was insignificant (P>0.5). The chicken body weight gain on R_0 (=978.00 g) was significantly higher compared to others (R_1=799.50 g, R_2=810.00 g and R_3=638.25 g). In conclusion, application of banana peel on 20% level to heat exposed chicken can increase nitrogen retention but it is not follow by body weight gain while haematological parameters for all treatment is remained the same.

Keywords: *banana peel, broiler chicken, heat stress, hematological parameters, nitrogen retention.*

INTRODUCTION

Broiler chickens have a lot of positives, for example it fast growth, could be harvested in a matter of time, but it also have a negative issues such as fragile body defence to natural cause, like nutrition, climatology and managerial (Ensminger, 1991). As homeoterm organism, broiler chickens will always constantly maintain its body temperature, but chickens have few sweat glands. These circumstances often lead to problems that obstruct the process of removing heat, so some of the heat accumulates in the body (Sugito, 2008) which in turn will trigger a stress.

Indonesia is a tropical country where the weather is characterized by high temperature and humidity, which ranged between 27.7 to 34.60^{0}C and humidity ranged between 55.8 and 86.8% (BPS, 2010), but the optimum temperature for raising broiler chickens 21˚C (Soeharsono, 1976) or in the temperature range 18-22^{o}C (Charles et al., 2002). The high temperature of the environment canal so lead to oxidative stress in the body, resulting in excessive free radicals (Miller et al., 1993; Aruoma, 1999), which can cause membrane to undergo lipid peroxidation, so that free radicals can attack DNA and cell protein (Rahman, 2003).

To maintain body temperature, chicken will trying to improve heat loss through evaporation (Campbell et al. 2004) and reduce the formation of body heat by reducing the consumption of rations, to change the behaviour and physiological activity (Indriani, 2008). When this condition occurs, chicken will suffer micronutrient deficiencies in the body and in turn will be manifested by inefficient use of feed and growth impairment (Donkoh, 1989; Mashaly et al., 2004). These circumstances resulted in unequal nitrogen and minerals in the body.

The farmers often supplement with macro minerals such as K, Na and Cl to replace lost ions in the event of stress directly even though this action will not ease the stress or discomfort of the chicken.

Ration plays an important role in supporting the growth of chicken, considering the growth cannot be separated from consumption, which in turn reflects the ration nutrients consumed anyway (Soeharsono, 1976) is shown by the quality of the feed is lost after digestion, absorption, and metabolism. While retained nitrogen is the food that not excreted in the

faeces and urine. Nitrogen is nitrogen is derived from protein ration so that retention of nitrogen can be used to assess protein ration.

Indonesia's rich flora can be utilized to meet the nutritional needs of broiler chickens, including natural ingredients found in many industrial waste, such as banana peels. Banana peel contains macro mineral (Margen, 2002), and a number of active compounds such as tannins, saponins (Anhwange, 2001), vitamin A, B, C and E (Kanazawa and Sakakibara, 2000)

Research experts from Chung Shan Medical University Taichung, Taiwan (Anonymous, 2009), banana skin is rich in vitamin B6, vitamin C, vitamin E, potassium and Cl, besides, many contain serotonin which plays a very vital to balance mood, for prevention of stress and depression in humans. The purpose of this study to determine the extent of the use of banana peels in the ration to reduce the impact of heat stress in broilers.

MATERIALS AND METHODS

Animal experiments were 80 broiler chickens, final stock 21 days old Ross strain weighing 450 g, and the coefficient of variation of 5.83%. Straight run system rearing. Cages are used as much as 20 units with stage system, a length, width and height of 1 x 0.5 x 0.75 cm (for five chickens). Enclosure temperature is maintained between 28-34^{o}C, each cage has two incandescent bulbs (60 watt), and thermometer for easy record keeping.

Banana peels used in this experiment are Ambon Banana (*Musa sapientum sp*.) It is first dried and then made into flour by machine. The content of nutrients and metabolic energy feed ingredients making up the ration can be seen in Table 1.

Ration prepared in accordance with the recommendation of Daghir (1995). The metabolic energy content of 3000 kcal/kg and 22% crude protein are presented in Table 2.

Table 1. Composition of the formula ration

Ration Ingredients	CP	CF	CFi	Ca	P	Lysine	Met	Cystine	ME
	……%.....								Kkal/kg
Soybean Meal	48.00	0.90	6.00	0.30	0.29	2.90	0.65	0.67	2240.00
Coconut Meal	18.58	12.60	15.38	0.21	0.20	0.64	0.29	0.30	2212.00
Fine Bean	12.00	13.00	12.00	0.12	0.21	0.71	0.27	0.40	160.00
Yellow Corn	8.60	3.90	2.00	0.02	0.30	0.20	0.18	0.18	3370.00
Coconut Oil	0.00	100.00	0.00	0.00	0.00	0.00	0.00	0.00	8600.00
Fish Flour	61.00	9.00	1.00	5.50	2.80	0.65	1.80	0.94	3080.00
Bone Flour	0.00	0.00	0.00	24.00	12.00	0.00	0.00	0.00	0.00
Top Mix	0.00	0.00	0.00	40.00	0.00	0.30	4.00	4.00	0.00
Banana Peel Flour	10.30	4.02	17.62	0.29	0.07	-	-	-	2915.00

Note : CP: Crude Protein CFi: Crude Fiber P:Protein
CF: Crude Fat Ca: Calsium ME: Metabolic Energy

Table 2. The nutrient and metabolizable energy in ration

The Nutrients	Ration				
	R0 (BP 0%)	R1(BP10%)	R2(20%)	R3(30%)	Needs
Crude Protein (%)	22.00	22.02	22.17	22.14	22.00
Crude Fat (%)	7.15	6.84	6.53	6.18	≤8.00
Crude Fiber (%)	4.08	5.28	6.46	7.51	≤8.00
Calsium (%)	1.07	1.09	1.11	1.13	0.90-1.00
Phosforus (%)	0.64	0.62	0.60	0.58	0.45-0.80
Lysine (%)	0.76	0.72	0.70	0.68	0.65-0.72
Methionine (%)	0.48	0.46	0.44	0.42	0.40-0.50
Cystine (%)	0.39	0.37	0.34	0.32	0.40
EM (Kkal/Kg)	3011	3023	3024	3020	3000

Parameters observed:

1. Haematology	:	a.	Number of of erythrocytes was calculated using hemocytometer Improve Neubaeur
		b.	Hemoglobin was measured using Sahli hemometer
		c.	Hematocrit was calculated using methods of microhematocrit
		d.	MCHC (Mean Cellular Hemoglobin Capsular) is calculated by comparing the amount of hemoglobin by the number of erythrocytes X 100%
2. Nitrogen Retention	:	Measurements conducted on nitrogen retention 36 days old chickens, collecting method to accommodate faeces for three days. Calculation of nitrogen retention by using Maynard and Loosli (1962), namely: $$RN = \frac{NI - (NF + NU) \times 100\%}{NI}$$ Description : RN = Nitrogen Retention (%) NI = Amount of Consumed Nitrogen (g) NF = Amount of Nitrogen in Feces (g) NU = Amount of Nitrogen in Urine (g)	

1. Ration Consumption : Consumed ration counts in 21 days of experiment with units of grams
2. Body Weight Gain : Weight gain is done by calculating the difference between weight loss-weight end of the week earlier in the week for three weeks of study with units of grams

Experiment methods

Research was conducted in experiment using a completely randomized design (CRD). Four treatments were used in which each treatment contained different percentage of banana peel in the given ration. For practical purpose, treatments were named R1, R2 and R3. R1 treatment contained 10% of banana peel in the ration, meanwhile R2 contained 20% of banana peel and R3 contained 30% of banana peel. These treatments were repeated five times so that there are twenty experimental units, each unit consisting of four broiler chickens. Other than the four treatments, another treatment was used as comparison. That treatment named R0 which contained no banana peel on the ration. Data were analyzed statistically (Gasperz, 1995), and the differences between treatments performed by Duncan Multiple Test distance.

RESULTS AND DISCUSSIONS

Haematological level

Erythrocytes, haemoglobin, hematocrit and MCHC

Erythrocytes play a role in the transport of oxygen by haemoglobin assistance. The ability of blood to carry oxygen depends on the level of haemoglobin in the blood and chemical characteristics of haemoglobin (Cunningham 2002). Haemoglobin fully is one-third of the components of erythrocytes (Reece, 2006). About 400 million haemoglobin molecules are inside the erythrocytes (Jain, 1993). Under normal circumstances erythrocytes, haemoglobin and hematocrit in parallel are observed.

Observations using a banana peel in the ration of broiler chickens exposed to heat are presented in Table 3 and illustration 1.

In Figure 1 it appears that erythrocytes from each treatment giving a banana peel (R1, R2 and R3) in the ration showed a higher tendency of treatment without banana peel (0%) erythrocyte count range 1.84 - 2.29.10^6/mm^3. Haemoglobin from each treatment (R1, R2 and R3) showed a decrease compared to R0, i.e. in the range of 7.8 – 8.54 g%. Hematocrit of each treatment (R1, R2 and R3) showed a declining trend compared to R0. While MCHC (mean capsular Haemoglobin Concentration) seems to treatment (R1, R2 and R3) tends to decrease as compared with the treatment of R0, MCHC number range 28.80 - 35.41%.

Table 3. Levels of haematological in blood of broiler chicken heat exposed

Treatments	Variables			
	Erythtrocytes (10^6 mm^3)	Hemoglobin (Gr %)	Hematocryte (%)	MCHC (%)
R0	1.824	7.8	27.2	28.8
R1	2.292	8.1	25.7	33.41
R2	2.177	8.54	24.2	35.41
R3	2.291	8.54	24.9	34.78

Description : R0 = 0 % BP; R1 = 10 % BP; R2 = 20 % BP; R3 = 30 % BP
BP = Banana Peel

Haematological levels still within the normal range, which is about $3.10^6/mm^3$ for erythrocyte (Bell, 2002); 7.0 to 13.0 g/dL for haemoglobin (Jain, 1993); 24-43% (Smith, 1988) and 30-33% for hematocrit (Swenson, 1984) and normal range MCHC is 26-35% (Hodges, 1977).

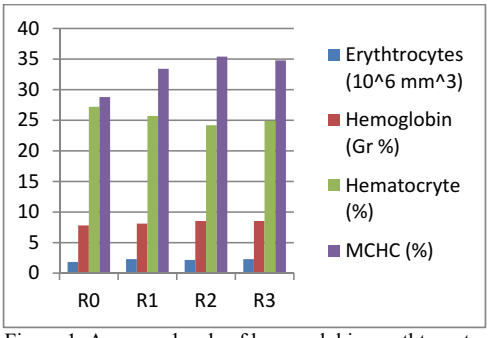

Figure 1. Average levels of haemoglobin, erythtrocytes, hematocyte and MCHC

Statistical analysis showed that the erythrocytes, haemoglobin, hematocrit and MCHC did not differ significantly (P>0.05). This suggests that the provision of rations containing banana peels (0, 10, 20 and 30%) in chickens exposed to heat does not affect the value of erythrocytes, haemoglobin, hematocrit and MCHC.

Erythrocytes, haemoglobin, hematocrit and MCHC are very susceptible to changes in environmental temperature and nutrient levels (Roth, 1970). In hot conditions, chickens will show changes in behaviour, metabolism aimed at maintaining the balance of the milieu interior of physiological and biochemical processes that keep it running. Chicken behaviour of the most prominent is the increase in water consumption, decreased feed consumption, improving the metabolism. In hot temperatures, the cell will be damaged structure and function due to excessive production of free radicals, which in turn will damage the enzyme haemoglobin (especially the sulfhidryl) and membrane lipids. Membrane oxidative damage can lead to intravascular hemolysis or eritrofagositosis and erythrocyte life span is shorter.

Giving a banana skin can maintain relatively normal hematologic level, allegedly contains active compounds, vitamins (A, B, C and E), β - carotene (Kanazawa and Sakakibara, 2000) and phenolic compounds such as catechin, epicatechin, lignin and tannins (Someya et al., 2002) work synergistically. Pantothenic acid, vitamin B2, B12 and folic acid play a role in the formation of erythrocytes. Vitamin B_2 is responsible for the turn folic acid into coenzyme, vitamin B_{12} plays a role in the maturation of erythrocytes (Pilliang and Djojoseobagio, 2006). Vitamin C and E act as antioxidants to protect the membrane from damage by preventing oxidation (peroxide). In addition, vitamin C acts as a reducing agent (antioxidant) in aqueous solutions such as blood and in cell (Gropper et al., 2005).

Red blood cells with normal haemoglobin concentration called normochromic (Nordenson, 2007). Therefore, the role of haemoglobin, erythrocytes in the circulatory system carries oxygen to run properly. Normal, haemoglobin levels indicate the adequacy of oxygenated to circulate throughout the body tissues of chicken is physiologically meaningful in good health. Using banana peels to the level of 30% peel until level 30% were able to maintain normal levels of haemoglobin, erythrocytes, hematocrit and MCHC or in other words no chicken physiological disorder. The content of active compounds from banana peels are prominent tannins (24 ± 0.27 mg/g) and saponin (6.84 mg/g) (Anhwange, 2001) is still below the limit of tolerance, ie 2.6 g/kg ration (Kumar, 2005) and saponin 3.7 g/kg ration (FAO, 2005), so that the hematologic level can be maintained within normal limits.

Ration consumption, nitrogen retention and body weight gain

A growth rate of chicken is a sensitive index of protein quality. Weight gain is comparable to the addition of essential amino acids in the body. Average consumption of ration, nitrogen retention value, and weight gain of broiler finisher phase exposure to heat from each treatment sequence ranges: 2900-3600 g, 74.03

to 82.28%, and 638.00 to 978.00 g presented in Table 4 and to clarify the effect of BP on ration consumption, the value of nitrogen retention and weight gain depicted in Figure 2.

Table 4 shows that the highest ration consumption obtained at R0 treatment and the lowest in R1 treatment. Highest nitrogen retention values obtained in treatment R2 and the lowest in R3 treatment. The highest weight gain on treatment R0 and the lowest in R3 treatment. Ration treatment R2 significantly ($P<0.05$) had the highest nitrogen retention compared to the other two treatments.

Table 4. Feed consumption , nitrogen retention and body weight gain on broiler chickens heat exposed

Treatment	Parameter		
	Feed Consumption	Nitrogen Retension	Body weight gain
	.....(g/e/p).....	.....(%).....	.....(g/e/p).....
R0	3,600 d	75.33	978.00
R1	2,900 a	74.03	799.50
R2	3,300 c	82.28	810.00
R3	3,120 b	75.29	638.25

Description : R0 = 0 % BP; R1 = 10 % BP; R2 = 20 % BP; R3 = 30 % BP
BP = Banana Peel

To clarify the data, Table 4, Figure 2 describe the tendency of the effect of the banana peel in the ration on feed consumption, the value of nitrogen retention and weight gain. It was a decrease in feed consumption and body weight gain by increasing the provision of scene percentage in the ration, while the highest value was shown nitrogen retention ration R2.

Chicken is a homeoterm animal that always maintain body temperature which is relatively constant. Changes in the external environment will affect the function of organs in the body, so that there is always a balance between heat production and heat loss through thermal regulation. Chickens suffering from heat stress showed increased activity of evaporation, the increase in respiratory rate in certain circumstances even look for behavioural changes such as hoops, and in addition an increase in heat dissipation are taken with urine (Hoffman and Walsberg, 1999; Ophir et al., 2002). Behavioural changes such as increased respiratory rate impact on the concentration of electrolytes in the body is the electrolyte flow or expenditures as Na^+ and K^+ from the body (Indriani, 2008), the impact on the balance of electrolytes in the intracellular and extracellular fluid. In this condition, much chicken micronutrient deficiencies in the body and in turn manifested by inefficient use of feed, and growth impairment (Donkoh, 1989; Mashaly et al., 2004).

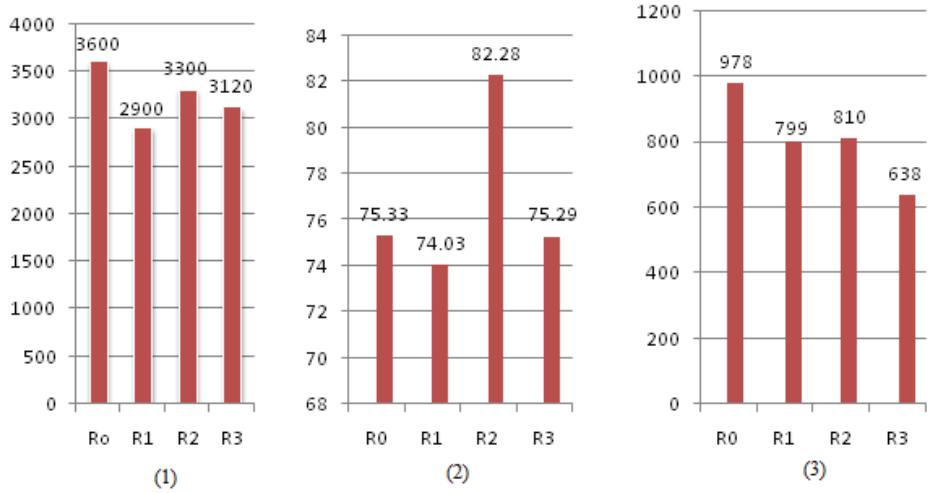

Figure 2. (1) Consumed Ration in Heat Exposed Broiler Chicken
(2) Nitrogen Retention Mean Value of Heat Exposed Broiler Chicken
(3) Weight Gain of Heat Expossed Broiler Chicken

Banana peel has a lot of nutrients, one of which is a high mineral content such as Na and K (Margen, 2002). The use of 20% of banana peel in ration providing the highest nitrogen retention value (82.28%). It gives the sense that is the optimal levels of the mineral needs of Na

and K, when we use 20% banana peels in the ration.

Banana peel can replace lost electrolytes in broiler chickens exposed to heat. This is in line with Borges et al. (2004) which shows that the presence of Na and K minerals play an important role in the absorption of nutrients in the small intestine and increases nitrogen retention (Nor and Yusoff, 2008).

The low body weight gain in the treatment of R1, R2, and R3 caused by the amount of the consumption ration lower than R0 treatment. Total consumption of ration at finisher phase normal circumstances (temperatures at thermoneutral zone) is 4000 g/head (Cheng et al., 1997). Ration consumption in this study is 3600 g/head for the treatment of R0, and other treatments were lower (R1 = 2900 g/head; R2 = 3300 g/head, and R3 = 3120 g/head).

Alleged lack of feed consumption would come on the composition of the ration, the results are reported on a banana peel contains a many macromineral, vitamins and some active compounds such as tannins and cyanide, oxalic acid, phytat (Ahnwange, 2001) and saponins (Tartrakoon, 1999). Tannin content by 24 ± 0.27 mg/g peel bananas and 0:37 ± 6.84 mg saponin/g banana peel, so the ration R1 (10% BP), R2 (20% BP) and R3 (30% BP) sequentially contains tannin 0.69; 1.37 2.05 g/kg and saponin 2.40; 2.48; 7.20 g/kg. Associated with tolerance limit (2.6 g/kg ration (Kumar et al, 2005), third ration were below the limit of tolerance. The content of saponin in rations R3 (30% BP) has a tolerance limit of 0.37 g/kg ration (FAO, 2005) seems to ration R3 already well above the limit of tolerance. Tannins are polyphenolic compounds with high molecular weight and has the ability to bind to the protein. Actually, tannin is a means of protection from animal attacks, bacteria and insects, the attack will soon give rise to a sense of being protected by Sepat, namely the interaction of tannins with salivary proteins (Cheeke, 1989; Widodo, 2000). Transient attacks of microorganisms and insects protected by means turn off the protease enzymes from bacteria and insects in question (Cheeke, 1989), tannins will bind feed protein in the digestive tract making it difficult to digest. While saponin with nature such as soap (foaming) will clean up the materials attached to the intestinal wall and increase the permeability of the intestinal wall (Francis et al., 2002), but also have a negative effect in feed intake that is caused intestinal damage and lack of protein digest. Provision of rations containing 30% BP showed a reduction in feed consumption affecting body weight gain.

CONCLUSIONS

In conclusion, application of banana peel on 20% level to heat exposed chicken can increase nitrogen retention, but it is not follow by body weight gain while haematological parameters for all treatment is remained the same.

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THE EFFECT OF FERMENTED FEED SUPPLEMENT ON MEAT pH AND TENDERNESS OF BROILER

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Abstract

The study of the effect of fermented feed supplement on meat pH and tenderness of broiler was aimed to determine the effect of the feed fermented with Aspergillus niger. Meat tenderness may be influenced by changes occurring during muscle conversion to meat and these changes may be controlled, to improve meat quality. The feed supplement is a mixture of solid coconut oil and solid tofu waste, which were fermented by Aspergillus niger. With this feed was fed to 120 Cobb-strain day old chicks for 5 weeks. And then the meat was boiled at 60^{o}C and checked the meat pH and tenderness. This experiment was used Completely Randomized Design (CRD) 6 x 4; consisted of six treatments{(R-0)0% fermented feed supplement, (R-1) 5%; (R-2) 10%; (R-3) 15%; (R-4) 20%; and (R-5) 25%}; and each treatment was repeated four times. Statistical tests performed by analysis of variance and the differences between treatments effect were examined using Duncan's multiple range test. The results indicated that usage up to 25% fermented feed supplement in the ration will increase the meat tenderness and the pH.

Keywords**:** *broiler, fermented feed supplement, meat pH, meat tenderness*

INTRODUCTION

The fermentation process, the controlled action of selected micro-organisms is used to alter the texture of feed. The main advantages of fermentation processing is the use of mild conditions of pH and temperature which maintain and improve the nutritional properties and sensory of the food. The separation of enzymes from microbial cells, for use in vitro in the processing is a more recent development (Fellows, 1990). The mild conditions used in fermentation produce few of the deleterious changes to nutritional and sensory quality. Microbial growth causes complex changes to the nutritive value of fermented products by changing the composition of proteins, fats and carbohydrates. Many of fungi are beneficial, and nearly all species of molds are harmless. Yeast a by-product in the manufacture of various items, furnish an excellent protein and vitamin supplement. The species of Aspergillus, vary somewhat in color, some are black. The *Aspergillus niger* is used in commercial citric acid production (Gebhardt, 1970). One of the advantages in fermentations, the controlled action of selected microorganism is used to alter the texture, which increase the quality and value of raw materials. *Aspergillus niger* is a type of mold that is used commercially unimproving the quality of agricultural processing wastes, because of the easy handling, its ability to grow quickly and it is not harmful because it does not produce mycotoxins. This fungus can produce enzymes such as a-amylase, amylase, cellulase, gluco-amylase, catalase, pectinase, lipase, and galactosidase (Ratledge, 1994).

The pH of IVRIN (the plant proteolytic enzyme, was isolated from unripe fresh fruits of *Cucumis pubescens W)* treated meat was decreased significantly ($p<0.01$) and the effect was more pronounced in breast than thigh muscle (Sinku et al, 2002). The pH of meat plays important role in maintaining the quality of meat. The pH of broiler meat around 5.95 – 6.00. The toughness of meat occurs even at low pH.

It was observed that the fall in pH of breast muscle was more rapid as compared to thigh muscle in treatment group. An ultimate pH near 5.7 is desirable for maintaining quality of poultry breast meat (Khan et al., 1970).

The meat tenderness may be influenced by changes occurring during muscle conversion to meat. These changes may be controlled, to improve the meat quality. The influence of diet on meat properties is minor importance if there are non nutritional deficiencies. Any feeding practice which alters the quantity of glycogen stored in muscles can influence the ultimate meat properties. Some of the physical properties of fresh meat are difficult to measure objectively. Many factor within muscles, such as intra muscular fat content, can contribute to these physical properties. The tenderness is one of palatability factor that has received more research study (Abrele, 2001). The tenderness of meat is distinctly important to the consumer, having much to do with the pleasure derived from eating meat. Many factors influence tenderness. Certain feeding programs are known to increase the proportion of connective tissue in meat. Feeding may, therefore, have relative direct influence on tenderness, in addition to the fattening effect (Acker et al., 1991). According to the laboratory and consumer studies, it has shown that tenderness is the most important sensory attribute of meat. The tenderness and juiciness are closely related, the more tender the meat, the more quickly the juices are released by chewing it. The deposits of fat in muscle, add to the juiciness and flavor of meat, when it was cooked. The tenderness of meat is measured as the force required to cleave a standard cross sectional area of cooked meat across the muscle fibres (Davey et al., 1988). The heating of meat is accompanied by changes in appearance, flavor, texture and nutritive value. The most drastic changes in meat during heating, such as shrinkage and hardening of tissue, release of juice and discoloration are caused by changes in the muscle proteins. The heating of muscle tissue as well as of myofibrils results in an increase of pH which depends on the initial pH and starts at about 30^0C, the maximum pH increase was observed to be between 40 and 60^0C. Simultaneously, the pH of minimum water holding capacity of the myofibrilar proteins is shifted from 5.0 – 6.0 after heating at 80^0C. The most drastic changes of the myofibrillar and sarcoplasmic proteins occur between 30 and 50^0C, reaching almost completion at 60^0C (Schmidt , 1988).

MATERIALS AND METHODS

Materials: 120 chickens were used for 24 experimental units, each unit were 5 chickens. After slaughter, the meat was put in the polyethylene bag and boiled at 60^oC for 10 minutes and then was check the tenderness using Universal penetrometer 1/10 TH MM DV and pH meter Jenway 3310.

Methods: This research used Completely Randomized Design (CRD) 6x4; consisted of six treatments: (R-0) diet + 0% fermented feed supplement, (R-1) diet + 5% fermented feed supplement; (R-2) diet + 10% fermented feed supplement; (R-3) diet + 15% fermented feed supplement; (R-4) diet + 20% fermented feed supplement; and (R-5) diet + 25% fermented feed supplement; and each treatment was repeated four times. Statistical tests performed by analysis of variance and the differences between treatments effect were examined using Duncan's multiple range test.

RESULTS AND DISCUSSIONS

The effect of treatment on meat pH

In Table 1, there are the results of treatment on meat pH.

Table 1. The effect of treatment on meat pH

Replication	R-0	R-1	R-2	R-3	R-4	R-5
I	6.36	6.36	6.54	6.25	6.70	6.64
II	6.40	6.40	6.63	6.50	6.58	6.73
III	6.26	6.24	6.51	6.64	6.64	6.67
IV	6.30	6.56	6.20	6.73	6.60	6.76
Total	25.32	25.56	25.98	26.12	26.52	26.80
Average	6.34	6.39	6.495	6.53	6.63	6.70

Notes : (R-0) diet + 0% fermented feed supplement,
(R-1) diet + 5% fermented feed supplement
(R-2) diet + 10% fermented feed supplement
(R-3) diet + 15% fermented feed supplement
(R-4) diet + 20% fermented feed supplement and
(R-5) diet + 25% fermented feed supplement.

The means on pH of meat broiler has not significance, even the more fermented feed supplement, gave more pH and this results indicated that there is insignificantly, because the carcass has the same treatments and the pH was measured after the meat was boiled in 60^{o}C. The pH of meat plays important role in maintaining the meat quality. The thoughness of the meat occurs at low pH. In this treatment, the pH of the meat are between 6.34 – 6.70. The meat pH level rise as the fermented feed supplement percentage in the ration was higher. It means that the fermented feed supplement has effect on pH level. So there is an activity of fermented feed supplement towards the meat pH, even there is insignificantly on this treatment. In R-0 (diet without fermented feed supplement), the pH is 6.34, and the meat pH will increase when the level of fermented feed supplement more higher; in R-1(diet + 5% fermented feed supplement), the pH 6.39; and in R-2 (diet + 10% fermented feed supplement) the meat pH is 6.495; the R-3 (diet + 15% fermented feed supplement; meat pH is 6.53); R-4 (diet + 20% fermented feed supplement; the meat pH 6.63); and in R-5 has the highest meat pH is 6.70 (diet + 25% fermented feed supplement). This results are more better compared to the pH meat of culled layers are 5.63 (Sinku et al., 2002), and an ultimate pH near 5.7 as desirable for maintaining quality of poultry breast meat (Khan et al., 1970). The broilers that given fermented feed supplement which consists of solid coconut oil and tofu waste fermented with *Aspergillus niger* have more meat pH, compared to the broiler give no fermented feed supplement. The influence of diet on meat properties is minor importance if there are no nutritional deficiencies. The influence of diet on the physical properties of muscle, as long as no serious nutritional deficiencies, the feeding practice in ante mortem period which alters the quantity of glycogen stored in muscles can influence the ultimate physical properties of meat (Aberle et al., 2001).

The effect of treatment on meat tenderness

In Table 2, there are the results of fermented feed supplement in ration, to the broiler carcass tenderness.

Table 2. The effect of treatment in ration on meat carcass tenderness (mm/g/10 sec)

Replication	R-0	R-1	R-2	R-3	R-4	R-5
I	12.60	12.10	12.80	13.20	15.90	19.50
II	12.40	14.50	14.20	13.40	16.80	17.10
III	13.60	15.20	15.50	15.40	16.00	16.60
IV	14.50	14.70	17.20	19.30	18.90	21.10
Total	53.10	56.50	59.70	61.30	67.60	74.30
Average	13.275	14.125	14.925	15.325	16.90	18.575

Notes : (R-0) diet + 0% fermented feed supplement,
(R-1) diet + 5% fermented feed supplement
(R-2) diet + 10% fermented feed supplement
(R-3) diet + 15% fermented feed supplement
(R-4) diet + 20% fermented feed supplement and
(R-5) diet + 25% fermented feed supplement.

The meat tenderness values were between 13.275 mm/g/10 sec to 18.575 mm/g/10 sec. The highest meat tenderness (18.575 mm/g/10 sec) was get from the broiler that fed R-5 (diet + 25% fermented feed supplement) and the lowest (13.275 mm/g/10 sec) was get from the broiler that fed R-0 (diet + 0% fermented feed supplement). The meat tenderness was significantly better in the groups which consumed fermented feed supplement. The tenderness will increase when the fermented feed supplement in the ratio level percentage are higher. It means that the meat from broilers that given only ration without feed fermented supplement has the lowest tenderness. The more fermented feed supplement in the ration will results the more tenderness of the meat. In R-0 (diet without fermented feed supplement), the tenderness are 13.275 mm/g/10 sec, and the meat tenderness will increase when the level of fermented feed supplement more higher; in R-1 (5% fermented feed supplement), 14.125 mm/g/10 sec; and in R-2 (10% fermented feed supplement) the tenderness is 14.925 mm/g/10 sec; the R-3 (15% fermented feed supplement; meat tenderness 15.325 mm/g/10 sec); R-4 (20% fermented feed supplement; the tenderness 16.90 mm/g/10 sec); and in R-5 has

the highest tenderness (18.575 mm/g/10 sec, 25% fermented feed supplement). It means that the fermented feed supplement has influenced the meat tenderness. The influence of diet on meat properties is minor importance, if there are no nutritional deficiencies. The influence of diet on the physical properties of muscle, as long as no serious nutritional deficiencies, the feeding practice in ante mortem period which alters the quantity of glycogen stored in muscles can influence the ultimate physical properties of meat (Aberle et al., 2001).The broilers that given fermented feed supplement which consists of solid coconut oil and tofu waste fermented with *Aspergillus niger* have more tenderness meat, than the broilers only consumed normal diets. It means that the *Aspergillus niger* has an effect to the tenderness in the meat that feed fermented supplement (Lengkey et al., 2013).

CONCLUSIONS

The broilers that given fermented feed supplement which consists of solid coconut oil and tofu waste fermented with *Aspergillus niger* have more higher meat pH and more tenderness meat, than the broilers only consumed normal diets. It means that the *Aspergillus niger* fermented feed supplement has an effect to the pH and tenderness in the meat broiler that fed with fermented feed supplement. And using up to 25% of fermented feed supplement in the ration will increase the meat tenderness and the pH.

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RESEARCHES CONCERNING THE EFFECTS OF SUPPLEMENTARY FEEDING OF BEES FAMILIES DURING AUTUMN, WINTER, SPRING

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Abstract

The purpose of the paper is to track the production of honey, its chemical analysis and determination of the economic efficiency of supplementary feeding of bee families during the fall, winter and spring. Chemical analysis of honey indicated in most cases according to its characteristics, supplementary trace evidence of industrial products and is noticeable only at the four experimental batches, where they found traces of industrial glucose. Although experimental batches recorded higher output compared to the control batch, however, due to expenses related to supplementation, only 3 groups, namely those who received Apiinvert, Melisol and glucose-fructose syrup recorded higher profits of 9%, respectively 31.6% compared to the control batch, retrieving other lower profits, the lowest value being noticeable in the experimental batch that was used as a semisolid food supplement, which had a deficit of 11.52% compared to the control batch.

Keywords*:* bees, chemical analysis, honey, profit, supplementary feeding.

INTRODUCTION

The most important problem that beekeeper must solve for the bee family is to provide all the time food and its health. During an apian year there are periods during which bee pollen and nectar are missing from nature, the beekeeper being forced to interfere by feeding stimulation or supplementation (Warring et al., 2012). An early feeding stimulation can prepare the beehive to harvest the nectar or to allow proper preparation of the queen bee for laying (Czirjak et al., 2012). Sugar syrup or syrups made from fructose-glucose administered in small doses and frequencies will act as supplements to the family (Marghitas, 2008; Bura et al., 2005).

MATERIALS AND METHODS

The experiments were performed in its own apiary on a number of 35 bee families (6 frames per family) divided into 7 batches of 5 families, including a control batch and 6 experimental batches (Table 1).
The 6 experimental batches were given different energy supplements, as well as carbohydrate composition (Table 2) additional periods being, August 15 to September 15, respectively March 15-April 15 for the batches E2-E6 and September 1 - November 30, January 7 to April for the batch E1.

Table 1. Experimental scheme

Batch	Treatment	Number of frames average intervals	Season treatment	Following objectives
Control	15 kg honey	6	-	Evolution of honey production The economic efficiency of feed supplementation Determination of humidity and honey diastase index Dosage of hidroximetilfurfurol (HMF) Identification of industrial glucose
E1	15 kg honey + 13 kg semisolid food	6	01 Sept – 30 Nov 07 Jan – 15 Apr	
E2	15 kg honey + 13 kg Apiinvert	6	15 Aug – 15 Sept 15 Mar – 15 Apr	
E3	15 kg honey + 13 kg Dulcofruct	6	15 Aug – 15 Sept 15 Mar – 15 Apr	
E4	15 kg honey + 13 kg Melisol	6	15 Aug – 15 Sept 15 Mar – 15 Apr	
E5	15 kg honey + 13 kg Apimera	6	15 Aug – 15 Sept 15 Mar – 15 Apr	
E6	15 kg honey + 13 kg glucose-fructose syrup	6	15 Aug – 15 Sept 15 Mar – 15 Apr	

Table 2. The chemical composition of supplements used for experimental batches (% of dry matter)

Product	Glucose	Fructose	Sucrose	Maltose
Semisolid food*	8	12	80	-
Apiinvert	31	39	30	-
Dulcofruct	35	55	-	10
Melisol	50	45	-	5
Apimera	40	50	-	10
Glucose-fructose syrup	55	35	-	10

* The semisolid food was made by mixing 840 g sugar Farin (powder) and 160 g of polyfloral honey.

Taking the supplement consisted in the introduction of 1 kg from each product in very thin bags, they being introduced inside the beehive, over the bee family frames, the quantities being of 7 kg supplement in the first period and 6 kg in the second period.
The supplement doses were administered after previous dose consumption.

RESULTS AND DISCUSSIONS

The entire experimental period was followed both the degree of consumption of administered supplements, some features of honey (Table 3), the main harvest recorded productions, as well as the profit differences between the control and the experimental batches.
In terms of dry matter content, we find that the highest value (83.2%) was met at the control batch, while the lowest one at the experimental batch E4 (78.8), which denotes a difference of 5.6%. The other batches recorded intermediate values.
This HMF presence is within the limits shown in the specialized literature (up to maximum 4%), except for the experimental batch 6.

Table 3. Features characteristics of honey

Batch	Dry matter (%)	HMF (mg%)	Diastase index	Industrial glucose
M	83.2± 0.33	0.38± 0.04	13.9± 0.51	None
E1	81.5± 0.41	1.23± 0.09	11.2± 0.29	None
E2	79.2± 0.27	1.25± 0.08	10.9± 0.60	None
E3	80.3± 0.34	2.53± 0.12	6.5± 0.44	Present
E4	78.8± 0.50	3.81± 0.32	6.5± 0.41	Present
E5	81.2± 0.27	3.99± 0.36	6,5± 0.38	Present
E6	82.9± 0.39	5.13± 0.69	10.9± 0.52	Present

Diastase index values confirm the natural characteristic of honey, all the recorded values being above 6.5, being considered as minimum by specialized literature.
Industrial glucose is present under the form of traces only at batches E3-E6, lacking at the control batch and experimental batches E1 and E2.
By analyzing the obtained average honey production per family (Table 4), we find that the highest efficiency was at the experimental batch 6, which had a higher production with 18.1 kg compared to the control batch, 14.6 kg compared to the experimental batch 1, 8.2 kg compared to the experimental batch 2, 13.4 kg compared to the experimental batch 3, 9.6 kg compared to the experimental batch 4, 13.8 kg compared to the experimental batch 5.
Moreover, it can be noticed that all experimental batches recorded higher productions compared to the control batch.

Table 4. The honey production

Harvest	Control batch		E1 batch		E2 batch		E3 batch		E4 batch		E5 batch		E6 batch	
	Total/ batch	Average/ family	Total /batch	Average/ family	Total/ batch	Average/ family	Total/ batch	Average/ family	Total/ batch	Average/ family	Total/ batch	Average/ family	Total/ batch	Average/ family
Acacia 1	49.0	9.8± 0.34	60.5	12.1± 0.41	71.0	14.2± 0.39	58.4	11.7± 0.27	72.5	14.5± 0.34	61.6	12.3± 0.21	76.8	15.4± 0.72
Acacia 2	52.0	10.4± 0.54	57.0	11.4± 0.36	70.0	14.0± 0.22	57.1	10.4± 0.51	70.6	14.1± 0.22	61.5	12.3± 0.24	81.7	16.3± 0.85
Lime	42.0	8.4± 0.51	43.5	8.7± 0.20	47.5	9.5± 0.31	51.7	11.3± 0.58	49.0	9.8± 0.29	47.5	9.5± 0.20	58.6	11.7± 0.25
Sunflower	74.0	14.8± 0.66	73.5	14.7± 0.25	78.0	15.6± 0.33	73.6	14.7± 0.47	77.5	15.5± 0.31	68.1	13.6± 0.31	90.6	18.1± 0.25
Total	217.0	43.4± 1.89	234.5	46.9± 0.62	266.5	53.3± 1.08	240.8	48.1± 1.11	269.6	53.9± 1.35	238.7	47.7± 1.06	307.7	61.5± 1.05

Table 5. Income and expenses quantification

Batch	Honey production (kg)	Average price (lei/kg honey)	Income (lei)	Supplement administrated (kg)	Supplement price (lei/kg)	Expenses with supplementation (lei)	Profit (lei) % compared to the control
M	43.4	13	564.2± 24.62	-	-	-	564.2 100%
E1	46.9	13	609.7± 8.07	13	8.5	110.5	499.2 88.48%
E2	53.3	13	692.9± 14.03	13	6.0	78.0	614.9 109%
E3	48.2	13	626.6± 14.45	13	5.0	65.0	561.6 99.5%
E4	51.9	13	674.7± 17.59	13	4.5	58.5	516.2 109.2%
E5	47.7	13	620.1± 13.83	13	4.6	59.8	560.3 99.3%
E6	61.5	13	799.5± 13.68	13	4.4	57.2	742.3 131.6%

Table 6. The testing of the differences significance of followed characters

Specification	Honey production/family						Profit/family					
Batch	Control	E1	E2	E3	E4	E5	Control	E1	E2	E3	E4	E5
Control	-	IS	DS	S	DS	S	-	S	S	IS	S	IS
E1	IS	-	S	IS	S	IS	S	-	DS	S	DS	S
E2	DS	S	-	S	IS	S	S	DS	-	S	IS	S
E3	S	IS	S	-	S	IS	IS	S	S	-	S	IS
E4	DS	S	IS	S	-	S	S	DS	IS	S	-	S
E5	S	IS	S	IS	S	-	IS	S	S	IS	S	-
E6	VS	VS	S	DS	S	DS	VS	VS	DS	VS	DS	VS

IS –insignificant; S – significant; DS – distinct significant; VS – very significant

Although the experimental batches recorded higher productions compared to the control batch, however, due to expenses from the supplementation (Table 5), only 3 batches, namely E2, E4 and E6, recorded higher profits of 9%, respectively 31.6% than the control batch, the others bringing lower profits, the lowest value being noticeable at the experimental batch E1 (Table 6), which had a deficit of 11.52% compared to the control batch.

CONCLUSIONS

Following further feeding with different carbohydrate preparations of bee families the following facts have been observed:

1. Honey production for the batches which received energy supplements registered increases compared to the control batch, although different depending on the preparation used, as follows:

- Syrup made up of glucose and fructose determined an increase in the production of 41.7% compared to the control batch, being the closest (15.4%) for this indicator to the batch that received as supplement product Apiinvert;

- The weakest experimental results were recorded at the experimental batch E1, followed by the experimental batch E5, at which the differences from the control batch were of 8.1% and 9.9%

2. In terms of profits obtained from the sale of production it can be found that it was registered only for half of the experimental batches, the others recording lower values that the control batch.

3. The chemical analysis of honey indicated in most cases its characteristics of conformity, evident traces of supplementary with industrial products, this being noticeable only at the four experimental batches, where industrial glucose traces were found.

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ALTERATION IN RUMINAL FERMENTATION: THE EFFECT OF *MEGASPHAERA ELSDENII* INOCULATION ON SUBACUTE RUMINAL ACIDOSIS (SARA) *IN VITRO*

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Abstract

SARA is a serious herd problem in intensive dairy and beef operations because of triggering other metabolic disorders and causing lactation-fertility losses. SARA was induced in vitro to evaluate the effectiveness of Megasphaera elsdenii inoculation. Rumen fluid was collected from 2 ruminally cannulated Holstein heifers. Medium was prepared by mixing macromineral (200 ml), micromineral (0.1 ml), buffer (200 ml), reduction (40 ml) and resazurin (1 ml) solutions as well as distilled water (400 ml). The media were then added with 1) a test diet consisting (g/kg) of 550-soluble starch, 260-glucose, 60-cellulose, 70-cellobiose and 60-tripticase, 2) ground wheat and 3) ground corn, at levels of 10, 15, 20, 25, 30, 40, 50, 70 and 100 g/l. After determining their levels causing SARA as reflected by pH (~5.3) in preliminary experimentation, the substrates (test diet, 40 g/l; wheat, 30 g/l; corn, 50 g/l) were incubated with presence of 0, 10^5, 10^7, and 10^9 cfu M. elsdenii per ml at 39°C for 24 h. Rumen parameters were analyzed by 2-way ANOVA. There was substrate, but not inoculum level and substrate by inoculum interaction effects on measurements. The data confirm that increasing level of starch-rich feedstuffs leads to acidosis as reflected by decreased pH and the Ac:Pr ratio and increased lactate concentration. However, addition of M. elsdenii into media, one of the predominant lactate-utilizing bacteria failed to reverse SARA in vitro.

Keywords*: subactute ruminal acidosis, Megasphaera elsdenii inoculation, rumen fermentation, in vitro.*

INTRODUCTION

Introducing high energy diet after parturition as well as low absorptive capacity of rumen epithelium increase risk for subacute ruminal acidosis (SARA). About 97% of lactate resulting from starch fermentation is utilized by *Megasphaera elsdenii* (Piknova et al., 2004). Both *in vivo* and *in vitro* studies ascertained critical role of *M. elsdenii* in preventing lactic acid accumulation during transition to high-grain diet (Greening et al., 1991; Kung and Hession, 1995). *M. elsdenii* is reported to reduce adaptation period by 5-7 d to high-grain diet when introduced gradually (Klieve et al., 2003).

Kung and Hession (1995) reported that inoculation of 10^5 and 10 *M. elsdenii* cfu per ml *in vitro* elevated pH and reduced lactate concentration as compared to non-inoculation. They also reported that lactate concentration was <5mM during incubation with *M. elsdenii*. Greening et al. (1991) also showed that *M. elsdenii* inoculation increased pH and decreased lactic acid concentration in beef subjected to experimentally induced acidosis. Lactic acid-utilizing bacteria (*M. elsdenii* and *Selenomonas ruminantium*), alone or in combination alleviates adverse effect of rapidly fermentable carbohydrate introduction by slowing down pH reduction and lactate accumulation (Nocek et al., 2002; Wiryawan and Brooker, 1995). This experiment was carried out to demonstrate the effect of *M. elsdenii* on rumen fermentation characteristics in SARA *in vitro*.

MATERIALS AND METHODS

Prior to morning feeding rumen fluids were collected from 2 ruminally cannulated Holstein heifers. In pressure-resistant Pyrex tubes, test diet (550 g soluble starch + 260 g glucose + 60 g cellulose + 70 g cellobiose +60 g tripticase),

wheat, and corn were mixed at different amounts in 20 ml rumen fluid and 30 ml buffer at 39°C for 20 h. pH and lactic acid concentration were determined (Sung et al., 2004) to assess amounts of substrates necessary to induce acidosis *in vitro*.

Test (40 g/l), wheat (30 g/l), and corn (50 g/l) diets were mixed with 20 ml rumen fluid and 30 ml buffer. Then, media were added with 0, 10^5, 10^7, and 10^9 cfu/ml *M. elsdenii*. Gas production, VFA, lactic acid, and NH_3-N, and pH were measured at 2, 4, 6, 8, 10, 12, and 24 h relative to incubation. Using Real-Time PCR *S. bovis* and *M. elsdenii* were counted. Amount of gas was calculated based on pressure, which was determined by digital manometer (with sensitivity of 0.2%; Keller Leo 1, Switzerland), in 100 ml bottle (Lopez et al., 2007).

The linear model included the effect of substrate, day, and sampling time as well as their interaction in data analysis using one-was ANOVA (SAS, 2002). Significance was declared at $P<0.10$.

RESULTS AND DISCUSSIONS

All rumen response variables are summarized in Table 1. pH decreased in test diet, whereas remained unchanged in wheat and corn diets. Lactic acid concentration was lower for test diet than for others. In SARA lactate-utilizing bacteria convert it to VFA (Nagaraja and Chengappa, 1998). Lactic acid concentration is 0.17-0.74 m*M* in SARA (Chıquette, 2009; Fulton et al., 1979, Oetzel et al., 1999; Plaizier et al., 1999). That is, there was no lactic acid accumulation in media. There was *M. elsdenii* inoculation effect on pH and lactic acid concentration.

NH_3-N concentration was the highest in wheat diet, and it was not affected by *M. elsdenii* inoculation. Gas production varied by the substrate, but not *M. elsdenii* inoculation. Substrate type affected total VFA and VFA profiles. However, *M. elsdenii* inoculation did not alter fermentation profile. Media containing wheat and corn diets as substrates had the highest number of *S. bovis* and *M. elsdenii*, respectively.

Table 1. Responses of rumen parameters to addition of *M. elsdenii* (10^{power}/ml) into media containing different substrates.

Trt*		Response variables										
S	I	*M. elsdenii* (log/ml)	*S.* bovis (log/ml)	pH	N-NH_3 (m*M*)	Lactate (m*M*)	Gas (ml)	Ac (%)	Pr (%)	Bu (%)	ΣVFA (m*M*)	Ac:Pr
TD		8.88^{ab}	9.49^{b}	5.93^{b}	7.2^{b}	0.90^{a}	50.3^{c}	32.0^{a}	30.4^{a}	21.1^{b}	192^{b}	1.19^{b}
WD		8.70^{b}	11.43^{a}	6.14^{a}	10.3^{a}	0.69^{b}	63.2^{a}	28.2^{b}	27.5^{b}	29.0^{a}	224^{a}	1.05^{b}
CD		9.18^{a}	9.12^{b}	6.15^{a}	7.4^{b}	0.57^{c}	60.8^{b}	31.9^{a}	26.1^{b}	28.6^{a}	211^{a}	1.47^{a}
	0	8.86	9.87	6.10	8.2	0.70	58.4	30.5	27.9	26.5	220	1.14
	5	8.96	10.21	6.10	8.6	0.71	59.7	30.2	28.0	27.2	210	1.23
	7	8.88	10.03	6.11	8.6	0.69	59.2	30.8	27.5	26.6	207	1.30
	9	8.90	9.97	6.09	8.5	0.68	59.8	30.6	27.1	27.8	209	1.32
TD	0	9.08	8.60	5.95	7.0	0.90	50.5	32.5	30.5	20.8	196	1.09
	5	8.52	9.98	5.93	7.3	0.90	50.5	31.6	30.3	21.0	189	1.08
	7	9.03	9.72	5.91	7.3	0.89	50.1	33.2	31.0	20.8	191	1.10
	9	8.93	9.60	5.93	7.4	0.93	50.0	30.6	29.8	21.9	193	1.50
WD	0	8.51	11.31	6.12	10.2	0.70	61.1	27.3	28.3	28.7	251	1.03
	5	9.16	11.53	6.16	10.5	0.71	65.3	28.0	27.3	29.5	221	1.03
	7	8.32	11.20	6.15	10.4	0.69	62.8	28.0	26.8	28.8	213	1.06
	9	8.74	11.72	6.15	10.2	0.64	63.5	29.4	27.6	29.1	211	1.09
CD	0	9.04	9.60	6.17	7.0	0.58	60.2	32.3	26.1	27.7	205	1.27
	5	9.16	8.89	6.15	7.6	0.58	59.5	31.7	27.4	28.6	210	1.53
	7	9.34	9.17	6.18	7.6	0.56	61.2	32.0	25.9	28.1	211	1.66
	9	9.14	8.84	6.12	7.6	0.57	62.0	31.7	25.0	30.0	216	1.43
SEM		0.26	0.39	0.12	0.4	0.06	8.1	1.1	1.0	1.4	15	0.13
Effect		----------					*P* > F	----------				
S		0.04	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.004	0.002
I		0.99	0.84	0.82	0.36	0.94	0.82	0.78	0.72	0.23	0.61	0.55
S x I		0.12	0.24	0.23	0.96	0.93	0.72	0.30	0.72	0.91	0.39	0.67

*Trt = treatment; S = substrate; I = inoculant.

TD = test diet, 40 g/l (550 g soluble starch + 260 g glucose + 60 g cellulose + 70 g cellobiose +60 g tripticase)/kg. WD = wheat diet (30 g/l), CD = corn diet (50 g/l).

CONCLUSIONS

Wheat favored *S. bovis* growth and corn favored growth *Elsdenii* growth. Both feedstuffs did not affect medium pH and had low lactic acid concentration in medium as compared to test mixture. *M. elsdenii* inoculation did not affect fermentation parameters in media containing test diet, wheat diet, and corn diet.

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EFFECT OF THE DIETARY DRY GRAPE POMACE ON THE PERFORMANCE AND HEALTH STATE OF FATTENING STEERS

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Abstract

Potential source of nutrients and also a strong antioxidant, grape pomace is currently in the focus of the animal scientists. This 3 months trial used 14 Romanian Spotted fattening steers, with an average initial weight of 256 kg, assigned uniformly to two groups. The groups differed by the absence or presence of dry grape pomace in the compound feed, next to the bulk forage, alfalfa haylage. Feed intake of the two groups was similar both as concerns the bulk feed and as concerns the compound feed. The weight gain was also similar, 1306 g/steer/day in the control group (no dry grape pomace in the compound feed) and 1301 g/steer/day in the experimental group (20% dry grape pomace which replaced most of the barley used for the control group). The replacement of barley by dry grape pomace in the compound fed for fattening steers didn't have a significant influence on feed conversion ratio and feeding efficiency; no health problems were noticed either, as shown by the plasma profile of the experimental animals.

Key words: *steers, alfalfa haylage, dry grape pomace, feed intake, weight gain, feed conversion, biochemical profile*

INTRODUCTION

The dry grape pomace is one of the supplemental, or newly identified feed sources, with less known feeding potential that can be fed to ruminants. The dry grape pomace is a by-product from wine making, or from table grape processing. In a dry form, it can be preserved for a longer period, just like the hay Şerban, 2013); from a ton of fresh grape pomace result about 140-150 kg dry grape pomace (M. Bahcivanji et al., 2012), which is consumed with pleasure by the animals due to its sourish taste.

It appears that the volume of research on the dietary dry grape pomace is limited, both in Romania and worldwide, regarding the efficiency of nutrient utilization, the economics of feeding dry grape pomace and the health state of the animals treated with dry grape pomace as supplemental feed for ruminants.

The dry grape pomace is a true "mine" of resveratrol, one of the most powerful antioxidants identified so far, which has strong therapeutic properties. Resveratrol is found mainly in the skin and pulp of the red grapes. It is regarded as the most efficient natural cardiovascular protector, being 50 times more powerful than vitamin E. Recent studies conducted by researchers at Harvard University, USA, show that resveratrol, the magic antioxidant, changes considerably the quality and length of life. These scientific facts support the fact that grape pomace, a by-product from grape juice extraction, is actually a medicine with major health benefits.

The variation of the blood biochemical parameters can be used (Jain, 1986; Bush, 1991; Awah and Nottidge, 1998) to estimate the nutritional, physiological and pathological state of the animals; it can also be used to determine the influence of feeding, age, sex, housing, environment, stress and transportation on the animals both in tropical and in temperate regions (Ogunrinade et al., 1981; Bush, 1991; Ogunsanmi et al., 1994).

The grape pomace is given to animals mixed with other forages; its feeding value is rather modest, about half of the feeding value of the hay and it has a high level of hardly-digestible matter (Şerban, 2013). Starting from these facts, this experiment aimed to determine the effects produced by the dietary grape pomace given to fattening steers on animal performance and health state.

MATERIALS AND METHODS

The trial was conducted on Romanian Black Spotted (BNR) fattening steers with an average initial weight of 256 kg, assigned uniformly to a control group and an experimental group with

7 steer each. The diets were formulated according to the new IBNA system (Burlacu et al. 1991; 2002) of feeding value evaluation, and they were designed to have similar energy and protein levels. The diets consisted of alfalfa haylage as single bulk forage and a concentrate feed made of corn, barley, sunflower meal, monocalcium phosphate and salt (control diet). 82% of the barley was replaced by dry grape pomace in the formulation for the experimental group. A specific premix was used in both diets, adequate to the species and weight category, which provided a balanced supply of vitamins and minerals. Table 1 shows the compound feeds formulations.

Table 1. Compound feeds formulation (%)

Specification	Control	Experimental
Corn	33.00	40.00
Barley	24.50	-
Wheat	27.00	26.50
Dry grape pomace	-	20.00
Sunflower meal	12.00	10.00
Monocalcium phosphate	1.00	1.00
Salt	1.50	1.50
Premix	1.00	1.00
Total	100.00	100.00

The animals were housed in a fattening house for steers, in collective stalls with slatted concrete floors and with a feeding alley. Feed intake and the leftovers were measured. The animals had free access to the water supplied by constant level drinkers.

The experiments run for 91 days, which included a period of accommodation of the animals with the new diet formulations. During the actual experimental period we monitored daily the feed intake and the body weight (the animals were weighed in the beginning and at the end of the trial), and we calculated the average daily feed intake, feed conversion ratio and the economic efficiency of feeding.

At the end of the trial we collected blood samples from the jugular vein from the entire group of animals. The blood samples were assayed for blood sugar, cholesterol, triglycerides, DHL, LDL, total protein, albumin, total bilirubin, creatinine, urea, alkaline phosphatase, gamma globulin, LDH, creatine kinase, calcium, phosphorus, magnesium and iron, to determine the health status of the animals. All biochemical assays were performed with a semiautomatic **BS-130 Chemistry analyser** (Bio-Medical Electronics Co., LTD, China).

All data concerning animal performance and the biochemical assays were processed statistically; STAT VIEW: Anova and T Test were used to determine the significance of the differences between groups.

RESULTS AND DISCUSSIONS

The chemical composition of the feed ingredients was determined with the modified Weende design (Criste et al., 2003). The alfalfa haylage had 526 g DM, 121 g CP, 357 g CF and 17.39 MJ GE per 1000 g DM. The compound feed ingredients (Table 2) had the following chemical composition (g/kg DM): protein – 88 g for corn, 104 g for barley, 124 g for wheat, 158 g for the dry grape pomace and 358 g/kg DM for the sunflower meal; crude fibre – 35 g for corn, 85 g for barley, 36 g for wheat, 292 g for the dry grape pomace and 229 g/kg DM for the sunflower meal. Research by Bahcivanji et al., 2012, show quite similar values with our findings for the chemical composition of the dry grape pomace for most nutrients. Comparable values were also reported in the recent paper by Coşman et al., 2012.

The chemical composition of the compound feed which included 20% dry grape pomace had several difference in the organic components compared with the control compound feed. These differences were due to the different amount of barley (24.50%) in the formulation of this compound feed, as shown in the table below.

Table 2. Chemical composition of the feed ingredients and of the compound feed (g/kg feed/1000 g DM)

Item	DM	OM	CP	EE	CF	NFE	Crude ash	GE (MJ)
Alfalfa haylage	526	473	64	9	188	212	53	9.17
	1000	899	121	17	357	404	101	17.39
Corn	850	837	75	29	30	703	13	15.50
	1000	985	88	34	35	828	15	18.23
Barley	890	867	93	15	76	683	23	15.92
	1000	974	104	17	85	768	26	17.89
Wheat	908	888	113	20	33	722	20	16.41
	1000	978	124	22	36	796	22	18.07
Dry grape pomace	812	735	128	27	237	77	343	14.56
	1000	905	158	33	292	95	422	17.93
Sunflower meal	890	826	319	15	204	288	64	17.01
	1000	928	358	17	229	324	72	19.11
Compound feed, control	922	881	126	16	93	646	41	16.43
	1000	956	137	17	101	701	44	17.83
Compound feed, experimental	928	886	108	20	110	647	42	16.55
	1000	955	116	22	119	697	45	17.83

The chemical composition date were used to calculate the feeding value of the feed ingredients and of the compound feeds (with and without dry grape pomace), expressed in meat feed units (mFU), IDPN (intestinally digestible protein allowed by nitrogen supply), IDPE (intestinally digestible protein allowed by energy supply), calcium (Ca) and phosphorus (P), as shown in Table 3. The energy and protein feed values of the studied forages and of the compound feeds are generally within the range of values admitted by the literature (Burlacu et al., 2002).

Table 3. Calculated feeding values of the dietary ingredients and of the compound feeds

Item	DM (g/kgDM)	mFU /kg DM	IDPN (g/kg DM)	IDPE (g/kg DM)	Ca (g/kg DM)	P (g/kg DM)
Alfalfa haylage	526	0.62	77	70	12.38	3.75
Corn	850	1.54	70	121	1.02	4.48
Barley	890	1.26	67	90	1.02	4.39
Wheat	908	1.54	82	103	1.02	5.01
Dry grape pomace	812	0.78	102	88	7.35	3.84
Sunflower meal	890	0.79	231	119	4.03	12.61
Monocalcium phosphate	900	-	-	-	380	-
Salt	900	-	-	-	-	-
Premix	900	1.3	80	100	0.20	2.40
Compound feed. control	922	1.27	94	107	7.89	8.49
Compound feed. experimental	928	1.16	80	98	8.93	7.93

Throughout the experimental period we measured on a daily basis the feed intake and the leftovers for each group of animals, expressed both in gross kg, and in dry matter, thus showing the proportion of the compound feed (Table 4). The feed intake was rather similar in both groups, both as proportion of the bulk feed (alfalfa haylage) and of the concentrate feed (CF) taken as such or as dry matter. For instance, the intake of alfalfa haylage (free access) was 11.56 kg/steer/day for group C and 12.49 kg for group E, or 6.08 and 6.57 kg DM/steer/day, respectively. The differences were not statistically significant, as shown in the table below.

Table 4. Average daily feed intake (kg/steer/day and kg DM/steer/day)

Item	Romanian Black Spotted steers	
	Control	Experimental
Alfalfa haylage – gross	11.56	12.49
Compound feed – gross	3.74	3.76
Alfalfa haylage – DM	6.08	6.57
Compound feed – DM	3.46	3.48
Total DM	9.54	10.05
Bulk forage of the total DM (%)	63	65
Compound feed of the total DM (%)	37	35

The estimated feeding values and the feed intake were used to determine the daily supply of major nutrients (energy, protein, calcium and phosphorus) and how much were they supplied related to the feeding norms; this was estimated according to the average body weight, average daily weight gain and nutrient supply of the diets (Table 5). There were very small differences between groups in terms of energy and protein supply. Thus, the animals from the control group consumed in average on a daily basis 8.16 mFU, 780.40 g IDPN and 750.95 g IDPE, while the animals from the experimental group consumed in average on a daily basis, 8.11 mFU, 810.17 g IDPN and 779.88 g IDPE. This shows that the energy supply of the diet met the energy requirement of the animals from the two groups (C and E) in a very similar proportion (102.15 – 102.79%); this was valid for too IDPN (115.44 – 119.85%) and IDPE (111.09 – 115.37%).

On the other hand, the situation was different concerning the supply of calcium and phosphorus salts, which increased (not significantly, however) in both groups, oscillating between 100.23% and 113.78% for calcium, and between 117.75 and 118.41% for phosphorus, but in general, the results fall within the range of values reported by the literature.

Table 5. Nutrient supply of the diets and how much of the requirement was met

Item	mFU	IDPN (g)	IDPE (g)	Ca (g)	P (g)
Control					
Dietary supply	8.16	780.40	750.95	56.03	38.24
Requirement	7.94	676	676	52.91	32.29
Supply/requirement (%)	102.79	115.44	111.09	100.23	118.41
Experimental					
Dietary supply	8.11	810.17	779.88	60.20	38.02
Requirement	7.94	676	676	52.91	32.29
Supply/requirement (%)	102.15	119.85	115.37	113.78	117.75

The recorded feed intakes were used to calculate the average daily weight gains, which were similar for both groups (1306.12 g for group C and 1301.41 g for group E). Table 6 shows that animal performance resembled in both groups (group C with no dry grape pomace treatment and group E where the 20% dry grape pomace replaced completely the barley.

Table 6. Body weight gain and average daily weight gain*

Item	Romanian Black Spotted steers	
	Control	Experimental
Average initial weight (kg)	250.86±38.98	255.00±23.22
Average intermediary weight	306.43±44.49	313.14±23.59
Average final weight (kg)**	369.714±35.76	373.86±23.27
Total gain (kg/steer)	118.85	118.43
ADG (g/steer)**	1.306.12[a]±136.80	1.301.41[a]±66.83

*means plus standard deviation

** non-significant differences between groups (P≥0,05)

Feed conversion, correlated with animal performance, expressed in dry matter (DM) meat feed units (mFU), IDPN (intestinally digestible protein allowed by nitrogen supply), IDPE (intestinally digestible protein allowed by energy supply), shows that the experimental group used slightly higher amounts of DM (7.54 kg), mFU (6.26), IDPN (621.00 g) and IDPE (601.00 g) compared to the control group (7.13, 6.18, 593.00 and 571.00, respectively), which means that the experimental group made a poorer use of the dietary energy and protein to make one kg of gain, compared to the control group, as shown in Table 7.

Table 7. Feed conversion ratio

Item	Romanian Black Spotted steers	
	Control	Experimental
Kg DM/kg gain	7.13	7.54
mFU/kg gain	6.18	6.26
g IDPN/kg gain	593.00	621.00
g IDPE/kg gain	571.00	601.00

Analysing the expenditure with animal feeding, expressed in lei/steer/day and in lei/kg gain (Table 8) we may notice that the diet with dry grape pomace didn't improve feed conversion ratio and didn't make the conversion more efficient. The cost of feeds per kg gain is just 0.9% higher in the experimental group compared to the control group, correlated with a slightly lower average daily weight gain and, implicitly, a slightly higher feed conversion ratio in the experimental group, but with no significant difference, however.

Table 8. Feeding cost

Item	Romanian Black Spotted steers	
	Control	Experimental
Lei/animal/day	3.334	3.352
Lei/kg gain	2.553	2.576
Cost lei/kg gain - % compared to the control	100	100.9

The health state of the animals, shown by the blood plasma concentration of glucose, cholesterol, triglycerides, HDL and LDL, shows normal values for the particular species and category of production. Statistically, as shown in Table 9, the differences are significant ($P \leq 0.05$), both the cholesterol and for the HDL (high density lipoproteins, the "good" cholesterol), with higher levels for the dry grape pomace treatment. These differences are due to the structural particularities of the fat from the dry grape pomace, also associating the fact that a higher amount of dietary fat leads to a higher level of serum cholesterol (Abrams, 1980). On the other hand, the differences for the values recorded for glucose and triglycerides are not significant ($P \geq 0.05$). Highly significant ($P \leq 0.001$) differences were noticed for the LDL (low density lipoproteins, the "bad" cholesterol), the control group recording higher values than the experimental group, but, nevertheless, within the normal limits allowed for this species of animals.

Table 9. Plasma energy profile of the fattening steers*

Parameters	Control	Experimental (grape pomace)	Value of P
Glycaemia (mg/dL)**	69.94 ± 12.71	71.70 ± 7.91	0.7557
Cholesterol (mg/dL)***	86.73 ± 23.84	129.59 ± 29.91	0.0118
Triglycerides (mg/dL)**	13.64 ± 5.30	10.01 ± 1.80	0.1119
HDL direct (mg/dL)***	46.57 ± 16.55	73.57 ± 16.00	0.0091
LDL (mg/dL)****	38.29 ± 2.16	17.82 ± 8.45	0.0001

*means and standard deviation
**not significant differences between groups ($P \geq 0.05$)
***significant differences between groups ($P \leq 0.05$)
****highly significant differences between groups ($P \leq 0.001$)

Table 10 shows that the plasma protein profile (total protein, albumin, total bilirubin, creatinine and urea) falls within the range of normal physiological values for these parameters and for the category of the experimental animals, being very similar with

the results reported by Kaneko et al., 1997. Only for urea the difference is significant (P≥0.05), with higher values for the experimental group compared to the control group, knowing that the concentration of serum urea can be influenced by the diet formulation too (Royakkers, 2011).

Table 10. Plasma protein profile of the fattening steers *

Parameters	Control	Experimental (grape pomace)	Value of P
Total protein (g/dL)**	6.76 ± 1.57	6.99 ± 0.72	0.7231
Albumin (g/dL)**	3.80 ± 0.50	4.11 ± 0.33	0.1974
Total bilirubin (mg/dL)**	0.35 ± 0.12	0.36 ± 0.11	0.8044
Creatinine (mg/dL)**	1.01 ± 0.35	1.18 ± 0.21	0.2694
Urea (mg/dL)***	13.71 ± 4.27	20.14 ± 3.19	0.0077

*means and standard deviation
**not significant differences between groups (P≥0.05)
***significant differences between groups (P≤0.05)

Plasma mineral profile. The parameters of the plasma mineral profile generally range within the normal limits both for the control and for the experimental group. Thus, the concentration of serum calcium is within the normal physiological limits for the age and weight category of the animals in both groups. The concentration of serum magnesium, however, exceeded the normal physiological limits (1.5- 2.9 mg/dL) reaching the value of 4.00 mg/dL in the control group, possibly due to the dry grape pomace, existing a trend to influence the reference values (P>0.05 up to 0.10). Knowing that in the young animals, the bones are more vascularized and the mineral exchange is more intense, with higher values of the plasma magnesium, we think that this is the situation from our experiment, as shown by the table below. The plasma phosphorus concentration ranged within the normal physiological range for both groups of animals, with slightly (but not statistically significant) higher values for the control group. The serum iron concentration too falls within the normal physiological values for the age and category of animals in both groups, which confirms that iron acts synergistically with magnesium.

Table 11. Plasma mineral profile of the fattening steers *

Parameters	Control	Experimental (grape pomace)	Value of P
Calcium (mg/dL)**	9.72 ± 3.45	10.25 ± 1.20	0.7100
Phosphorus (mg/dL)**	6.47 ± 1.54	7.34 ± 0.56	0.1829
Magnesium (mg/dL)***	1.94 ± 0.26	4.00 ± 2.56	0.0555
Iron (ug/dL)**	1489.53 ± 170.89	1413.79 ± 238.90	0.5080

*means and standard deviation
**not significant differences between groups (P≥0.05)
***P>0.05 up to 0.10 = trend to be influenced

Plasma enzyme profile. The common characteristics of the enzymatic compounds are rather variable because of the instability of the blood biochemical indicators, as shown by Şogorescu et al., 2008. However, the concentration of transaminases (alkaline phosphatase, Gama GT and creatine kinase), indicators of the liver function, revealed normal values for the category of steers used in the experiment. In this case too, same as with the magnesium concentrations, there is a trend of influence on the reference values (P>0.05 to 0.10) for the alkaline phosphatase in the group treated with dry grape pomace (Table 12).

Table 12. Plasma enzyme profile of the fattening steers*

Parameters	Control	Experimental (grape pomace)	Value of P
Alkaline phosphatase (U/L)***	67.94 ± 28.90	93.77 ± 22.61	0.0872
Gama GT (U/L)**	14.84 ± 6.07	13.54 ± 3.36	0.6292
Creatine kinase (U/L)**	397.14 ± 177.66	381.71 ± 154.59	0.8653

*means and standard deviation

**not significant differences between groups (P≥0.05)

***P>0.05 up to 0.10 = trend to be influenced

CONCLUSIONS

- The inclusion of 20% dry grape pomace in the compound feeds for fattening steers, replacing most of the barley (82%) used for the control group, didn't influence CF intake or the total feed intake (compound feeds + alfalfa haylage). The resulting weight gains were rather similar in the control (no grape pomace) and the experimental (dry grape pomace treatment) groups, the differences not being statistically significant (P≥0.05): 1306 g/steer/day for the control group and 1301 g/steer/day for the experimental group.
- The replacement of the barley by dry grape pomace in the compound feeds for fattening steers didn't yield any significant differences in terms of feed conversion ratio, feeding efficiency and it didn't cause any health problems in the experimental animals, as shown by the determinations of the biochemical parameters from the blood plasma of the animals.

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THE EFFECT OF SUBSTITUTION OF FISH MEAL BY BLACK SOLDIER FLY(*Hermetia illucens*) MAGGOT MEAL IN THE DIET ON PRODUCTION PERFORMANCE OF QUAIL *(Coturnix coturnix japonica)*

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Abstract

Black Soldier Fly (BSF) maggot is the larvae of a fly Hermitia illucens, which hatch in four days, very well used as a source of protein feed ingredients for poultry and could be used to substitute fish meal (FM) where still a lot of imported. BSF maggot meal contains 46.58% crude protein, crude fiber 4.32%, 23.56% crude fat and metabolizable energy 3457 kcal/kg and the amino acid profile has similarities with the amino acid profile of fish. This experiment was carried out to study the effect of fish meal substitution meal by black soldier fly (Hermetia illucens) maggot meal in the diet on quail (Coturnix coturnix japonica) production performance. One hundred female Japanese quails at 6 weeks of age were raised in cages until 20 weeks old. The treatments were 5 types of diets, i.e., R_0 (100% FM), R_1(75% FM + 25% BSF), R_2 (50% FM + 50% BSF), R_3 (25% FM + 75% BSF) and R_4 (100% BSF). Completely Randomized Design (CRD) was used 5 treatments, replicated four times, and if there are any significant effect then followed by orthogonal contrasts test. The results showed that treatment substitution of fish meal (FM) protein with maggot meal (BSF) protein in quail diet was significant effect on feed consumption, feed conversion and egg weight but was no significant effect on egg production. The average consumption of R_4 treatment (100 % BSF maggot level) was lower than the R_0 treatment (100% FM level) in production of quail egg. This indicated that black soldier fly (BSF) maggot meal can be used to alternative protein source of feedstuff to substitute fish meal protein in quail diet.

Keywords: *Fishmeal, black soldier fly maggot meal, egg production, Coturnix coturnix japonica.*

INTRODUCTION

Fish meal is a conventional source of animal protein in poultry feed, because it has been valued for its balanced amino acids, vitamin content, palatability and growth factors. The use of fish meal as a source of animal protein ranged from 10-15%, or one-third of the protein ration (Anggorodi, 1985; Amrullah, 2003). However, the constraint on the use of fish meal as the feed material is evaluable commodity imported in puts that are relatively expensive. Such conditions, it would be a threaten to the poultry industry facilities especially feed meal production.

Dependence on imported feed ingredients need to search for alternative sources ingredients are cheap and do not compete with humans and nutritional content worth transform and utilized. Black soldier fly (BSF) maggot is the larvae of a fly *Hermetia illucens*could be used as an excellent source of protein feed ingredients for fish meal substitute a lot recently imported. Black Soldier fly maggot (*Hermetia illucens*), which are larvae from housefly, grow easily on poultry droppings or any organic waste and the larvae matured within in a short period of 3 to 4 days and were harvested, dried and milled to form maggot meal (Olivier, 2000; ESR, 2009).

BSF maggot meal contains 46.58% crude protein, 4.32% crude fiber, 23.56% crude fat, 2.39% calcium, 1.03% phosphorus, and 3457 kcal/kg metabolizable energy (Science and Technology Laboratory, IPB, 2008), and the amino acid profile are similarities with the amino acid of fish meal profile (Newton et al, 2009; Gunawan, 2012). BSF maggot meal turns lower in protein than fish meal protein (46.58% vs. 60%). Thus, if fish meal partially substituted by maggot meal the nuse in the composition of the ration will be effectively. According Akpodiete et al. (1998) concluded that maggot meal can nutritionally and productively replace fish meal in layer diet without adverse consequences on performance

and egg quality characteristics. Awoniyi et al. (2003) reported that the performance of broilers was not affected by treatment ration substituting fish meal by maggot meal. Agunbiade et al. (2007) reported that maggot meal can substitute a 50% fish meal and did not have negative effect on egg production and shell thickness. While Okah and Onwujiariri (2012) showed that the replacement of a 4% dietary fish meal in finisher broiler chickens diet with 50% maggot meal showed superior performance characteristics to the basal diet, and also proved to be more economically option.

Therefore, to evaluate the protein quality of maggot meal must be tested against the test object, and quail are highly responsive animal experiments. This study aimed to evaluate the replacement of maggot meal value for fish meal in quail diets on quail production performance (*Coturnix coturnix japonica*).

MATERIALS AND METHODS

One hundred female Japanese quails at 6 weeks of age were raised in cages until 20 weeks old, with the average of body weight was 117 grams (Coefficient variance 7.41%). The birds kept in cage system, as much as 20 cages, and each cage consisted of 5 quails. The ration was made by corn meal, fish meal, rice bran, soybean meal, bone meal, $CaCO_3$, maggot meal and premix as additive feed in 20 percent protein and 2900 kcal/kg of metabolizable energy (NRC, 1994). The composition, nutrient and metabolizable energy contents are showed in Table 1 and Table 2. The experiment rations were:

R_0 Ration contained 100% fish meal
R_1 Ration contained 75% fish meal and 25% maggot meal
R_2 Ration contained 50% fish meal and 50% maggot meal
R_3 Ration contained 25% fish meal and 75% maggot meal
R_4 Ration contained 100% maggot meal

Completely Randomized Design (CRD) was used with 5 treatments, and each treatment was replicated 4 times. The data were analyzed by using analysis of variance and, if there are any significant effects, then followed by orthogonal contrasts test. The analyzed variable were feed consumption, egg weight, egg production (quail day) and feed conversion.

Table 1.Composition of the formula rations (%)

Ingredients	**Ration**				
	R0	**R1**	**R2**	**R3**	**R4**
Yellow corn meal	53.0	50.0	50.0	52.0	51.0
Soy-bean meal	21.0	22.0	23.0	22.0	23.0
Rice bran meal	10.0	12.0	11.0	10.0	10.0
Fish meal	10.0	7.5	5.0	2.5	0
Maggot meal	0	2.5	5.0	7.5	10.0
Bone meal	2.0	2.0	2.0	2.0	2.0
$CaCO_3$	3.5	3.5	3.5	3.5	3.5
Premix	0.5	0.5	0.5	0.5	0.5
Total	100.00	100.00	100.00	100.00	100.00

Table 2. The nutrient and metabolism energy content in the rations

Nutrients	**R0**	**R1**	**R2**	**R3**	**R4**
Crude Protein (%)	20.26	20.51	20.67	20.09	20.27
Crude Fat (%)	6.10	6.39	6.70	7.13	7.51
Crude Fiber (%)	4.90	5.11	5.10	5.30	5.20
Calcium (%)	2.26	2.77	2.24	2.30	2.31
Phosphorus (%)	0.69	0.69	0.69	0.68	0.68
Metabolizable Energy (Kcal/kg)	2.900	2.900	2.900	2.900	2.900

RESULTS AND DISCUSSIONS

The effect of dietary treatment on feed consumption, egg weight, quail day and feed conversion of quail (*Coturnix coturnix japonica*) is shown in Table 3.

Table 3. The average of feed consumption, egg weight, quail day and feed conversion

Variable	R0	R1	R2	R3	R4
Feed consumption (g/day)	17.90[a]	19.08[b]	19.23[b]	17.85[a]	17.18[a]
Egg weight (g)	9.25[a]	10.12[b]	10.12[b]	9.41[a]	9.41[a]
Quail day (%)	74.80[a]	75.26[a]	75.19[a]	75.09[a]	74.21[a]
Feed conversion	2.44[a]	2.27[b]	2.33[b]	2.42[a]	2.54[a]

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

Feed Consumption

Feed consumption were variations, from the lowest R_4 = 17.18 gram to the highest R_3 = 19.23 gram (Figure 1).

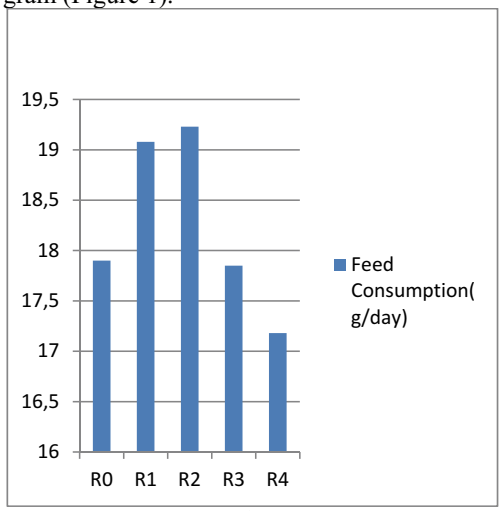

Figure1. Feed Consumption

In Table 3 shows that feed consumption reduced with increase the level of BSF maggot meal in the diets. The analysis of variance showed that substitution of fish meal by maggot meal has significant effect ($P<0.05$) on quail feed consumption. The difference is due to the consumption of palatability diet containing fish meal better than a diet containing BSF maggot meal. The decrease of feed consumption in the diet containing 100% BSF maggot meal (R_4) is caused by high levels of fat contained in the maggot meal, so the ration R_4 which has the highest fat content is equal to 7.51%. High fat content in the diet determined a decreased feed consumption, it is because of fat can produce high energy, so that quail will stop eating when energy needs has been reached. In line with the opinion of Zouand Wu (2005) which states that fat supplementation or increased energy ration will reduce feed intake and improve feed conversion of laying hens. At R_1 treatment (25%) and R_2 (50%) of substitution between fish meal with BSF maggot meal has reached the proper composition, thereby affecting the palatability of feed, quail consequently will consume more feed. Awoniyi et al. (2000) reported that BSF maggot meal was not nutritionally inferior to fish meal.

Egg Weight

The egg weight of each treatment is showed in Table 3. The average of egg weight was 9.25 – 10.12 g (Figure 2).The results of the analysis that the substitution treatment of fish meal by BSF maggot meal significantly affect on egg weight. This suggests that the substitution of fish meal by maggot meal gave a positive response on the weight of quail eggs. Orthogonal contrasts test results indicate that the substitution of fish meal by BSF maggot meal until 50% in the diet resulted in weight of eggs was significantly higher compared with control diet (without BSF maggot meal). According Agunbiade et al. (2007) that the maggot meal can replace 50% of fish meal with no negative effect on egg production, egg weight and egg shell strength. In the research substitution of fish meal by BSF maggot meal as much as 75% produce the same weight of eggs with control diet (R_0).

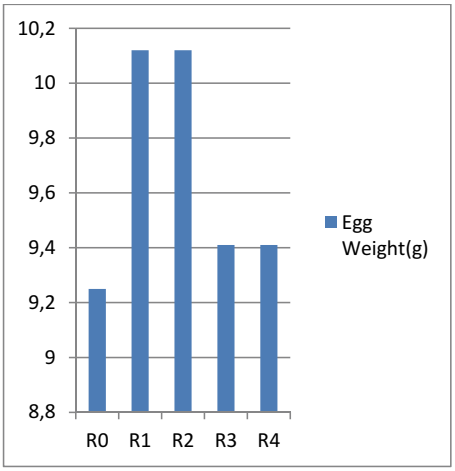

Figure 2. Egg Weight

The treatment of R_1 and R_2 produce egg weights were significantly higher than R_0, because of the relationship with feed intake were significantly higher, so the protein consumed will be higher resulting in an increase on weight of eggs produced. BSF maggot meal can replace fish meal, because maggot meal has a protein with characteristics of the amino acid profile relatively similar to fish meal (Newton et al., 2009).

Quail Egg Production

Substituting fish meal with maggot BSF meal produces a range egg production 74.21 – 78.19 percent, this suggest that utilize maggot meal as good as fish meal (Fig. 3).

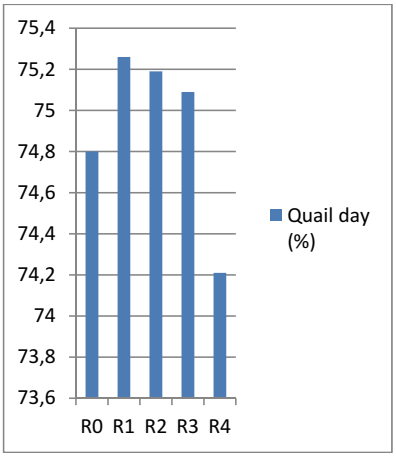

Figure 3. Quail Day (%)

The results of analysis of variance showed that fish meal substitution by BSF maggot meal into the ration was significantly ($P<0.05$) effect on quail egg production (Table 3). Increased levels of maggot meal in the ration had no effect on egg production. This proves that the similarity in the quality of fish meal protein with BSF maggot meal. Despite the reduction in feed intake with increasing levels of fish meal substitution by maggot meal which resulted on low protein intake, but egg production can still be maintained. This result is consistent with research Akpodiete et al. (1998) reported that the replacement of fish meal by maggot meal had no effect on egg production.

Feed Conversion

The feed conversion ratio of quails fed BSF maggot meal diets were better ($P<0.05$) than for those control diet fed (without maggot meal). Therefore the use of maggot meal in the quails diets enhanced nutrient utilization than fish meal based diet. By treatment of substitution fish meal by BSF maggot meal until 50% in the ration gave the best result of feed conversion ratio and significantly different ($P<0.05$) from other treatments

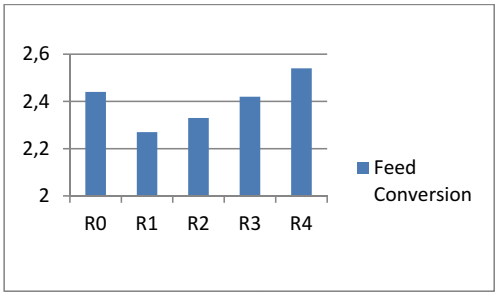

Figure 4. Feed Conversion

The result was parallel on feed consumption those was no significant different among the treatment R_1 (25% BSF maggot meal) and R_2 (50% BSF maggot meal) and significant different to R_0, R_3 (75%) and R_4 (100%). Its mean that BSF maggot meal from 25% until 50% in the ration did not influence palatability of ration and quail appetite. The similarity in amino acid profile maggot meal with fish meal showed that quail can utilize BSF maggot meal as well as fish meal. According to Agunbiade et al. (2007) that maggot meal can replace 50% of fish meal with no negative effect on the layer performance.

CONCLUSIONS

It can be concluded that the substitution of fish meal by black soldier fly (*Hermetia illucens*) maggot meal until 50% levels in the ration were still able to support a good result on quail production performance *(Coturnix coturnix japonica)*.

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SPECIFICITY OF MEMBRANE-BOUND ENZYMES ACTIVITY OVER THE CRYOPRESERVATION OF FARM ANIMALS SPERM

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Abstract

The intensity of the vital processes in spermatozoa in addition to the transmembrane defects is determined through activity of membrane-bound enzymes. The purpose of our research was to study the activity of membrane-dependent enzymes in the sperm of different animals species at cryopreservation. In experimental studies were used physiological, cryobiological and statistical methods that were held on the plasmatic membranes of sperm of the boar of Large White breed and the roosters of Rhode Island Red breed. The obtained results in the study of the activity of Mg^{+2} (Na^{+} + K^{+}) - ATPase in the membranes of spermatozoa of the diluted sperm, and after freezing and thawing of sperm of rooster and boar attest substantial destruction of the plasmatic membranes of these breeds. Activity of 5'-nucleotidase in isolated rich fractions of plasmatic membranes of native spermatozoa of rooster and boar not suffer essential changes at cryopreservation of sperm these species. Our research revealed minor changes in the activity of alkaline phosphatase in the membranes of native and frozen-thawed rooster and boar spermatozoa. The study of the above enzymes of plasmatic membranes of spermatozoa of rooster and boar are certain theoretical and practical interest. They allow obtaining new data on the chemical composition of the membranes, reveal the specific relationship between the content and ratio of the structural components of membranes and the resistance of rooster and boar spermatozoa to low temperatures.

Keywords: *membrane, spermatozoa, freezing, enzymes, farm animals*.

INTRODUCTION

Increase of productive and reproductive indices of farm animals can be achieved by improving methods of artificial reproduction. But this requires the availability of high-quality sperm, the indicators of which are reduced in the process of cryopreservation. Stabilization of functional parameters of reproductive cells is possible on the basis of fundamental research at the subcellular level. The intensity of the vital processes in spermatozoa besides the transmembrane defects (Белоус et al., 1982) is determined also on the activity of membrane-bound enzymes.

Previously completed studies on enzymes characteristic for plasmatic membranes of gametes males have showed that their activity depends on the type of animals. For example, in the membranes of spermatozoa from native sperm of bull the activity of ATPase is almost 10 times higher than in the membranes of spermatozoa of ram, the activity of which in the technological processing of sperm decreases in plasmatic membranes of both species of animals. Thus observed the same tendency of activity of ATPase in the membranes of sperm cells of the bull, which is almost 10 times higher than in the membranes of spermatozoa of the ram after thawing of sperm. Investigation of alkaline phosphatase in the native membranes of spermatozoa showed that the activity of this enzyme also varies depending on the species. At the cryopreservation of sperm the activity of this enzyme increases in plasmatic membranes of spermatozoa of bulls, whereas in the membranes of gametes of ram is a slight decrease (Борончук et al., 2008).

Based on the above, the purpose of the paper was to study the activity of membrane-dependent enzymes in the sperm of different species of animals during cryopreservation.

MATERIALS AND METHODS

The experimental investigations were carried out using the plasmatic membranes of spermatozoa of the boar of Large White breed and the roosters of Rhode Island Red breed.
Native sperm was divided into two parts. The first part (without dilution) was subjected to analysis served as control. The second part was diluted with synthetic mediums of corresponding type of animal. Both parts were cooled and freezed as granules on the surface of fluoroplastic plates at a temperature of minus 100-110°C. Defrost of sperm was carried out at 40°C, using a specially designed aerodynamic devices that can reduce the contact between the solid and liquid phase formed when heated. The selection of plasmatic membranes was performed using a polymer system, consisting of dextran with a molecular mass 500000 D and polyethylene glycol - 6000 D (Ivanov et al., 1981).

Determination of activity of membrane enzymes Mg^{+2} (Na^+ + K^+) - ATPase (CE 3.6.1.3), 5`-nucleotidase (CE 3.1.3.5) and alkaline phosphatase (CE 3.1.3.1) was performed in accordance with recommendations by the same authors. All the processing of the membranes was carried out at 4°C.
The obtained data were processed using the Student's t-test.

RESULTS AND DISCUSSIONS

Based on the above were continued research to elucidate the activity of Mg^{+2} (Na^+ + K^+) - ATPase, 5`-nucleotidase and alkaline phosphatase of the plasmatic membranes. They showed that the cooling, freezing and thawing of rooster and boar sperm have different effect on them.
However, in isolated plasmatic membranes of thawed spermatozoa of both species was observed a significant reduction the activity of these enzymes (Table 1).

Table 1. The activity of Mg^{+2} (Na^+ + K^+) - ATPase, 5`-nucleotidase and alkaline phosphatase of isolated plasmatic membranes of spermatozoa at cryopreservation of rooster and boar sperm (µmol/h/mg protein)

Stage of technological processing	Mg^{+2} (Na^+ + K^+) - ATPase	5`-nucleotidase	Alkaline phosphatase
Rooster			
Dilution (control)	16.23±2.20	8.09±0.86	1.19±0.08
Freeze-thawing	9.57±0.56*	7.39±0.59	1.17±0.06
Boar			
Dilution (control)	28.03±1.30**	4.49±0.98**	3.02±0.36**
Freeze-thawing	18.85±1.43*. **	3.17±0.66**	2.48±0.37**

*The difference is statistically authentic
** Statistically authentic differences breed

The results obtained in the study of activity of Mg^{+2} (Na^+ + K^+) - ATPase in the spermatozoa membranes from the diluted sperm, and after freezing and thawing of rooster and boar sperm attest substantial destruction of the plasmatic membranes of these breeds. For example, in the process of cryopreservation of sperm the activity of this enzyme is reduced from 16.23±2.20 to 9.57±0.56 µmol/h/mg protein at rooster and from 28.03±1.30 to 18.85±1.43 µmol/h/mg protein at boar. And since this enzymatic system performs transport function and the role of the transformer of energy, accumulated in ATP for active transport of Na^+ and K^+ on the membrane, the revealed changes of active transport of Mg^{+2} (Na^+ + K^+) - ATPase shows the large deenergization of the plasmatic membranes of rooster and boar spermatozoa. Further analysis of the data shows that in the membranes of boar spermatozoa in the process of freeze-thawing of sperm occurs most significant decrease of activity of Mg^{+2} (Na^+ + K^+) - ATPase, which indicates more labile relation of this enzyme with membrane of boar spermatozoa ($P<0.01$).
In addition, numerous studies show that treatment of ATPase with various detergents, phospholipases, and solvents leads to their

inactivation (Bagatolli et al., 2010; Болдырев, 1990). This highlights need for participation of lipids to the manifestation of mentioned enzymes activity. It should be noted that from all factions of phospholipids the greatest increased activity of ATPase causes negatively charged phospholipids - phosphatidylserine and phosphatidic acid (Болдырев, 1990; Ипатова, 2005). It follows that the one of possible causes of the substantial inactivation of Mg^{+2} (Na^{+} + K^{+}) - ATPase membranes boar spermatozoa after thawing in comparison with such indicators of membranes sperm of rooster, may be most lower content of phosphatidylserine at all stages of technological processing of the material (Наук, 1991).

Activity of 5`-nucleotidase in isolated rich fractions of plasmatic membranes of native rooster spermatozoa was higher than in the boar spermatozoa, which is 8.09±0.86 against 4.49±0.98 μmol/h/mg protein, respectively. In the process of cryopreservation of rooster and boar spermatozoa occurs a slight decrease of activity of this enzyme, namely 7.39±0.59 at rooster and up to 3.17±0.66 μmol/h/mg protein at boar. It follows that this enzyme is not suffer essential changes in the cryopreservation of sperm of these animals.

The representative of associated enzymes with the plasmatic membrane, is alkaline phosphatase, which participates in the mechanism of realization of the physiological action of cyclic AMP by releasing of orthophosphates and phosphorylated proteins substrates (Gotoh et al., 2007; Ипатова, 2005). Our research revealed insignificant changes in the activity of this enzyme in the membranes of native and freeze-thawed rooster and boar spermatozoa. So, alkaline phosphatase activity is respectively 1.19±0.08 and 1.17±0.06 μmol/h/mg protein in the membranes of native and thawed spermatozoa of rooster. At boar the activity of this enzyme is reduced from 3.02±0.36 in the plasmatic membranes of native spermatozoa to 2.48±0.37 μmol/h/mg protein in the membranes of thawed spermatozoa. The obtained data are consistent with research results of the functional indices of spermatozoa after freezing and thawing of bull and ram sperm (Борончук et al., 2008). The analysis of the received results allow to note that changing the activity of Mg^{+2} (Na^{+} + K^{+}) - ATPase, a 5`-nucleotidase and alkaline phosphatase during cryopreservation are the result of changes in the molecular organization of plasmatic membranes, the restructuring of which is carried out in the process of dilution, cooling, freezing and thawing of sperm of these animals breeds.

The comparative analysis of breeds features of the membrane-bound enzymes activity of spermatozoa of these species showed that the activity of Mg^{+2} (Na^{+} + K^{+}) - ATPase and alkaline phosphatase is higher in plasmatic membranes of boar spermatozoa. The activity of these enzymes constitute respectively in membranes of native spermatozoa 16.23±2.20 and 1.19±0.08 at rooster, 28.03±1.30 and 3.02±0.36 μmol/h/mg protein at boar. In turn, the activity of 5`-nucleotidase is higher in the membranes of rooster spermatozoa, which at the cryopreservation is reduced from 4.49±0.98 to 3.17±0.66 at boar and from 8.09±0.86 to 7.39±0.59 μmol/h/mg protein at rooster. However, the obtained data indicate that the membrane-bound enzymes of plasmatic membranes of rooster and boar spermatozoa have the species specificity, as their activity differs between them on a statistically significant difference. At the same time, for boar spermatozoa membranes are the most species-specific Mg^{+2} (Na^{+} + K^{+}) - ATPase and alkaline phosphatase, whereas for rooster – 5`-nucleotidase.

Thus, the study of the above enzymes of plasmatic membranes of rooster and boar spermatozoa are certain theoretical and practical interest. They allow obtaining new data on the chemical composition of the membranes, reveal the specific relationship between the content and ratio of the structural components of membranes and the resistance of rooster and boar spermatozoa to low temperatures.

This offers the possibility to limit the scope of searches conditions of cryoprotection and improve the quality of sperm after long-term preservation outside the body.

CONCLUSIONS

The researches allow making the following conclusions:

1. As a marker for the determination of the functional state of boar and rooster sperm may be used the activity of Mg^{+2} (Na^{+} + K^{+}) - ATPase, which is more labile.
2. The process of cryopreservation of boar and rooster sperm initiates a reduction of the activity of membrane-bound Mg^{+2} (Na^{+} + K^{+}) - ATPase, whereas the activity of the 5`-nucleotidase and alkaline phosphatase remains stable.

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CRYOGENIC CHANGES OF LIPID DURING PRESERVATION OF SPERM ANIMAL FARM

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Abstract

The solution of the problems of cryopreservation of sperm of farm animals is determined largely by the intermolecular interactions of cellular components of reproductive cells. Therefore, the aim of the research was the study of the contribution of the lipid components in the manifestation of adaptive-compensatory changes of gametes of animals in hypothermic conditions. Experimental investigations were carried out using sperm of the boars of Large White breed, the roosters of Rhode Island Red breed and the bulls of Black and White breed. Isolation of plasmatic membranes of spermatozoa was conducted according to the method developed by Ivanov and Porfiry (1981) in our modification (Наук et. al, 1993). The obtained results proved that the phospholipids in the process of cryopreservation of sperm of farm animals are exposed to the greatest changes, while the cholesterol content is more stable. Also, the molar ratio phospholipids:cholesterol was changed in the direction of value "1" after cooling and freezing of sperm. Decrease of the ratio phospholipids:cholesterol is one of the mechanisms in the system of adaptive-compensatory reactions of spermatozoa at the influence of low temperatures. The study of the adaptation mechanisms at the cellular and molecular levels attracted the attention of an increasing number of researchers, because in this direction is possible prospects of solving the problems of cryogenic changes during preservation of sperm.

Keywords: *lipids, cryopreservation, sperm, cholesterol, plasmatic membranes.*

INTRODUCTION

The stabilization of intermolecular interactions of cellular components of reproductive cells may be the solution of the problems of cryopreservation of sperm of farm animals. An important role in the vital functions of gametes belongs to the plasmatic membrane. Due to the strictly coordinated work of membrane mechanisms is supported cellular homeostasis, is carried out the fine regulation of functional activity in response to the impact of environmental factors and changes within the cells (Наук, 1991).

In the functional gametes must be supported the specific liquid-crystalline status of lipid systems (Болдырев, 1990). It is important for the cell that the status of lipid phase is determined by strictly specific chemical composition of lipids and could easily be broken in the changing conditions of environment. Such damage causes a number of adaptive-compensatory changes in the structure of biological membranes. These changes are very important for maintaining the structural and functional activity of gametes.

The foregoing was the motive of the research the contribution of the lipid components in the manifestation of adaptive-compensatory changes of gametes of animals in hypothermic conditions.

MATERIALS AND METHODS

The experimental investigations were carried out using sperm of the boars of Large White breed, the roosters of Rhode Island Red breed and the bulls of Black and White breed. The animals were housed in vivarium conditions with observance of veterinary requirements. The isolation of plasmatic membranes of spermatozoa was conducted according to the method developed by Ivanov and Porfiry (1981) in our modification (Наук et. al, 1993). Cholesterol was determined by the method of Ilka (Покровский, 1969), at wavelength 665 nm using a spectrophotometer SF-46. Principle of the method is based on the degree of degradability of biocomplexes and extraction

from them of loosely bound cholesterol with organic solvents.

The study of phospholipids of spermatozoa were conducted by the method of Keith (Кейтс, 1975) with the use of plates 9x12 cm, which is applied the mixture of silicogel L and LSL firm Chemapol in the ratio 1:0,6. The extract of lipids was received according to the technique described above, then dried at a rotary evaporator and the sediment was dissolved in 0,5 ml of a mixture of chloroform:methanol (1:1). The received mixture of lipids used for thin-layer chromatography, and their separation was carried out in the system chloroform:methanol:water (65:25:4).

Statistical processing of data was performed using a Student's t-test.

RESULTS AND DISCUSSIONS

In stabilization of biological structures at the stages of low temperature preservation of great importance have the protein-lipid interactions. In this connection it is of interest to study the biochemical structure of plasmatic membrane to clarify the reasons of different stability of gametes at the effect of low temperatures (Table 1).

Table 1. Cryogenic changes of the ratio protein:lipid in plasmatic membranes of gametes of farm animals

Animal species	Ratio of protein:lipid of membranes		
	Native gametes	Cooled gametes	Thawed gametes
	$x \pm s_x$	$x \pm s_x$	$x \pm s_x$
Bull	0.43 ± 0.014	0.3 ± 0.11	0.25 ± 0.019*
Boar	0.17 ± 0.009	-	0.20 ± 0.004*
Rooster	0.40 ± 0.042	0.4 ± 0.08	0.52 ± 0.041*

*Statistically reliable cryogenic changes

As the table shows, the highest ratio of protein:lipid in plasmatic membranes noted in the gametes of a bull, which is well endure freezing of cells (Наук, 1991). In the membranes of the gametes of the boar, which are characterized by high sensitivity to cooling and freezing, it was discovered lowest ratio of these components. The value of this indicator in the membranes of gametes of the rooster occupies an intermediate position.

Apparently, the decrease of protein:lipid ratio during defrosting is more beneficial for maintaining the usefulness than its increase, since the sperm of the bull in the membranes which reduced this ratio, it is better kept at cryopreservation than the sperm of other species of animal (Борончук et al., 2008).

In connection with the original structure of cholesterol and its ability to regulate viscosity of membranes in the following studies were studied cryogenic changes during the process of preservation of sperm of the bull and a boar. The results of these studies are presented in Table 2.

Table 2. The content of loosely bound cholesterol in the process of cryopreservation of gametes of farm animals

Animal species	The amount of cholesterol (mg/1mlrd) in gametes after:		
	Dilution	Cooling	Thawing
	$x \pm s_x$	$x \pm s_x$	$x \pm s_x$
Bull	415.6 ± 10.9	379.9 ± 10.6*	342.0 ± 10.6*
Boar	482.0 ± 4.0	456.4 ± 10.4*	424.2 ± 11.4*

*Statistically reliable cryogenic changes

From data of Table 2 it follows that the number of loosely bound cholesterol is greatest in the gametes of the boars and least - for the bulls. Therefore, the biological membranes of gametes of the boar are distinguished by a reduced elasticity, which is consistent with studies of Nauc (1991). The cooling process significantly affects at the concentration of loosely bound cholesterol in gametes of investigated species of animals that should be considered when developing of new synthetic mediums and techniques.

By the fundamental research in the field of cryobiology quite definitely was proved that

lipids play an important and sometimes decisive role in a number of processes flowing in the cell in norm and in pathology contributing to the stabilization of its functional homeostasis (Schäfer, 2011). In this regard, were investigated the cryogenic changes of phospholipids and cholesterol in gametes of the bull and of the boar (Table 3).

Table 3. Phospholipids, cholesterol, and their ratio in the process of cryopreservation of sperm of the bull and a boar

The studied parameters in gametes of:			
Bull		Boar	
After dilution	After thawing	After dilution	After thawing
$x \pm s_x$	$x \pm s_x$	$x \pm s_x$	$x \pm s_x$
Phospholipids (mol %)			
3.8 ± 0.06	2.3 ± 0.10*	3.6 ± 0.09	2.8 ± 0.04*
Cholesterol (mol %)			
1.1 ± 0.04	0.9 ± 0.04*	1.3 ± 0.05	1.1 ± 0.03*
The molar ratio of phospholipids:cholesterol			
3.54	2.62	2.86	2.56

*Statistically reliable cryogenic changes

The presented data show that after cryopreservation the amount of phospholipids is 60.5%, and cholesterol 81.8% of native bull sperm, while in the sperm of the boar the studied characteristics, respectively amounted to 77.7 and 84.6%.

The table also shows that the number of the examined lipid in diluted material is almost on the same level. The cryopreservation process leads to decrease of the specified indicator to statistically significant value which may be due to: 1) involvement of lipids in energy metabolism, 2) increasing the activity of phospholipases, 3) increased of free radical process of lipid peroxidation.

Reducing the amount of cholesterol in the spermatozoa of the bull and of the boar after cryopreservation, apparently, can occur as a result of his decompaction in the lipid bilayer membranes or by loosening of lipid micelles in the structure of membranes (Богач et al., 1979).

At similar change is exposed and phospholipid-cholesterol ratio. The results obtained clearly show that the molar ratio of phospholipids:cholesterol in spermatozoa is changed in the direction of value "1" after cooling and freezing of sperm, namely in the direction of ratio which eliminates the phase transitions of lipids, or at least moving it to the area of lower temperature. It should be noted that the tempo of approximation to value "1" is the most high in the case of experimenting with the sperm of the bull. This is another proof of predominance of its cryoresistance in comparison with the sperm of the boar.

Given the fact that the maximal activity of phospholipases refers for the temperature range of the phase transition of lipids (Белоус et al., 1982), and also that he is the initiator of the main biochemical changes, can be assumed that the decrease of the ratio phospholipids:cholesterol is one of the mechanisms in the system of adaptive-compensatory reactions of spermatozoa at the influence of low temperatures. However, it is necessary to admit that along with the magnitude of ratio of phospholipids:cholesterol or content of these components separately, an important role is played their dynamics in the process of cryopreservation of sperm, as an important mechanism of adaptation of spermatozoa to low temperatures (Polyansky, 2010). The positive effect of this mechanism can occur only in the presence of exogenous lipids, such as lipids of seminal plasma, yolk of chicken eggs etc. and can also be caused by the processes of synthesis or resynthesis of endogenous substrates. Thus, the inclusion of the system of phospholipases aimed at changing of the ratio of phospholipids:cholesterol can be considered as own protective function of cell.

Many researchers tried to explain the complex formation of cholesterol-phospholipids by hydrogen bonds between the hydroxyl group of sterol and the oxygen atoms of phospholipids. However, the relationship of this type is not

probable, since, firstly, the ether groups of phospholipids should be hydrated, secondly, the inclusion of cholesterol in different phosphatidylcholine liposomes not change the NMR spectrums (Богач et al., 1979). The structure of the molecule of sterol is unique because of its cyclic part and side chain has great opportunities for the manifestation of intermolecular interactions, different reactions and transformations. These include the ability of hydrogen atoms being replaced by various radicals. Additionally, the presence of double bonds, few of hydroxyl groups, carbonyl and carboxyl groups in various combinations define a spatial configuration of cholesterol. Therefore is possible the formation of a very large number of individual compounds with various specific properties, including to enter into intermolecular interaction (Борончук et al., 2008). Consequently, cholesterol can be used in composition of cryoprotective mediums.

Thus, at the study of the influence of cooling on the status of various cellular components, the important point is the account of cryogenic changes of proteins and lipids, as these changes result in complex adaptive-compensatory processes in cryobiological systems and the possibility of full or partial rehabilitation of the object after cryopreservation.

CONCLUSIONS

The researches allow making the following conclusions:

1. In the process of cryopreservation of sperm of farm animals to the greatest changes are exposed phospholipids, while the cholesterol content is more stable.
2. Cryogenic changes of the content of phospholipids and cholesterol are most pronounced in the sperm of the bull, while in the sperm of the boar studied indicators are less pronounced.
3. The content of phospholipids in the native spermatozoa of the bull and of the boar is practically at the same level, while the amount of cholesterol is higher in the spermatozoa of the boar ($P<0.05$).
4. High amount of cholesterol in the spermatozoa of the boar plumping biological membranes, making it more fragile, which can reduce their cryoresistance compared with those of bull's spermatozoa.
5. Enhancing the effectiveness of cryopreservation of sperm of farm animals is possible by the introduction in the synthetic mediums of exogenous lipids.
6. Stabilization of adaptive-compensatory reactions can be implemented by changing of the structure of lipid components of cryoprotective mediums.

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COMPARATIVE PHYSICOCHEMICAL AND BIOCHEMICAL CHARACTERIZATION OF BULL AND BOAR SEMEN

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Abstract

There are numerous specialized studies of the physicochemical and biochemical aspects of bull and boar semen, but this paper highlights additional enzymatic activity of superoxide dismutase and glutathione peroxidase enzymes that behave as sperm defense mechanism against of oxygen free radicals(the protection of membrane lipids against oxidative damage). Such a comparative study was performed from a physicochemical (pH, buffering capacity) and biochemical (seminal fructose content, total protein, total lipids, total cholesterol and lactate dehydrogenase enzymatic activity, glutamate dehydrogenase, superoxide dismutase, glutathione peroxidase and acrosin activity) point of view for 10 samples of boar and bull seminal plasma. The investigation showed smaller values of total protein, fructose, lipids and total cholesterol in the boar seminal plasma samples than in the bull seminal plasma samples (the decrease was significant, P <0.05). LDH and GDH activity in bull sperm is increased compared to the boar (P< 0.05), which leads to the idea that the anaerobic degradation of fructose (fructolise) in which LDH is involved, as well as the oxidation of the amino acids where GDH is involved are both processes that take place more intensively in bull sperm than in the boar one. Acrozine of boar sperm cells showed an increased activity compared with that of bull sperm (P< 0.05), because the intracellular pH of boar sperm (7.3-7.9) is closer to the optimal action pH for this enzyme (8,0). SOD activity in both the sperm extract and the seminal plasma of the bovines is increased compared to the ones from boar samples(P<0.01), which explains the higher resistance to lipid peroxidation of the bovine sperm compared to all other animal species. Regarding glutathione peroxidase activity, similarly to SOD activity, it is greatly increased in the extract of bovine sperm compared to bovine seminal plasma. In the boar samples, no traces of glutathione peroxidase could be found.

Keywords: *boar semen, superoxide dismutase, glutathione peroxidase*

INTRODUCTION

Advanced research activity in the field of domestic animals sperm biochemistry have been the subject of many specialized works which have shown the important role of biochemical semen quality on livestock birth growth ratio and prolificacy (Cristea, 1999).

On the other hand, biochemical assessment of sperm from different breeding individuals facilitates the understanding of many different transformations that occur during semen preservation until artificial insemination (Bailey, 2012). The need for introducing a physicochemical and biochemical control of sperm arises, along the usual microscopic control, in the standard spermiogram (Cristea, 1999; Diaconescu, 2001).

The most important physicochemical parameters of semen are: volume, color, pH and buffering capacity (determined and highlighted as a metabolic feature).

In order to move, sperm need energy that can be obtained either by anaerobic catabolism of carbohydrates (fructose) or by oxidation of substrates in the presence of oxygen or by endogenous lipid catabolism (Tamba, 1998; Bailey, 2012). Due to the partial electric charge of the amino acids, seminal plasma proteins contribute to the determination of the pH and to buffering it, and by the albumin the osmotic pressure is adjusted in the sperm, and it helps maintain the integrity of the membrane (Diaconescu, 2001). Many of the proteins have bio catalytic properties, so they are enzymes and they represent the key to intermediary metabolism. There is a wide variety of enzymes active in sperm, some having an important role in the energy generating processes (lactate dehydrogenase and sorbitol dehydrogenase), in the protein metabolism process (glutamate dehydrogenase and acrozine) and especially in the protective lipid peroxidation processes, such as superoxide dismutase (SOD) and glutathione peroxidase (GPX) (Dejica, 2000).

It is known that from all animal species, the bull sperm, followed by the boar one, is the

most resistant to freezing in liquid nitrogen (Bailey, 2012).

Therefore, this paper aimed to conduct a physicochemical study (pH, buffering capacity) and biochemical (seminal fructose content, total protein, total lipids, total cholesterol and enzyme activity of lactate dehydrogenase, glutamate dehydrogenase, superoxide dismutase, glutathione peroxidase and acrozinic activity) of the bull semen compared with the boar one.

MATERIALS AND METHODS

For the physicochemical and biochemical characterization, the following number of samples were used: 10 bull ejaculates (5 of 5 Friesian breed brown), 10 breeds Landrace boar ejaculate (5 samples), Hampshire (5 samples).

Semen samples were collected from clinically healthy animals and sperm harvesting was done with artificial vagina in both species.

Fresh semen was centrifuged at 2000 g for 20 minutes. Seminal plasma was decanted.

In order to obtain the extract of sperm, after removal of seminal plasma, gametes were washed two times with Ringer solution pH = 6.6, and then mixed/crushed with silica sand, and re-suspended in PBS buffer or saline solution, centrifuged at 25,000 g , at t = 4°C, for 20 minutes so that the supernatant contains 0.5 g% protein.

Both in seminal plasma as well as on the extraction of sperm following determinations were performed:

PH measurement: were performed obtaining potentiometric pH values with two decimal precision;

Buffer capacity measurement: in 0.15 ml of seminal plasma 0.05 ml 0.1 N HCl was added. The pH was measured before and after the addition of the acid. The difference is the buffering capacity;

The seminal fructose measurements based on its reaction with concentrated hydrochloric acid, the resulting hydroxymethyl - furfural which, with resorcino, forms a complex condensation whose intense red color is estimated by photocalorimetry at λ = 540nm (Diaconescu, 2001).

Measurement of total protein by Biuret method: over the variable seminal plasma volume a 5 ml of Biuret reagent was added and after 30 minutes the purple color intensity was estimated by photocalorimetry at λ = 570 nm using as base etalon a standard a solution of 1% bovine serum albumin (Merck). The results were expressed in grams of protein on 100 g seminal plasma (Iordăchescu and Dumitru, 1988)

Measurement of total lipid: total lipids were extracted from both plasma and sperm suspension by homogenization with a mixture of chloroform: methanol (2:1). From the methanol and chloroform extract, the total lipids were determined on the basis of the reaction with the compound fosfovanilina.

The red colored substances was estimated at λ = 530 nm (Diaconescu, 2001). Results were expressed as mg of total lipid /100 ml seminal plasma and sperm extract.

Measurement of total cholesterol: from the chloroform & methanol extract, the total cholesterol (free + esterified) was determined by the Zlatkis-Zak-Boyle method using ferric chloride. The red compound whose intensity of color was read at λ = 570 nm.

Results were expressed in mg colesterol/100 ml seminal plasma and sperm extract (Diaconescu, 2001).

Measurement of lactate dehydrogenase activity (LDH): LDH catalyzes the reduction reaction of pyruvate to lactate, by NADH means (H^+), the reaction rate is determined by subtracting the optical density at 340 nm following the oxidation of NADH (H^+). The results were expressed in $mUI/10^8$ sperm (Rajan, 2011);

Measurement of glutamate dehydrogenase activity (GDH): GDH catalyzes the reaction of oxidative deamination of α-glutamate to α-ketoglutarate. The reaction speed is determined by reducing the optical density at λ= 340 nm due to NADH oxidation (H^+). Calculation and expression of results were performed as in point 7 (Rajan, 2011).

Measurement of superoxide dismutase activity (SOD): SOD activity was determined by a method which is based on the ability of SOD to inhibit the reduction of the tetrazolium salt (NBT2+) due to the superoxide radicals, till it reaches blueformazan (solution). The

color intensity was estimated by photocalorimetry at λ = 560 nm. Results were expressed in UI/10^{10} spermatozoa (Michalski, 1996).

Measurement of glutathione peroxidase activity (GPX) is based on the Plagia&Valentine method (Iordăchescu and Dumitru, 1988);

Measurement of acrozine activity: was performed using the Schwert and Takenaka method. The results were expressed in UI/10^{8} spermatozoa (Strzezek et al., 1992).

RESULTS AND DISCUSSIONS

Physicochemical and biochemical parameters measured in seminal plasma of the studied species are shown in Table 1.

Table 1. Values for the main physicochemical and biochemical parameters determined in the bull and boar seminal plasma

Biochemical parameters	Species	
	Bull	Boar
PH ($\overline{X} \pm s_{\overline{X}}$)	6.87 ± 0.02	7.35 ± 0.04
Buffering capacity ($\overline{X} \pm s_{\overline{X}}$)	$1.17 \pm 0,02$	$1.89 \pm 0,07$
Protein (g%ml) ($\overline{X} \pm s_{\overline{X}}$)	$7.80 \pm 0,19$	$2.30 \pm 0,09$
Fructoze (mg/100 ml) ($\overline{X} \pm s_{\overline{X}}$)	713 ± 25.70	78 ± 1.20
Total lipids (mg/100 ml) ($\overline{X} \pm s_{\overline{X}}$)	59.10 ± 4.89	5.60 ± 0.10
Total cholesterol (mg/100 ml) ($\overline{X} \pm s_{\overline{X}}$)	25.49 ± 1.60	2.08 ± 0.16

*Values are shown as mean±standard deviation of 10 samples

By analyzing the pH values and those of buffering capacity, we can see that they fall within the limits described in the domain literature (7.57 for bull and 6.7 for boar);

In boar we found lower fructose values (P<0.001), knowing that boar spermatozoa have a predominantly respiratory activity and the fructolize index is very low compared to the bull.

The protein content had the highest value in bull samples (7.80 ± 0.19 versus 2.30 ± 0.09) and is correlated with the field research which revealed that 90 % of the total nitrogen of bull seminal plasma is of protein nature (Cristea and Rotar, 1999).

From the analysis of the total lipid concentration, large differences are observed between the bull and boar ($\hat{t} > t_{\alpha}$ = 0,01) and in general, low levels are recorded, that correlate with the field literature (67 mg/100 ml in bull and 5 mg/100 ml in boar samples) (Cristea and Rotar, 1999).

Studies have shown that seminal plasma lipids originate primarily from prostatic secretion and are concentrated mainly in the sperm membrane (23% in the tail, 6% in the intermediate piece and 7% at the ends) .As for the concentration of total cholesterol, boar seminal plasma has the lowest values compared with the bull one (the decrease was significant, P < 0.05), which is positively correlated with total lipid content.

Table 2 shows the values of intracellular enzyme activity for LDH, GDH and acrozine, in Brown and Friesian breeds for cattle and Landrace bread for swine.

Table 2. Values of intracellular enzyme activity for LDH, GDH and acrozine, in Brown and Friesian breeds for cattle and Landrace bread for swine

Biochemical parameters	Specie and breed		
	Cattle		Swine
	Brown $\overline{X} \pm s_{\overline{X}}$	Friesian $\overline{X} \pm s_{\overline{X}}$	Landrace $\overline{X} \pm s_{\overline{X}}$
LDH (mUI/10^{8}spz)	4.65 ± 0.14	4.10 ± 0.17	3.60 ± 0.06
GDH (mUI/10^{8}spz)	3.39 ± 0.13	2.80 ± 0.14	2.29 ± 0.06
Acrozine (N.F.U.I/10^{8}spz)	1787.78 ± 9.15	1766.70 ± 8.34	1893.01 ± 7.83

*Values are shown as mean ± standard deviation of 10 samples

Analysis of these experimental values revealed the following:

- activity of LDH, GDH and acrozine in the sperm did not differ significantly between Brown and Friesian cattle breeds ($\hat{t} < t_{\alpha}$ = 0,05 which $\hat{t}$ = 2,262) and are, in general, comparable with the literature (Cristea and Rotar, 1999);
- In the same breed (both in cattle and swine) the highest enzymatic activity is that of the LDH, which is correlated with the importance of this enzyme in fructolize (Kohsaka et al., 1992);
- Between species, the measurements showed

that LDH and GDH activities of bull sperm are increased compared to the boar ones ($P < 0.05$), which leads to the idea that anaerobic degradation of fructose involving LDH, as well as the oxidation of the amino acids involving GDH, are processes that take place more intensively in bull sperm than in the boar one. It is known that boar sperm have a predominantly respiratory activity and low fructolize index versus bull sperm. Meanwhile, acrozine of boar sperm showed increased activity compared to that of bull sperm ($P < 0.05$), because intracellular pH of boar sperm (7.3-7.9) is closer to the optimum activity pH of this enzyme (8.0), compared to the intracellular pH of bull sperm (6.4-6.5) (Cristea and Rotar, 1999).

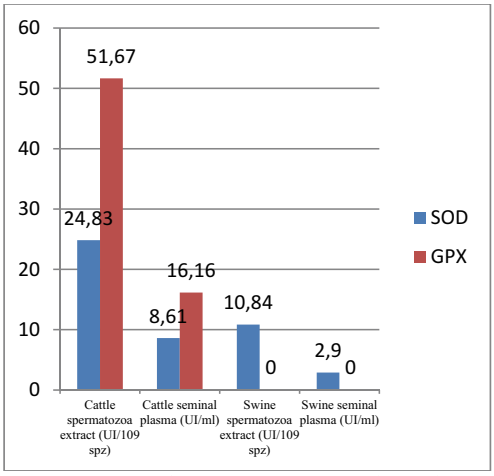

Figure 1. SOD and GPX activity measured in a plasma extract and in fresh sperm, from boar and bull. Values are shown as mean ± standard deviation of 10 samples

Figure 1 presents the main values of enzymatic activity for superoxide dismutase (SOD) and glutathione peroxidase (GPX), measured in a plasma extract and in fresh sperm, from boar and bull.

The analysis of the experimental results shows that the highest enzyme activity of SOD is the intracellular one, compared with the one from seminal plasma, both in the boar and bull, which confirmed that the SOD prevails in the cytoplasm and mitochondria of sperm.

Regarding GPX activity, like that of SOD, is greatly increased in the sperm extract compared to the seminal plasma for bull, and in boar the presence of glutathione peroxidase could not be revealed. Furthermore, research in this area revealed the presence of GPX only in bull, goat and ram sperm (Cristea and Rotar, 1999). The bovine seminal plasma shows a significantly increased GPX activity compared to SOD, which means that this enzyme performs an intense activity in bull sperm to reduce the harmful effect of H_2O_2.

CONCLUSIONS

The pH and the buffering capacity fall within the limits described in the literature for the two species investigated.

Research has shown values lower values of fructose, total protein, total lipids and cholesterol in all boar seminal plasma samples, compared with the ones of the bull (significant decrease of $P < 0.05$); LDH and GDH activity in bull spermatozoa were elevated as compared to the boar ones ($P< 0.05$), which correlates with the significantly lower index of fructose in the boar, versus the bull one;

Acrozine from boar sperm showed increased activity compared to that of bull spermatozoa;

SOD and GPX activity from both bovine sperm extract and seminal plasma is increased compared with the boar samples ($P < 0.01$), which explains the greater resistance to lipid peroxidation of bovine semen compared to all other animal species.

Data obtained from the comparative study of the physicochemical and biochemical parameters in bull and boar semen shows that these parameters are species particularities (related).

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THE IMPACT OF TREATMENT WITH INSULIN ON THE COMPOSITION OF PANCREATIC JUICE ENZYMES IN CHICKEN ORGANISM

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Abstract

The experiment was performed on seven weeks old chickens. Experimental and control groups have been made. The insulin was administrated in a dose of 4.00 U.I./kg body weight, once a day. The treatment was performed for 8 day and the chickens were periodically weighted. The pancreatic juice was obtained in an acute experiment. Than, pancreatic juice was volumetric measured and the flow *of trypsine and amylase was measured too. The results were statistically processed and the means and standard error of means were calculated. The statistic significance differences of the means between control and experimental group was searched by Student test. Following the experimental treatment of insulin on chickens, these results were obtained: 1. Insulin induced a juice flow of 387.5 microliter/kg body weight/30 minutes, insignificantly increased versus the control group. 2. Insulin increased the flow of pancreatic juice amylase and trypsine; that could explain the significant increase of the weight gain.*

Keywords*: amylase, chickens, insulin, pancreatic juice, trypsine*

INTRODUCTION

Insulin controls the carbohydrate metabolism and lipid and protein metabolism. It is important to note that the liver is the main target organ of insulin, in part because pancreatic venous flow enters directly into the liver (Mihalache, 2004).

The net effect of insulin action is lowering blood levels of glucose, fatty acids and amino acids to promote intracellular transformation of these compounds in their forms of storage: glycogen, triglycerides and proteins (Serban et al., 1993).

MATERIALS AND METHODS

Biological material was represented by the chickens aged 7 weeks (Cornish breed). It were set up two groups of chickens: a control group and a group treated with insulin. Both groups were fed ad libitum feed recipes for the stage and physiological status (growing youth) and benefited from a program conducted by artificial lighting (8 hours per day), according to technology growth.

The 18 chickens from the two groups were fed pelleted feed of prescription industrial code 21-3 (Table 1).

Table 1. Fodder recipe 21-3 code used to feed chickens

No.	Ingredients	Quantity (Kg)	Metabolizable energy (kcal)	Crude protein (%)
1	Corn	36	1212. 3	3.13
2	Barley	14	378	1.44
3	Wheat	16	476.6	1.90
4	Soybean cake	15	345	6.60
5	Sunflower cake	7	105	2.24
6	Meat flour	2	57.3	1.14
7	Oil	4.5	396	-
8	Premix methionine	0.9	18.8	0.50
9	Dicalcium phosphate	1.8	-	-
10	Calcium carbonate	1	-	-
11	Salt	0.3	-	-
12	Premix (MVP)	1.5	30	0.10
13	Total	100	3019.0	17.05

Legend: MVP = mineral-vitamin premix

A batch consists of nine chickens were treated with insulin, which was administered at a dose of 4.00 U.I. daily, once a day. Treatment duration was eight days. The second group (control group) remained untreated. Chickens of both groups were weighed every day during treatment (Table 2).

Table 2. Evolution of chicken weight (g) during the treatment with insulin

Specification	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8
Control group	2590	2590	2600	2630	2680	2680	2700	2730
Insuline group	2578	2590	2610	2650	2680	2700	2740	2760

In order to harvest pancreatic juice, each chicken was anesthetized with urethane 20% solution, administered intraperitoneally at a dose of 1.6 g per kg body weight. The abdomen was opened and spotted Wirsung canal in which a cannula was inserted. At the end of the cannula was connected to a capillary tube and gradually transparent. The basal secretion was collected pancreatic juice for a period of 10 minutes and secretion stimulated by secretin administration of an extract of duodenal mucosa, a period of 30 minutes. Every five minutes, the graduated tube was read the amount of pancreatic juice, which was then collected in a tube. They made these determinations: amylase activity and trypsin activity in pancreatic juice. Amylase activity was determined by the method of Smith and Roe, and trypsin activity was determined by the method of Schwert and Takenaka. Amylase and trypsin flows were calculated as the product between the average concentration of amylase or trypsin and the total pancreatic juice secreted a limited period of 30 minutes. The results were processed statistically and the significance of difference between groups was performed based on t test (Student test) (Tacu, 1968).

RESULTS AND DISCUSSIONS

In Table 3 presents the results regarding the total amount of pancreatic juice collected in basal conditions and in conditions of secretin stimulation of the pancreas in experimental group treated with insulin compared with controls.

Table 3. Effects of experimental treatment with insulin on volume secretion of pancreatic juice in chicken

Control group			Insulin group		
No. prove	Values		No. prove	Values	
	Basal (10 min.)	Stimulate (30 min)		Basal (10min.)	Stimulate (30min.)
1	0	52.5	1	4	70
2	0	80	2	11	47.5
3	7	87.5	3	0	42.5
4	11	52.5	4	7	87.5
5	4	65	5	16	140
$\bar{x} \pm s_{\bar{x}}$	4.4	67.5	$\bar{x} \pm s_{\bar{x}}$	7.6	77.5

It was found that administration of secretin stimulated pancreatic juice secretion in both groups, with different quantitative aspects according to the group. It is found especially stimulating effect of insulin on pancreatic juice volume: Basal (unstimulated) were higher than in controls, after 30 minutes of harvesting in conditions of acute experiment generated a total volume of 387.5 microliters of pancreatic juice from the 5 animals in experiment, which is a average of 77.5 microliters per 30 minutes compared with controls who had received only a total of 337.5 microliters pancreatic juice, with an average of 67.5 microliter per 30 minutes, distinct differences were statistically insignificant. In pancreatic juice samples were determined amylase activity and trypsin activity (Table 4).

Table 4. Enzymatic activity of pancreatic juice in chickens treated with insulin compared with control group

Control group			Insulin group		
	Values			Values	
No. prove	Amylase activity*	Tripsine activity**	No. prove	Amylase activity*	Tripsine activity**
1	351	44.0	1	555	11.1
2	210	23.5	2	540	31.0
3	659	52.2	3	319	33.0
4	640	24.9	4	877	14.5
5	195	36.9	5	74	20.4
- - $x \pm_s x$	411± 132.1	36.3± 2.0	- - $x \pm_s x$	473± 153	22.0± 7.5

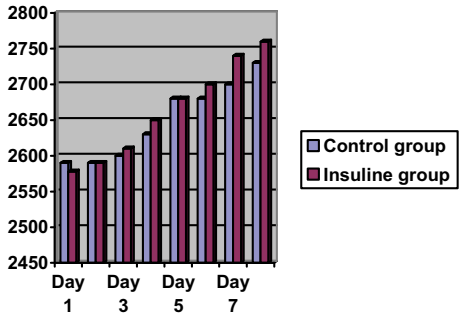

Figure 1. Evolution of chickens weight during the treatment with insulin

Legend

*- Activity expressed in AU/µl = units amylase-hydrolyzed starch amount in mg of the enzyme after its incubation on cooked starch substrate for 30 minutes at 37°C

**- nmoli BAEE (N-benzoil L-arginil etil ester) decomposed/ml/min. at 25°C/µl

So, the group treated with insulin, enzyme activity was stimulated both in terms of amylase, and trypsine. In this group, amylase activity was 473.0 UA from the control group the amylase activity was only 411 UA. Trypsine activity in the same group was 22.0 nmol BAEE (benzoyl ethyl ester arginil), lower than the control. Correlated with effects on growth ponderal, this stimulation of pancreatic enzyme flow explains the weight gain recorded in the experimental group treated with insulin (Figure 1).

CONCLUSIONS

Body weight of Cornish chickens treated with insulin evolved after a witness superior curve, although in other species, commonly, hipoglicemy cause conversely, a lower weight curve. So the average weight of 2578 g at the beginning of insulin administration, after 8 days of treatment it was 2760 g (an average daily gain of 22.75 g per day, compared to 20.00 g in controls). This beneficial effect appears to be due to anabolic protein action of insulin, which sometimes go beyond what was catabolised. Since it is not fat deposits, means that insulin stimulates the growth itself. The administered insulin to chickens Cornish induced pancreatic juice volume a little higher than the control - in 30 minutes the volume was 387.5 microliters, higher with 50 microliters pancreatic juice greater than in controls, differences were insignificant ($P>0.05$). Insulin, to the same type of chicken, stimulated enzyme activity, both in terms of amylase, as well as that of trypsin. Trypsine activity was 22.0 nmol BAEE (benzoyl ethyl ester arginil), higher than in controls. In this way stimulating the flow of pancreatic enzymes to understand why there is a weight increase registered in the group treated with insulin.

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THE EFFECTS OF HELIUM-NEON LASER WITH DIFFERENT ENERGY DOSES ON CRYOPRESERVED RAM SEMEN QUALITY IN VITRO EXAMINATION

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Abstract

The aim of the study was to investigate the effects of different energy doses of helium–neon (He–Ne) laser irradiation on the functions and functional quality of sheep spermatozoa during in vitro liquid storage. In the study of the quality of stored turkey semen was found to be improved significantly following He-Ne laser irradiation and irradiation with He-Ne laser prevented their in vitro liquid storage-dependent damage. It was found also in another study, that irradiation increased the sperm motility index, viability, and cell energy charge. Frozen ram sperm samples in the present study, were thawed in a water bath at 37°C for 30 seconds. Samples pool was divided into three aliquots: one represented the control and the others two was irradiated with He–Ne laser at two different energy dose (3.96 and 6.12 J/cm^2). Motility, viability, osmotic-resistance, acrosomial and DNA intactness were evaluated. The lower dose of laser energy resulted to be ineffective (P<0.05) than other irradiated samples and control. No significant difference between the control and the irradiated samples for viability and osmotic resistance, acrosome integrity and DNA integrity was found. However, the semen samples irradiated with 6.12 J/cm^2 showed a slight increase in sperm progressive motility, viability, osmotic resistance, acrosome and DNA integrity, respect to the semen samples irradiated at low energy doses and control semen samples. Further studies are needed to assess the effect of higher doses of He-Ne laser irradiation on the improvement of the quality ram semen after freezing-thawing process.

Keywords: *laser irradiation, DNA, semen, acrosome*

INTRODUCTION

The widespread application of artificial insemination (AI) and realization of its full potential in sheep depends largely on the use of frozen semen, and thus, on the availability of techniques that result in acceptable fertility (Donovan et al., 2004). Cryopreservation changes the behavioral and functional capacity of spermatozoa; leading to a reduction in motility (Ibrahim et al., 1982) reduced ability of sperm to traverse the cervix and decreased viability in the female reproductive tract (Salamon et al., 1995). This usually results in unacceptably low conception rates in ewes inseminated with frozen semen (Gillan et al., 1999). Thus development of new procedures aimed at improving the quality of cryopreserved spermatozoa is a suitable goal to be pursued. In previous studies it was showed that laser irradiation improved the quality of refrigerated semen in rabbit and turkey species (Iaffaldano et al., 2005; Iaffaldano et al., 2010). Iaffaldano et al. (2013) also found that the effects of post-thaw Helium–Neon (He–Ne) laser irradiation on mobility and functional integrity of frozen/thawed chicken, pheasant and turkey spermatozoa were investigated. Cytochrome C oxidase (COX) activity was also determined as a measure of the effect of irradiation on mitochondrial bioenergetics. The effects of post-thaw Helium–Neon (He–Ne) laser irradiation on mobility and functional integrity of frozen/thawed chicken, pheasant and turkey spermatozoa were investigated. Cytochrome C oxidase (COX) activity was also determined as a measure of the effect of irradiation on mitochondrial bioenergetics.

Every sperm cell consists of a head (acrosome), which contains tightly packed condensed DNA, followed by a short neck containing mitochondria (midpiece), and a thin tail (flagellum), which is responsible for the motility of the cells. The moving speed of a

spermatozoon depends upon energy supply. Spermatozoa maintain low energy consumption during storage in cauda epididymis. These cells are motile but unable to fertilize an egg. Enhanced adenosine-5′-triphosphate (ATP) production becomes critical at the time of fertilization. Motility is activated only upon ejaculation, and so-called "hyperactivation" takes place in the oviduct.

Activation of sperm flagella motility involves both energy metabolism in mitochondria and the motile apparatus of the cells. Mammalian spermatozoa can produce ATP both by anaerobic glycolysis and aerobic breathing. It is well documented that low- power laser irradiation of spermatozoa can increase their motility as well as the ATP amount in cells. Clearly evidenced study results showed that, human sperm motility as well as velocity can be improved by He-Ne laser irradiation. Second, it was found that the irradiation stimulated nonmotile and badly moving but live spermatozoa to move. Later, an important study in this particular field was done by (Breitbart et al., 1996). Stimulation of motility of bull, ram, mouse, and human spermatozoa as well as mouse oocytes by irradiation with visible light of laser and non-laser origin at 632.8, 660, and 780 nm as well as with broad band visible light 400–800 nm were studied. It was found that irradiation of human sperm with broad band visible light (400–800 nm) caused a significant increase in hyperactivated motility, but not in total motility, of human sperm.

A rapid increase in intracellular Ca^{2+} concentration and hyperactivated motility caused by irradiation were significantly reduced when voltage-dependent Ca^{2+} channel was blocked or when Ca^{2+}-deficient medium was used. Biochemical and topological analysis evidenced that fertilizing increased in irradiated spermatozoa.

The quality of stored turkey semen was found to be improved significantly following He-Ne laser irradiation 25 and irradiation with He-Ne laser prevented there *in vitro* liquid storage-dependent damage It was found, that irradiation increased the sperm motility index, viability, and cell energy charge. It was concluded that laser irradiation might be a useful technique for enhancing the quality of semen in long-term storage (Tiina, 2012).

Therefore, the aim of this study was to investigate whether and how two energy doses of laser irradiation (3.96 and 6.12 J/cm^2) can improve the qualitative characteristics and energetic profiles of ram spermatozoa after freezing-thawing process.

MATERIALS AND METHODS

20 sexual mature sheep bucks housed in a private farm were used. Semen from bucks was randomly collected via artificial vagina and pooled to avoid individual differences (5–10 ejaculates/pool; 1 pool/week). Semen was prediluted 1:1 with Tris–citric acid–glucose (TCG) extender (Tris: 250 mmolL^{-1}; citric acid: 88 mmolL^{-1}; glucose: 47 mmolL^{-1}). Frozen ram sperm samples were thawed in a water bath at 37°C for 30 seconds. Each pool was divided into three aliquots: one represented the control and the others two was irradiated with He–Ne laser at two different energy dose (3.96 and 6.12 J/cm^2). Motility, viability, osmotic-resistance, acrosomial and DNA intactness were evaluated (Table 1).

At the same time of storage, cytochrome C oxidase activity (COX) and energetic charge (EC) were assessed on control and irradiated samples to evaluate the energetic functions of spermatic cells.

COX activity was determined spectrophotometrically as Iaffaldano et al. (2010) by dual beam dual wave length system in lysated sperm cells. Energetic charge was measured, as Iaffaldano et al. (2013), by using high performance liquid chromatrography (HPLC) and was defined as the sum of ATP, ADP and AMP fractions, using the following equation:

$$EC = [ATP] + 0.5[ADP]/([ATP] + [ADP] + [AMP])$$

RESULTS AND DISCUSSIONS

The lower dose of laser energy resulted to be ineffective ($P<0.05$) than other irradiated samples and control (Table 1)

No significant difference between the control and the irradiated samples for viability (47.96 ± 2.18 vs. 45.77 ± 1.81 and 49.06 ± 1.66), osmotic resistance (37.94 ± 3.08 vs. 36.45 ± 2.85 and 39.43 ± 1.87), acrosome integrity

(37.89 ± 2.83 vs. 36.68 ± 2.68 and 40.68 ± 1.07) and DNA integrity (98.50 ± 0.29 vs. 97.79 ± 0.35 and 98.74 ± 0.20) was found. However, the semen samples irradiated with 6.12 J/cm^2 showed a slight increase in sperm progressive motility, viability, osmotic resistance, acrosome and DNA integrity, respect to the semen samples irradiated at low energy doses and control semen samples.

In parallel, the effect of irradiation on biochemical parameters of samples was evaluated by measuring the activity of cytochrome oxidase (COX) and the energetic charge (Figure 1).

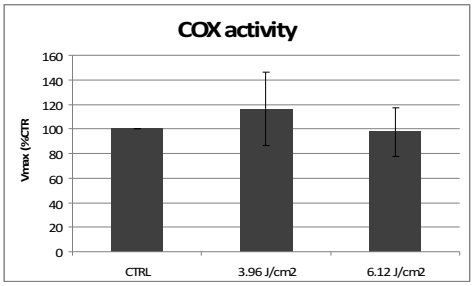
As for parameters reported in Table 1, no significant difference in mean values for both COX activity and Energetic charge between control and laser treated sperm samples was found. This could be mostly due to the extreme variability of semen samples which resulted in an unpredictable effect of laser treatment. Thus, to overcome such a problem, a comparison of all parameters obtained for each single pool is going on.

Also, Iaffaldano et al. (2005) showed an increased cell energetic charge in stored turkey semen after laser irradiation. Previous studies reported that He–Ne laser irradiation on isolated mitochondria resulted in the increase of ATP level (Passarella et al., 1984), RNA (Greco et al., 1989) and DNA synthesis (Pastore et al., 2000), generation of new mitochondria (Maxwell et al., 1996), enzyme activation (Faustini et al., 2004) and modifications in the substrate enzyme interaction. In sperm cells ATP, generated in the mitochondria, is primarily required for sperm motility. Thus the mitochondria can play roles other than just energy supply which are needed to maintain the contractibility of the tail, e.g. the regulation of the calcium flux and membrane potential.

Table 1. Sperm qualitative parameters (%) of cryopreserved and irradiated ram semen

Semen treatment	Semen parameters (%)					
	Mass motility	Progressive motility	Viability	Osmotic resistance	Acrosome integrity	DNA integrity
Control						
	53.17 ± 3,17a	44.5 ± 2.44a	47.96 ± 2.18a	37.94 ± 3.08a	37.89 ± 2.83a	98.50 ± 0.29a
Time 1 (3.96 J/cm^2)						
	43.67 ± 1,96b	36.50 ± 1.04b	45.77 ± 1.81a	36.45 ± 2.85a	36.68 ± 2.68a	97.79 ± 0.35a
Time 2 (6.12 J/cm^2)						
	52.83 ± 1.83a	45.17 ± 1.87a	49.06 ± 1.66a	39.43 ± 1.87a	40.68 ± 1.07a	98.74 ± 0.20a

a-b Different superscript letters within the same column indicate a significant difference ($P < 0.05$).



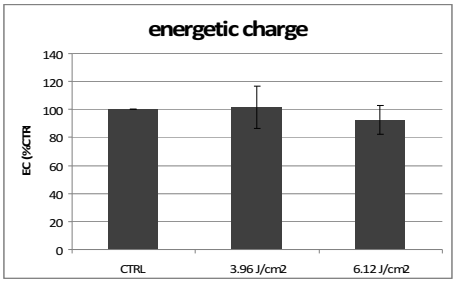


Figure 1. COX activity and Energetic charge of cryopreserved and irradiated ram semen

CONCLUSIONS

The lower dose of laser energy resulted to be ineffective than other irradiated samples and control. No significant difference between the control and the irradiated samples for viability and osmotic resistance, acrosome integrity and DNA integrity was found. However, the semen samples irradiated with 6.12 J/cm^2 showed a slight increase in sperm progressive motility, viability, osmotic resistance, acrosome and DNA integrity, respect to the semen samples irradiated at low energy doses and control semen samples.

Further studies are needed to assess the effect of higher doses of He-Ne laser irradiation on the improvement of the quality ram semen after freezing-thawing process.

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EFFECT OF Pb-ACETATE IN DRINKING WATER AND PHYTATE IN DIET ON CALCIUM, ZINC AND IRON IN BLOOD OF GROWING DUCK

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Abstract

The research was conducted at the Laboratory of Physiology and Biochemistry, Faculty of Animal Husbandry and the Laboratory of Chemistry Material and Environment, Faculty of Mathematics and Natural Science, Padjadjaran University. Treatment of Pb-acetate in drinking water was given during 16 weeks. The purpose of this study was to determine the effect of Pb-acetate in drinking water and phytate in diet on concentrations of calcium, zinc and iron in blood of growing duck. The experimental design used a completely randomized design (CRD) with a factorial pattern 3x3. Three levels of Pb-acetate in drinking water (0, 45 and 90 ppm) and dietary treatments containing different levels of phytate (0,12; 1,16 and 2,18%) with 3 replications were implemented. The results showed that there was no interaction between lead in drinking water and phytate in diet on calcium, zinc and iron of blood. The main effect of lead treatment up to 90 ppm increased calcium, zinc and iron in blood, but the main effect of phytate up to 2,18% in diet did not give significant difference.

Keywords*: phytate, lead, calcium, zinc, iron, duck*

INTRODUCTION

Duck's life cannot be separated from the water, both as a place to swim or as a source of drinking water. In fact water cannot be separated from pollution as a result of a wide variety of household and industrial waste (PPSDAL, 2004). Thereby, both the poor quality of water either directly or indirectly affect the growth of ducks, especially blood minerals. Many problems encountered in the field because ducks reared extensively that allows ducks exposed to contamination, especially lead (Pb). This pollution will ultimately lead to consumers who consume the products of duck.

Many studies reveal that the lead is a heavy metal that is most dangerous after mercury (Saeni, 1989; Darmono, 2001). The apparent effect on livestock, for example disruption of metabolism, is causing a decrease in body weight and high mortality. Basically, the lead enters the body through food or drink, the air and the skin. One of the efforts to minimize lead enters the body by way of phytate in the diet (Noor, 1992). How this is done because phytate has the ability to bind lead. Although phytate serves as anti nutrition substance, but it also serves as *chelating agent* for divalent metals, especially lead. Another way that is by changing the pattern of extensive maintenance becomes intensive in areas suspected of heavy metal contaminated lead.

In daily life, ducks also play a role in contributing to society through improved nutrition fulfilment of meat and eggs. On the farm, most of the ducks were reared extensively but lately with the increasing environmental pollution, especially lead, then the system needs to be improved so that maintenance of duck lead contamination either through food or drinking water.

The author tried to collect a variety of information field research the extent to which the effects of lead pollution on blood indicator minerals such as calcium, zinc and iron in blood. This laboratory study was conducted by simulating field conditions so that the physiological parameter that was used to provide a foundation in drawing a conclusion.

MATERIALS AND METHODS

In this experiment, 135 ducks aged 29 days with body weight between 212.5-306.55 grams. Due to they were as a continuation of the first experiment, thereby they had been treated lead-acetate since that time.

Ducks were kept in the same cage as in the first experiment and were treated for the next 12 weeks. At the end of the week sixteen, blood samples of ducks were taken randomly from each unit of the cage as a sample for analysis.

Phytic treatment used in this experiment were bounded in the bran, corn and soybean meal. Based on the analysis and calculations, phytic content of the experimental diets as follows: R0 = 0:12%, R1=1:16% and R2=2:18%.

Contaminant material was lead-acetate with molecular formula $(CH_3COO)+3H_2O_2$. Pb is given to duck through drinking water in *ad libitum*. Treatment concentrations of lead (lead-acetate) given in drinking water was made in the following manner: Pb0=0 ppm, Pb1=45 ppm and Pb2=90 ppm.

The experimental diet was a mash-shaped and made with 15% protein content and metabolizable energy 2800 kcal/kg.

The measured variables of this study were calcium, zinc and iron of blood, and the experimental design of this research was a completely randomized design with a 3x3 factorial pattern. The first factor was the phytate in the diet with 0.12% (R0); 1.16% (R1) and 2.18% (R2), while the second factor was the lead in drinking water with levels of 0 ppm (Pb0): 45 ppm (Pb1) and 90 ppm (Pb2). Data were analyzed with ANOVA followed by Duncan's test. The experiments were repeated three times with five individuals per sub-test.

RESULTS AND DISCUSSIONS

Effect of Lead and Phytate on Calcium, Zinc and Iron Blood

No interaction between phytic content of the ration and Pb in drinking water on calcium, zinc and iron of blood. Effect of Pb in drinking water showed significant effect ($P<0.05$), whereas phytate in the diet showed no significant effect on calcium, zinc and iron of blood.

Effect of Pb in Drinking Water on Calcium, Zinc and Iron of Blood

Duncan's multiple range test results regarding the effect of Pb in drinking water to calcium, zinc and iron duck blood grower phases can be seen in Table 1.

Table 1. Results of Duncan's Multiple Range Test Effects of Pb in Drinking Water on Blood Mineral of Growing Duck

Treatments	Ca (ppm)	Zn (ppm)	Fe (ppm)
Pb0	62.74^{a}	23.46^{a}	408.84^{a}
Pb1	63.63^{bc}	36.75^{b}	544.12^{b}
Pb2	76.78^{c}	38.18^{b}	547.69^{b}

Description: the same letter in the same column showed no significant difference

Table 1 illustrates that the blood calcium in the treatment of growing duck of Pb1 and Pb2 did not show significant differences, but the blood calcium in both the treatment significantly ($P<0.05$) higher than that of calcium in the treatment Pb0.

The high concentration of calcium in the blood of growing duck with increasing intake of Pb, most likely due to have occurred mobilization of calcium from bone into the blood. This is as a result of Pb deposition in the bone to encourage the release of calcium from the bone matrix due to parathyroid hormone action.

According to Klassen (1986), parathyroid hormone can mobilize bone mineral and its role in the synthesis of 1,25-dihidxyvitamin D. The action of this hormone resulted in the collection of citrate in bone. The citrate solution causes of woven bone mineral mobilization and move into the extracellular body fluids, subsequently resulting in bone mineral dissolution and transport of calcium citrate pass into the blood plasma. The citrate will undergo metabolism in plasma or excreted in the urine while the calcium remains in the blood plasma.

The pattern of the same relative increased calcium either in starting ducks of the experiment 1 or growing ducks (Kamil et al., 2011). This suggested that the starting duck was more sensitive than the growing duck to administration of Pb in drinking water. Therefore, a long time giving ducks Pb and age likely plays a role in maintaining the consistency of calcium to some extent Pb poisoning.

Table 1 illustrates that blood zinc in Pb1 and Pb2 treatment showed no significant difference but blood zinc in both the treatment was significantly higher than in the blood zinc treatment Pb0. It is interesting from an increase in blood zinc duck grower turned out to have the same pattern as it did of starting ducks (Kamil et al., 2011).

Increased blood zinc phenomenon was due to the strong influence of Pb given through drinking water that could encourage greater uptake of zinc. This was due to that the growing number of incoming Pb, most likely will change the pH of the small intestine is the major site for absorption. According Wahyu (1997), the pH of the small intestine has a pH in the range 5-6. Increased intake of Pb given through drinking water, most likely more acidic small intestine and result in an increased absorption of zinc so zinc in the blood increased.

Table 2 shows that the levels of iron in the treatment of growing ducks of Pb2 and Pb1 showed no significant difference, but the iron in the blood of the two treatments was significantly higher ($P<0.05$) compared with an average iron content on Pb0 treatment. These results had the same pattern as the increase in blood iron levels in starting ducks (experiment I).

Increased iron in the blood of both the growing and the starting ducks (experiment 1) treated with Pb through drinking water, it can be explained that the more incoming Pb, most likely will change the pH of the small intestine was the major site for absorption which increases the absorption of iron, thereby, the iron in the blood increased.

Another possibility is that the micromineral content must be enough in the food, so that iron remains high in the plasma. This was in line with the opinion of Kasperczyk et al. (2012).

Effect of phytate in the diet on calcium, zinc and iron of blood

Duncan's multiple range test results regarding the effect of phytate in the diet on calcium, zinc and iron of duck grower can be seen in Table 2.

Table 2. Results of Duncan's Multiple Range Test Effects of Phytate in Diet on Blood Mineral of Growing Ducks

Treatments	Ca (ppm)	Zn (ppm)	Fe (ppm)
R0	66.31[a]	30.26[a]	537.32[a]
R1	67.26[a]	38.12[a]	515.73[a]
R2	69.58[a]	30.05[a]	447.60[a]

Description: the same letter in the same column showed no significant difference

Table 2 shows that calcium, zinc and iron in the blood of growing ducks of R0, R1 and R2 did not show significant differences. This was due most likely to decompose phytate late, therefore the effect of phytate did not show significant differences on calcium, zinc and iron in blood. This was due to the effect of phytate on Pb which has a larger molecule showed that it was significant effect. Most likely others that needed higher phytate and purely to affect blood calcium, as seen from the content of phytate in the diet to 2.18% seen no significant effect on growing ducks though the molecular weight of Pb greater than calcium.

In addition, because the nutrient content in the form of micromineral is enough in food, then absorption will also be parallel as reported Kasperczyk et al. (2012).

CONCLUSIONS

The results showed that there was no interaction between lead in drinking water and phytate in diet on calcium, zinc and iron of blood. The main effect of lead up to 90 ppm increased calcium, zinc and iron in blood, but the main effect of phytate up to 2.16% in diet did not give significant difference.

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RESEARCH ON FEEDING DAIRY COWS IN PUERPERAL PERIOD

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Abstract

This paper aims to study the effect of feed rations structures and other factors on the reproductive performance and the yield in dairy cattle. It follows a study conducted between January 2013 and January 2014 on 60 animals from Arges County, using the comparative method. The animals were analyzed by groups, in a commercial type A farm and in GP system (household population) and by the period of the sexual cycles.

Key words*:cow feeding, puerperal period, milk cows.*

INTRODUCTION

Diet is one of the most important external environmental factors that contribute to high milk yields, namely the development and reproduction of animals. Poor nutrition during pregnancy, lack of exercise and polluted microclimate are directly related to the frequency of puerperal disorders.

In case of undernourishment, animals become susceptible to diseases and reproduction is disturbed by lowering their fecundity and prolificacy. Milk production and weight gain will also be reduced, which ultimately leads to the cost price raise of the products obtained and hence lower profitability of livestock.

Normal and pathological reproduction problems in farm animals are of great interest because they are affecting the livestock increasing, the breed structure improvement and production increasing, too. Studies with dairy heifers and older cows have shown little effect of maternal nutrition during the last month of gestation on calf birth weight or dystocia (Tapaloaga P., 2002). Two-thirds of fetal birth weight is occurred during the last trimester of gestation. Severe nutritional restriction during the last trimester, to the extent that the dam loses body condition, leading to reduced placental and foetal weight and pelvic area, can result in dystocia and stillbirth due to uterine inertia and inadequate relaxation of the pelvic ligaments (Tapaloaga, 2002). Overfeeding during the last trimester, to the point that dam body condition score is increased can result in fetal oversize and excess adipose deposition in the birth canal in heifers with consequent dystocia and stillbirth (Stoica, 1994).

MATERIALS AND METHODS

Results from this paper have been incurred by a survey conducted during January 2013 - January 2014 on a batch of 60 animals using the comparative method.

In order to analyze the environmental factors, food and microclimate, the used methods were documenting from scientific literature and the interpretation and analysis of the results was made in terms of quality and quantity.

The study followed 60 lactating cows from the Brown cattle breed between January 2013 and January 2014 in Arges County, as follows: 30 animals from the PG (population household) system and 30 animals from a commercial type A farm. Postpartum uterine involution was monitored for each animal and animal examination was performed every 3 times/day. The presence of clinical signs of oestrus was recorded in the individual gynaecological sheets. The 30 animals in the commercial farms were fed with a complete balanced ration for middle milk yield.

The 30 animals in the population households were fed differently, depending on the possibilities of each household.
The 30 cows in commercial type A farm were divided into groups depending the moment of the heat appearance after calving:

- Group I consists of 11 cows, they had estrus at the first sexual cycle, at 21 days respectively;
- Group II consists of 10 cows, they had estrus at the second sexual cycle, at 42 days respectively;
- Group III consists of 3 cows, they had estrus at the third sexual cycle, at 62 days respectively;
- Group IV consists of 4 cows, they had estrus at the fourth sexual cycle, at 84 days respectively.

The 30 cows belonging to the P.G. system (population household) were also divided into groups depending the moment of the heat appearance after calving, too:

- Group I consists of 6 cows, they had estrus at the first sexual cycle, at 21 days respectively;
- Group II consists of 10 cows, they had estrus at the second sexual cycle, at 42 days respectively;
- Group III consists of 7 cows, they had estrus at the third sexual cycle, at 64 days respectively;
- Group IV consists of 4 cows, they had estrus at the fourth sexual cycle, at 84 days respectively;
- Group V consists of 3 cow, it had estrus at the fifth sexual cycle, at 105 days respectively.

RESULTS AND DISCUSSIONS

The concept of animal fertility was approached by various researchers, depending on diet and other factors.
During **winter time**, animals should be given food rations with voluminous and succulent fodder satisfying 60-100% of the required values plus concentrates depending on the milk production.
Hay is a basic component of the food ration and an important source of proteins, minerals, vitamins, and the recommended amount of 1.8-2 kg/100 kg live weight. For dairy cows are recommended alfalfa hay, clover, mash and cultivated grasslands. In hilly areas, hay arerepresented by the mixture of grasses with legume, specific to the area. **Roughage** is recommended depending on milk production, reaching to 9-12 kg / head / day. For lactating cows are recommended alfalfa hay, clover, mash and cultivated grasslands (Dinescu, 2005).
Fodder concentrates are recommended in amounts of 0.25-0.35 kg/l milk for the cows with milk yield higher than 8-10 l in winter and 13-15 l in summer. Due to the fact that animals are fed with natural hay, it is imperative that the ration contains both wheat bran (25-50%) and grist (10-15%). At the end of the winter period, the best hay of legume, silage and concentrate mixtures with vitamin and mineral well-structured premixes are used in daily rations (Dinescu, 2005).

Summer feeding
During summertime, basic fodder is the grassland, from cultivated grasslands to alpine pastures. Additional concentrates (wheat bran) were added. Green fodder given to dairy cows averages 10-12 kg/100 kg live weight. Alfalfa and clover are recommended to be administered in withered form (Dinescu, 2007).
Following observations made on commercial type A farm, was found that:
Group I, consisting of 11 animals, received a balanced food ration, ensuring the necessary proteins, minerals and vitamins, microclimate was appropriate, postpartum uterine involution was monitored by clinical transrectal examination and was found that after 21 days, full morphological uterine involution was complete.
Group II, consisting of 10 cows, consists in animals with a medium milk yield with good reproduction results, even very well at artificial insemination.
Group III, consisting of the 3 cows, which had estrus at the third sexual cycle experienced unfortunately retention of the foetal covering. They have a much higher milk yield in comparison with the animals of groups I and II, and had a prolonged cold period to the group I and II cows.

Foetal coverings were removed; pessaries and antibiotics were administered for 3 consecutive days. Leakage and the general condition of the animal were also monitored.

Group IV consists in animals are primiparous, they accidently mated during grazing.

All these animals reared in commercial farms received balanced rations in vitamins, minerals, energy and proteins.

Animals that are part of the **population household (PG)** presented poor living conditions, they were fed with poor quality hay, without fodder concentrates and without ad libitum watering, while the animals from the commercial type A farm benefited during summertime from ad libitum cultivated pasture, combined fodder concentrates, combined semi silage perennial grasses, winter brewers draff in wintertime. At the 6 cows from **group I**, uterine involution was complete, animals were primiparous. Animals were monitored and examined daily and the changes that occurred were noted in individual gynaecological sheets.

Cows in **group II** presented poor living conditions and are at the third gestation and presented dystocia for two consecutive years and following placental retention. Lack of balanced rations and lack of appropriate microclimate led to consecutive dystocia, subsequently delaying the onset of sexual cycle.

Group III with an average of the sexual cycle of 60 days, are at the fifth gestation, two of them had twin births. At three of the animals mastitis and embryo death occurred, subsequently found due to the lack of microelements.

Group IV, the four cows had hoof conditions, consequence of hard floors.

Group V, 3 cows had retention of fetal covering for three consecutive years, and the last gestation was not carried to term because of a late miscarriage. Purulent discharge occurred and was administered Metrosept antibiotics. Uterine involution and leakage characteristics were monitored.

Table 1. Details about animal groups numbers and sexual cycle period

	Type A farm animals / group	Estrus in I, II, III, IV and V sexual cycles	Actual physiological sexual cycle period for type A farm animals group	Population Household (PG) animals / group	Estrus in I, II, III, IV and V sexual cycles	Actual physiological sexual cycle period for GP animals group
Group I	11	I	21 days	6	I	21 days
Group II	10	II	42 days	10	II	42 days
Group III	3	III	62 days	7	III	64 days
Group IV	4	IV	84 days	4	IV	84 days
Group V	-	-	-	3	V	105 days

CONCLUSIONS

It is clear that fodder rations are strictly related to reproductive performance, especially for dairy cows.

It was found that for the animals that received balanced rations and had a suitable microclimate, the health status has not been affected as much as those who received unbalanced rations.

Unqualified interventions in dystocia, unsanitary conditions, food without vitamins, minerals and proteins have led to delayed sexual cycles.

Cattle's breeding is harder than other species because deviations in fodder rations and living conditions have direct effects on the reproduction function (Tapaloaga, 2008).

The effects of uncontrolled puerperium that degenerate into pathological disorders are serious and with repercussions on reproduction and yield. The increased capability for milk production has been associated with a decline in fertility of lactating cows. Nutritional requirements increase rapidly with milk production after calving and result in negative energy balance (Stoica, 1994).

The negative energy balance delays the time of first ovulation through inhibition of LH pulse frequency

and low levels of blood glucose and insulin that collectively restrain oestrogen production by dominant follicles. It reduces serum progesterone concentrations and fertility. Diets high in crude protein support high milk yield, but are also associated with lower reproductive performance. High protein can result in elevated plasma urea concentrations that affect the uterine environment and fertility. Nutritional interactions resulting in poor fertility of high producing dairy cows include the antecedent effects of negative energy balance and effects of high dietary protein.

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THE INFLUENCE OF IRON AND COPPER OVER THE INTESTINAL ENZYMATHICAL ACTIVITY

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Abstract

The purpose of this study is the analysis of copper and iron microelements influence over the activity of intestinal amylase and maltase unfolding research on rats that the physiologically resemble swine, making it possible that the data obtained can be extrapolated. In most cases, the addition of microelements influence has manifested by inhibition of the enzymatic activity of intestinal maltase and amylase, growing with the increasing of concentration. A slight activation of intestinal amylase was observed with the administration of 600 ferric sulphate mg/kg food, maximal activation being of 104.3%.

Keywords: *copper, iron ,intestinal amylase, intestinal maltase.*

INTRODUCTION

Enzymes are biocatalysts, representing a special class of protein molecules that alter the kinetics of reactions which they catalyze, participating in small amounts in these reactions and do not undergone transformations during their development.

Enzymatic reactions can be influenced by various substances that act directly on the enzyme molecules. For example, salts of heavy metals, such as silver, copper, mercury or lead, in high concentrations, inactivate most of the enzymes. Some enzymes are very sensitive to even low concentrations of metal, such as for fructofuranosidase, which is inhibited by the silver salts.

The mechanism of inhibition is relatively well known, some authors suggesting that metallic ions combine with thiolic groups, while others do with the carboxyl groups.

Although the economic importance of the use of trace elements in animal rations is known, a relatively low number of data regarding their influence on the activity of various digestive enzymes is noticed.

Amylase is an enzyme that catalyzes the reaction for conversion of starch to dextrins and then to maltose. Amylase occurs in the digestion of carbohydrates, being secreted by the salivary glands, pancreas and small intestine mucosa.

Maltase is an enzyme secreted by the lining of the small intestine mucosa that breaks down the disaccharide maltose into two molecules of glucose.

Therefore, the aim of this study is the analysis of copper and iron microelements influence over the activity of intestinal amylase and maltase, the biological material being represented by rats, knowing from the metabolically point of view the rat specie resembles the swine, which will make possible extrapolation of data obtained in swine.

MATERIALS AND METHODS

In order to study the action of the microelements trace of copper (Cu^{2+}) and iron (Fe^{2+} and Fe^{3+}) the metal sulphate (cupric sulphate, ferrous sulphate, ferric sulphate)was used in these, being added to the vitamin and mineral premixes.

The biological material was represented by Wistar rats. They were fed a compound food similar to the one given to the young swine (Table 1). The batches of animals received premixes with a variable content of iron and

copper (Table 2). In a first stage 50 rats were used, being assigned to 10 batches, that have received as a supplement in premix divalent iron as ferrous sulphate which provided a content of 150, 300, 450, 600, 750, 900, 1050, 1200, 1350 mg/kg feed. In the second phase another 50 rats received mixes in which the trivalent iron content in the form of addition of ferrous sulphate varied, providing 150, 300, 450, 600, 750, 900, 1050, 1200, 1350 mg/kg feed. In the third step another 10 batches of 5 rats were given copper in premixes under the form of copper sulphate, providing 10, 20, 30, 40, 50, 60, 70, 80, 90 mg/kg.

Table 1. Composition and nutrients of diet

Ingredient	Composition (%)
Maize	65.50
Soya meal	20
Sunflower meal	10
L-lysine	0,37
DL-methionine	0,08
Threonine	0,05
Calcium carbonate	1,21
Dicalcium phosphate	0,86
Salt	0,50
Vitamin-mineral premix	1,00
Total	100.00
Nutrients level (calculated)	
Metabolizable energy (Mj/kg)	3126
Crude protein (%)	17,95
Lysine (%)	1,19
Methionine+cystine (%)	0,61
Calcium (%)	0,85
Total phosphorus (%)	0,48
Available phosphorus (%)	0,29

Table 2. Content of iron and copper of the administrated ratio

Experimental batch	Iron (mg/kg feed)	Copper (mg/kg feed)
E 1	0	0
E2	150	10
E3	300	20
E4	450	30
E5	600	40
E6	750	50
E7	900	60
E8	1050	70
E9	1200	80
E10	1350	90

The action of the two microelements has been studied using samples collected from the intestinal mucosa of rats after slaughter. Portions of the mucosa were homogenized in distilled water (4 ml per 1 g of tissue), after which they were kept for 24 hours at 4^{o}C and then centrifuged for 15 minutes.

The determination of enzyme activity was performed by dosing of a substrate of enzymatic reaction (starch) in the presence of the enzyme (amylase and maltase) that catalyze these reactions. The enzymatic activity represents the difference between the transformed substrate concentration in reaction product by enzyme and unchanged substrate concentration.

The quantitative expression of the enzymatic activity is done by activity units (U). U is the amount of enzyme that catalyzes the conversion of 1 μM substrate/min/l under optimum conditions of temperature and pH (Diaconescu, 2004). In current mode, the enzymatic activity is expressed as mU/ml. One unit of amylase or maltase activity is defined as the amount of enzyme that catalyses the conversion of 1 μg of substrate, namely glucose, per 1 minute/l at a pH of 7.

RESULTS AND DISCUSSIONS

The results concerning the influence of iron and copper microelements on the enzymatic activity of amylase and maltase are presented in Table 3.

The action of ferrous sulphate on intestinal amylase activity is materialized by a low reduction (3.1%) until the concentration of 300 mg/kg feed, while at the amount of 1050 mg/kg the decreased activity is of 24.7%, at 1200 mg/kg is of 38.5% and at 1350 mg/kg it reaches 56.3%.

Rat intestinal amylase is easily activated in the presence of small amounts of ferric sulphate, the maximal activation (104.3%) being found in a concentration of 600 mg/kg feed. At the following amount of 750 mg ferric sulphate / kg feed, there is a slight decrease from the maximum activity (with 15.7%). An almost total reduction of the enzyme (9.3 Uml^{-1}) occurs at a concentration of 1350 mg/kg feed.

From the analysis of the influence of copper sulphate over the action of intestinal amylase on rats, a slight decrease in activity up to the concentration of 30 mg/kg feed is observed. The noticeable reduction is of about 5.3%. At an increase in the amount of copper sulphate in feed above this value, a strong reduction of amylase activity about 57.3% in the amount of 70 mg/kg feed, 80.6% at 80 mg/kg and 94.2% at 90 mg/kg are observed.

Table 3. Influence of iron and copper of the intestinal enzymatic activity

Content in microelements of feed (mg/kg feed)	Amylase Uml^{-1} %	Maltase Uml^{-1} %
Divalent Iron		
0	15.57±1.12 100	13.93±1.12 100
150	15.18±1.04 97.5	13.70±2.42 98.4
300	15.09±0.87 96.9	13.44±1.37 96.5
450	14.23±1.65 91.4	12.70±1.63 91.2
600	14.18±1.53 91.1	11.92±1.93 85.6
750	14.14±1.84 90.8	11.86±2.01 80.5
900	13.45±0.97 86.4	10.43±1.11 74.9
1050	11.72±1.35 75.3	9.79±0.86 70.3
1200	9.57±1.74 61.5	9.48±1.04 68.1
1350	6.80±0.53 43.7	8.98±0.64 64.5
Trivalent Iron		
0	20.17±2.12 100	12.41±0.79 100
150	20.47±3.07 101.5	12.42±1.17 100.1
300	20.85±2.52 103.4	12.41±2.04 100.0
450	20.91±1.95 103.7	12.41±1.89 100.0
600	21.03±2.75 104.3	12.43±0.99 100.2
750	17.00±1.29 84.3	12.45±2.10 100.4
900	15.18±1.49 75.3	12.39±1.49 99.9
1050	10.51±1.11 52.1	12.41±1.75 100.0
1200	6.99±0.48 34.7	12.38±1.36 99.8
1350	1.87±0.07 9.3	12.39±1.92 99.9
Copper		
0	17.34±1.93 100	14.35±1.37 100
10	17.25±1.14 99.5	14.69±1.59 102.4
20	16.52±1.47 95.3	14.91±2.00 103.9
30	16.42±1.57 94.7	15.11±1.26 105.3
40	13.94±1.04 80.4	16.14±1.42 112.5
50	13.14±0.85 75.8	14.29±0.95 99.6
60	7.40±0.34 42.7	10.80±0.54 75.3
70	3.36±0.08 19.4	6.63±0.25 46.2
80	1.85±0.02 10.7	4.33±0.08 30.2
90	1.00±0.01 5.8	1.35±0.01 9.4

L. Fang et al. (2012) observed that pancreatic amylase was more sensitive than salivary amylase to small amounts of copper chloride.

Li et al. (2007) noticed that the addition of copper to hybrid tilapia reduced the activity of intestinal protease and inhibited the activities of amylase in intestine and hepatopancreas. Addition of iron reduced the activities of amylase in the intestine by 47.9%, but had no effect on amylase activities in the hepatopancreas.

Intestinal maltase in rats shows a steady decrease in activity related to the Fe^{2+} ion concentration, inactivation being more than 35.5% for an amount of 1350 mg ferric sulphate/kg feed.

Analyzing the influence of ferric sulphate (Fe^{3+}) over the activity of intestinal maltase it was found that Fe^{3+} ions had no effect over the studied enzyme, its values remaining constant at the variation of the amount of administered ferric sulphate.

Intestinal maltase undergoes a further activation in the presence of copper sulphate up to the amount of 40 mg/kg feed, when the activation was of 12.5%. For 50 mg copper sulphate/kg feed, the intestinal maltase activity is slightly inhibited, and for the subsequent increase in the amount of copper sulphate, the maltase activity is inhibited by 53.8% at 70 mg/kg and by 69.8% at 80 mg/kg. For the maximum concentration analyzed (90 mg/kg) it was found to inhibit the enzyme activity, which reaches only 9.4% of the initial activity.

Studying the effects of heavy metals over the intestinal maltase activity for rats, it was observed that the average amount of added copper ions, the concentration of enzyme was moderately inhibited.

CONCLUSIONS

From analysis of the presented results, it follows that the addition of divalent or trivalent iron and copper influence the activity of intestinal amylase and maltase in rats that resembles the physiologically with swine, which allows the data obtained to be extrapolated from them.

In most cases, the addition of microelements influence has manifested by inhibition of the enzymatic activity of intestinal maltase and amylase, growing with the increasing of concentration.

A slight activation of intestinal amylase was observed with the administration of 600 ferric sulphate mg/kg food, maximal activation being of 104.3%. Further, with the increase of the addition of divalent iron source a nearly complete inactivation of the enzyme (9.3 Uml^{-1}) at a concentration of 1350 mg/kg feed is achieved.

The addition of copper sulphate until the amount of 40 mg/kg of feed determines an activation of intestinal maltase, so that, afterwards, at increased amounts of copper sulphate to lower the enzyme activity, reaching the maximum concentration analyzed (90 mg/kg), the inhibition the enzymatic activity to be of 90.6%.

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PHYSIOLOGICAL AND MOLECULAR ASPECTS OF HEART OF RAT FED WITH CHOLESTEROL REACH DIET - THE IMPACT OF PROCAINE TREATMENT

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Abstract

The aim of this study was to investigate the effect of ischemia reperfusion(I/R) upon cardiac physiological parameters(CF) coronary flow,(HR) heart rat as well as LVPD(Left ventricle pressure developed) as well as on apoptosis in adult Wistar rats feed on Cholesterol diet and treated with Procaine. Material and methods:18 male Wistar rats aged 12 months have been used in our experiment divided into 3 groups of 6 rats each: group A Controls, Group B Cholesterol feed rats, group C Cholesterol feed rats treated with Procaine (20mg/kg body weight for 8 weeks). High cholesterol diet (lard mixed with chew) has been used to feed rats for 8 weeks. At the end of treatment the hearts have been excised and mounted in Langendorff reperfusion system. A 45 minutes ischemia has been followed by 120 minutes reperfusion on isolated rat heart in order to measure heart rate, coronary flow and left ventricle developed pressure as well as to assay left ventricle for apoptosis .Our data have pointed out modifications in physiological parameters and the presence of apoptosis in cholesterol treated rats while the Procaine seems to have a benefic effect on these parameters and on DNA integrity.

Keywords*: ischemia reperfusion, rat heart, coronary flow, heart rate, apoptosis*

INTRODUCTION

The heart muscle is largely dependent on uninterrupted blood flow which guarantees delivery of substrates and washout of harmful products of metabolism (Braunwald et al., 1992). The death of cardiac cells during ischemia and reperfusion is partially mediated by apoptosis (Sato et al., 2004; Kaul, 2001).
The aim of this study was to investigate the effect of ischemia reperfusion (I/R) upon cardiac physiological parameters (CF) coronary flow,(HR) heart rat as well as LVPD (left ventricle pressure developed) as well as on apoptosis in adult Wistar rats feed on Cholesterol diet and treated with Procaine (20 mg/kg body weight for 8 weeks.

MATERIALS AND METHODS

The 18 male Wistar rats aged 12 months have been used in our experiment divided into 3 groups of 6 rats each: group A Controls, Group B Cholesterol feed rats, group C Cholesterol feed rats treated with Procaine.
High cholesterol diet (Lard mixed with chew) has been used to feed rats for 8 weeks.
Group C have received also Procaine treatment (20 mg/kg body weight) for 8 weeks.

HEART PREPARATION FOR PERFUSION

The male Wistar rats have been anesthetized with Na Phenobarbital (20 mg/kg body) slowly administrated into the tail vein, together with 250 U heparin. When deep anesthesia has been fully installed, the thoracic cage has been opened and the heart has been quickly removed by excision of big vessels and then passed into cold perfusion, weighted and then mounted on a canula in Langendorff apparatus for retrograde perfusion in 2 minutes maximum.

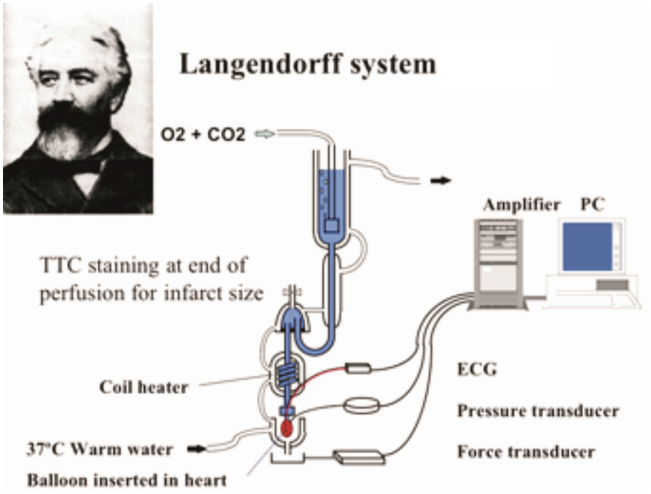

Figure 1

MOUNTING OF HEART IN LANGENDORFF PERFUSION SYSTEM

The hearts have been perfused in a noncirculant system with Krebs Henseleit (KHB) medium aired with 95% O_2 and 5% CO_2 in order to obtain a pH 7.35-7.40. The intracardiac temperature has been permanently monitorised by means of a thermocouple and maintained at 37°C with a thermostat bath.
The pressure developed by the left ventricle (LVPD) has been permanently recorded with a isovolumetric balloon positioned in the left ventricle through an incision of the left atrial apex. The balloon volume has been adjusted at the beginning of the experiment in 8-19 mm Hg. The CF (coronary flow) has been measured during the experiment
We used an ischemia (45 minutes) followed by 120 minutes reperfusion model of isolated rat heart in Langendorff retrograde perfusion.
TACS Apoptotic DNA laddering kit has been used to assay heart cells for apoptosis.
PRINCIPLE OF ASSAY
TACS Apoptotic DNA laddering kit has been used to assay tissues for apoptosis by detecting internucleosomal DNA fragmentation and displaying DNA laddering.

EXPERIMENTAL PROCEDURE
The heart tissue fragments (left ventricle) has been minced into small pieces and frozen in liquid nitrogen, then 0.2-1 g of frozen tissue has been grinded into the powder and then resuspended in 200 µl sample buffer.
20 µl of 10X tissue buffer has been added and incubated at 50°C for 12-14 hours with a gentle shaking.
DNA isolation has been done according with the instruction guide.
Etd.Br. labelling and detection of apoptosis
1 µl DNA has been diluted in 9 µl DNA free water. 2 µl gel loading buffer has been added and the next steps on electrophoresis have been done according with the instructions. Then the gel has been stained for 15 minutes in 0.5 µg/l Etd.Br.
The visualisation of DNA stained with Etd.Br has been done using UV transiluminator. The Photographs have been processed with KodakWratten 22A filter (Yellow).

RESULTS AND DISCUSSIONS

The oxidative stress imposed by 45 minutes ischemia followed by 120 minutes reperfusion intensifies the deleterious effects of pathological state on heart imposed by cholesterol treatment (Ambrosio, 1999; Brar et al., 2000; Braunwald et al., 1992). The effects are reflected upon Coronary flow, heart rate and left ventricular developed pressure.

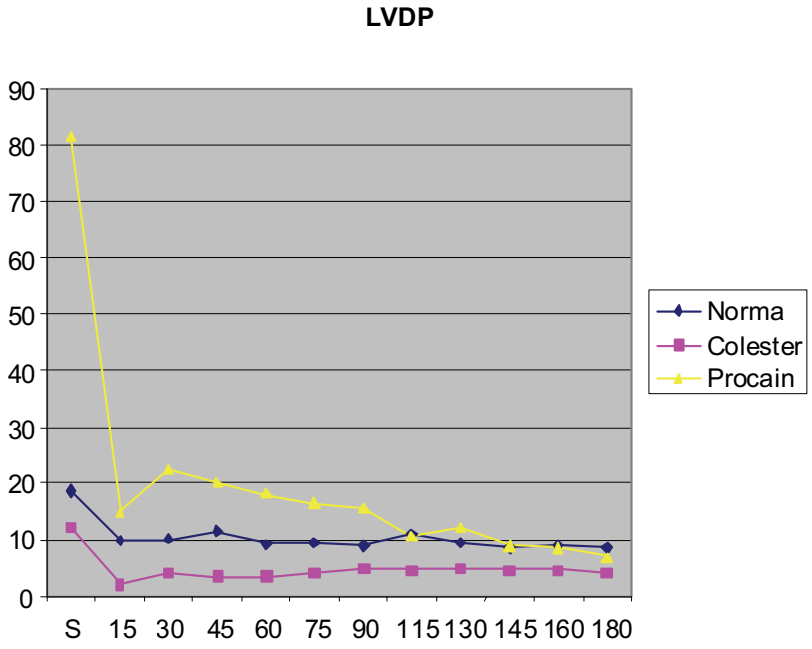

Figure 2

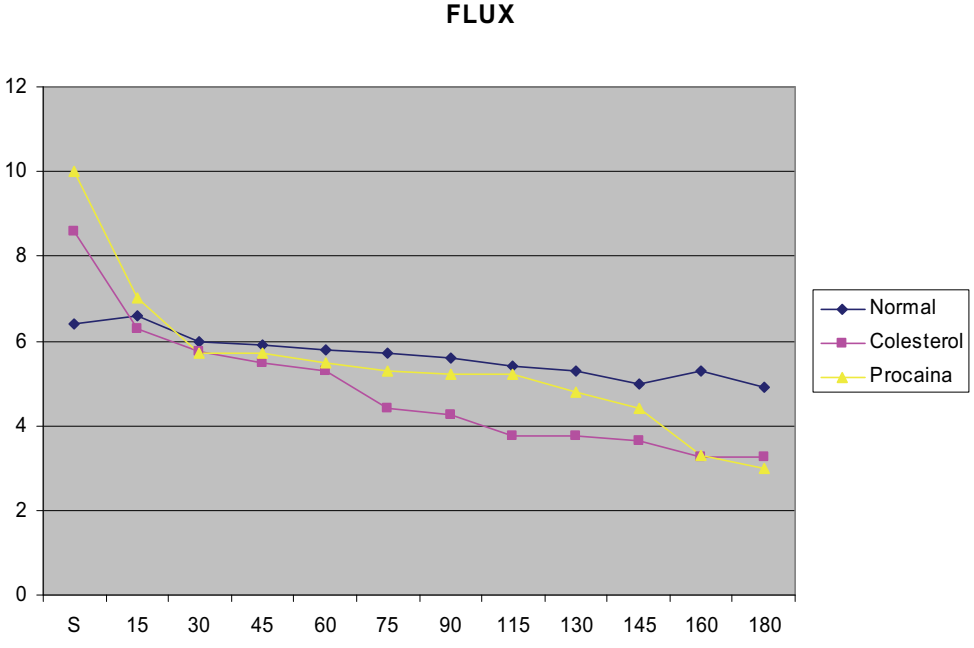

Figure 3

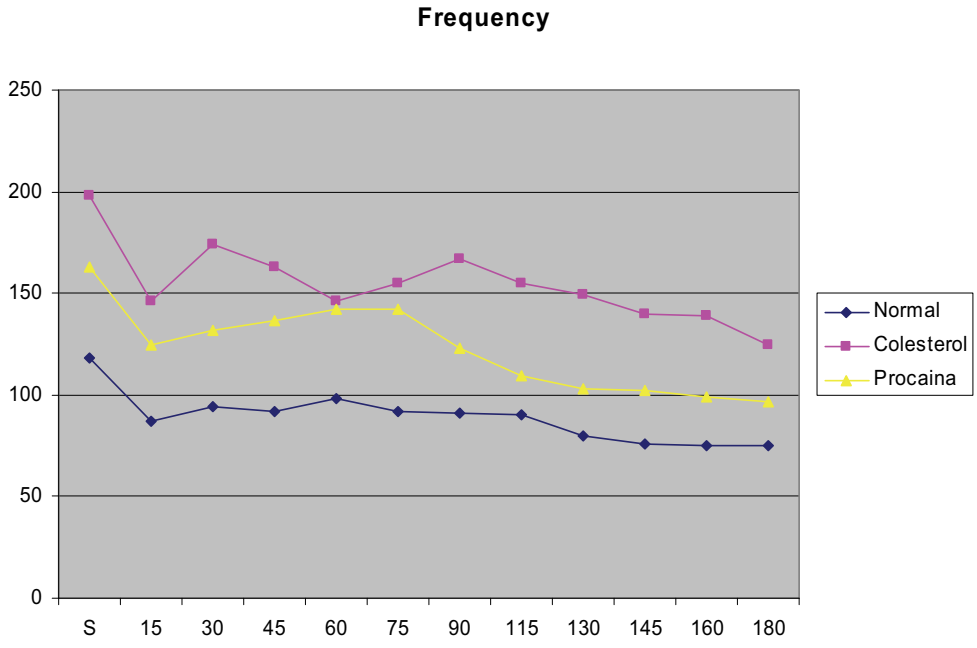

Figure 4

Figure 5. The image of DNA extracted from 12 month old rat ventricle fed on cholesterol reach diet cholesterol and procaine treated

Figure 6. DNA extracted from 12 month old rat ventricle fed on

Cholesterol reach diet induces a pathological state at the level of heart influencing the values of physiological parameters (Braunwald, 1992; Carmeliet, 1999).

The cholesterol diet influences the physiological parameters of heart as we have seen in our results. C.F.(cardiac frequency) which is increased in comparison with Controls. Our results are in accordance with the literature data (Cebbai et al., 1994; Derek et al., 2007).

The effect of treated rats feed with Procaine on Cholesterol diet leads to a decrease in cardiac frequency, approaching the values from the Controls.

The C.F. (coronary flux) is decreased in cholesterol feed rats versus Controls, while in Procaine treated rats the values of C.F. approaching the values from the Controls.

Concerning LVPD (Left ventricle developed pressure), in cholesterol feed rats, the values are decreased versus controls while in Procaine feed rats the values of LVPD are very much increased.

Reperfusion injuries (Kaul, 2001; Opic, 1989; Sato et al., 2004) due to free radicals generated during ischemia associate with 120 minutes reperfusion superimposed on a pathological condition generated by high reach cholesterol diet, generated severe injuries also at the molecular level expressed by internucleosomal fragmentation of DNA.

DNA laddering is present in rat heart feed on cholesterol diet, while in procaine treated rats feed on cholesterol diet, this is absent.

CONCLUSIONS

Our data have pointed out the negative impact of ischemia reperfusion associated with pathological state generated by cholesterol feeding upon heart contractility parameters as well as upon DNA integrity.

Procaine treatment seem to protect cardiovascular system from deleterious effects of cholesterol treatment at the physiological heart level as well as at the molecular level.

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THE IMPACT OF ISCHEMIA REPERFUSION UPON THE PHYSIOLOGICAL PARAMETERS OF RABBIT HEART WITH EXPERIMENTAL HYPERTHYROIDISM

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Abstract

The aim of this study was to evaluate physiological parameters of cardiac contractility in rabbit heart with hyperthiroid condition experimentally induced with T3. Our study has been done on 10 male rabbits treated with T3 (i.p. injections 4.5 mg/kg body weight) fo 4 weeks. Rabbit hearts have been mounted in a Langendorf retrograde perfusion system and after 30 minutes ischemia followed by 60 minutes reperfusion with Krebs Hanseleit bicarbonate-buffered saline at 37°C, pH 7.6 supplemented with 10 mM glucose, left ventricular developed pressure (LVDP), heart rate (H.R.) and coronary flow (C.F.) have been measured. Our data have pointed out that as far as LVDP parameter is concerned, in T3 treated rabbits, cardiac recovery is achieved at higher values above those from controls. Cardiac frequency is not different in T3 treated rabbits in comparison with controls. There is a decrease in coronary flow in hyperthyroid rabbit heart in comparison with controls, in both cases there is a decrease of this parameter during reperfusion with lower values in controls and a tendency of reaching a plateau value.

Keywords: *ischemia-reperfusion, left ventricular developed pressure, heart rate, coronary flow, cardiac frequency, hyperthiroid rabbit heart*

INTRODUCTION

Cardiovascular pathologies are frequent observed in hyper and hypothyroidism conditions. Cardiac performances could be directly correlated with serum levels of thyroid hormones. Cardiac contractility, heart rate, ejection fraction and coronary flow are known to have increased values in hyperthyroid condition, while vascular peripheral resistance is concomitantly reduced. These modifications are accompanied by cardiac hypertrophy, leading finally to cardiac insufficiency. On the other hand, thyroid hormone deficit is associated with a decrease in cardiac contractility and cardiac dilatation; both modifications may be reverted by reducing the thyroid hormone balance.

Despite the fact that relation between thyroid pathology and cardiovascular hemodynamics is well known, biochemical basis of triiodotironin (T3) action in heart has been intensively investigated in the last 20 years. Modifications induced by T3 in cardiac function may result from the direct or indirect effects. (Cabai et al., 1994).

It has been observed that T3 may act as vasodilatator or innotrop.

It has been open the way to new strategies of treatment in cardiac insufficiency.

An understanding of thyroid hormone action upon heart and peripherial vascularisation is essential for implementation this hormone as therapeutic agent. It is suggested the possibility that T3 to increase the cardiac load and decrease vascular systemic resistance offering in such a way a new therapeutic option for cardiovascular pathology treatment (Brar et al., 2000).

Taking into account the research done on the effects of T3 at the cardiovascular level, our paper is aimed to complete the studies regarding modifications following experimental hyperthyroidism at the level of mechanic and biochemical parameters of rabbit heart.

MATERIALS AND METHODS

The biological material (10 rabbits), kept in standard biobase conditions have been treated for 4 weeks with T3 intraperitoneal injections (4.5 mg/kg body weight).
After the last day of treatment, the animals have been anesthetised with Na Pentobarbital to which has been added heparin, and after opening the thoracic cage the aorta has been excised and the heart quickly mounted in Langendorff retrograde reperfusion system and perfused with Krebs Hanseleit buffer at 37^{o}C, in order to evaluate physiological parameters (Ambrosio et al., 1999)

Determinations of physiological parameters of perfused heart

Langendorff system is designed to fix the heart by means of a canula inserted in aortic cross at the valve level on the isolate heart in a perfusion system with a liquid with a composition identical with that of plasma. The recipient with this liquid is suspended a certain level in order to assure a pressure of 85 mmHg, and the liquid circulates through coronary system and leave the heart through right atrium (Carmeliet, 1999).
In left ventricle is introduced a latex balloon which is connected with a pressure transducer. The balloon is inflated inside the ventricle and this records perfectly the contraction movements of ventrice wall. In such a way the pressure may be recorded on a pressure monitor as pressure developed by left ventricle (**LVDP**). The monitor permits the recording of cardiac frequency (**HR**). By collecting the perfusion liquid which leave the heart in a given time (1 min) in a beaker it can be determined the coronary flow (**CF**). These parameters have been determined from the hyperthyroid hearts versus controls (Kloner, 1989).

RESULTS AND DISCUSSIONS

After the heart has been mounted in Langendorff, system the heart has been perfused for 30 minutes with perfusion liquid during this time the heart arrives in a normal regimen of functioning (**stabilization period**). Then the heart is ischemised for 30 minutes by interruption of perfusion, and then reperfused for 60 minutes (reperfusion period). It have been recorded physiological parameters at the end of stabilization period and during reperfusion in order to see the capacity of heart recovery after a medium interval of ischemia. Our data pointed out that:
- The pressure developed by left ventricle (**LVDP**)

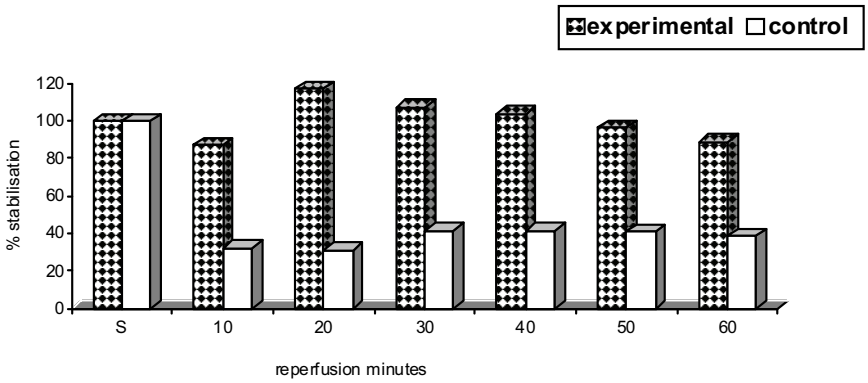

Figure 1. Evolution of pressure developed by the left ventricle in 60 minutes reperfusion in rats treated with T3 and in Controls

The values are given as % from the recorded value at the end of stabilising period. It can be observed that in the case of treatment with thyroid hormones, cardiac recovery, even does not exceed much the initial values (stabilization period), is realized at values much above the control heart. In both cases there is atendency of reaching a plateau value in function of left ventricle. The obtained differences between the two cases point out a high level of significance.

- Cardiac frequency (**HR**)

Cardiac frequency, measured by means of cardiac monitor, does not manifest a different allure in case of treated animals with T3, versus controls. Even, after a medium ischemia, the values do not reach those from stabilization, the obtained differences between the two cases are totally insignificant.

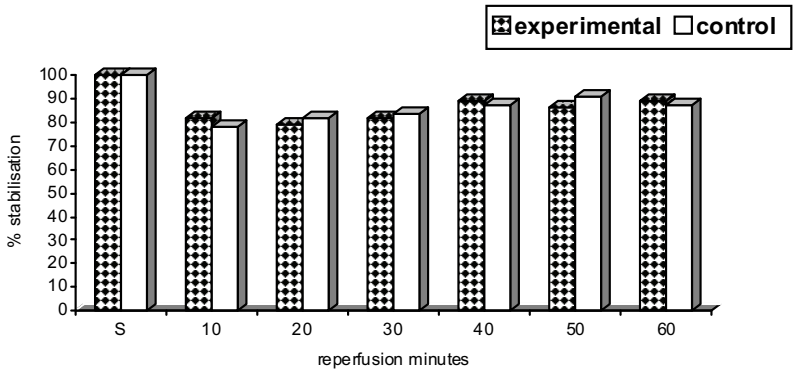

Figure 2. Heart rate (H.R.) in treated rats and in controls

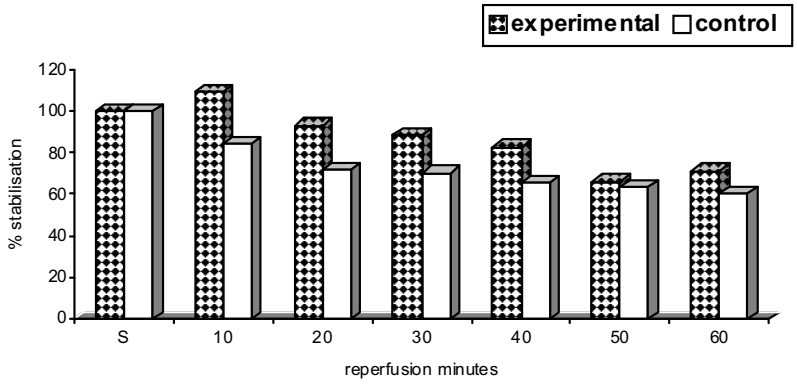

Figure 3. Coronary flow (C.F.) measured in treated rats and in controls

The evolution in time of coronary flow in T3 treated rabbits, comparatively with controls show that in both cases, a decrease in this parameter during reperfusion, with lower values in controls and with atendency to reach a plateau value.

Because heart is a major target organ for hormone action, many studies have examined modifications which appear in case of hyperthyroid condition. It has been established that (T3) influences amino acids, sugar and calcium transport through cell membranes. T3 stimulate the synthesis of myosin enzyme which present an increase ATP-ase activity, which results in an increased contraction speed of rabbit heart. Despite the fact that in heart there is an increased consumption of ATP, less from the chemical energy of ATP is used for contraction and much energy is directed for heat generation, which leads to a low efficiency of contraction in hyperthyroid heart (Braunwald et al., 1992).

On the other hand, one of the characteristics of arterial hypertension is deregulation of arterial hemodynamics. Every definition of arterial hypertension must take into account that fluctuations which appear during cardiac cycle, fluctuations in systolic pressure and diastolic

which accompany the heart dysfunction and result in pathological values of arterial hypertension. These fluctuations are determined by the ventricle ejection, by elasticity of arterial walls and by temporization of reflected waves in arteries (Opic, 1989).

CONCLUSIONS

The experimental model of arterial hypertension induced by means of T3 presents the following characteristics:
Cardiac frequency is measured by means of cardiac monitor and this does not manifest a different allure in case of treated animals versus controls. Even after a medium ischemia, the values do not reach those from stabilization period, differences obtained between the two groups are insignificant. Evolution in time of coronary flow in hyperthyroid hearts versus controls shows that in both cases there is a decrease of this parameter during reperfusion, with lower values in controls and with a tendency to reach a plateau.

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THE EQUINE COLOSTRUMS OF MILK TREATMENTAGAINST PATHOGENIC AGENT

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Abstract
Neonate losses are very important case in young animals in Indonesia, which is due to the virus, bacteria or parasites in conjunction with failure of passive transfer (FPT). Consequently, it is very urgent to break down these problems by realizing a scientific strategy on immunoglobulin-G (IgG) passive transfer in neonates. The existence of neonates life naturally depends on environmental conditions included to the nutrients supplied by their mother. The proteins immunoglobulin contained in the mother's colostrums are essential nutrients for immunity system of the newborn. This article aims to present the use of protein immunoglobular in colostrums through passive transfer of antibodies as a strategy in response to the high mortality rate of neonate animals in Indonesia, especially to goat farm in traditional farm maintenance system. In a farm system like this, it has a high risk of pathogenic infection. Limitations on the immune system of a new born occurred because in the uterus, a variety of antibody molecules from mother circulation cannot be transferred through the placenta to embryo. Consequently in the new ex-utero environment, such individuals are very susceptible to various infections of pathogenic micro-organisms.

Keywords*: IgG; colostrum; goat, neonate; pathogenic agent.*

INTRODUCTION

Food and nutrition for livestock neonates are very important to get attention, because it will determine its ability to defend itself against pathogenic environment and to the needs of his physical development. In the early stages of postpartum, nutrients for neonates are naturally derived from colostrum. However, the failure to obtain colostrum from its mother could happen due to various causes, such as newborn individuals in a weakened condition, less colostrum production, or improper handling during the birth process. These conditions result in a high mortality rate in individuals' neonatal period. Failure to maintain life in the newborn organism is generally caused by anoxie (hypoxy), hypothermic, and distocie (Grongnet, 1996). This situation of FPT (failure of passive transfer) relating to microbial infection pathogens from ex-utero environment. Therefore it is often associated with conditions where antibodies cannot be transferred from the parent to her newborn. Failure of Passive Transfer is generally associated with a lack of antibodies in the body of the neonate.

The Condition of Traditional Livestock

Decline in population and productivity of the Indonesian buffalo is generally caused by the pattern of traditional maintenance, reduced grazing land, males productive slaughter that reduced quantity of males, productive female buffaloes slaughter, the limitations of feed (dry season), high young buffalos mortality and decreased productivity (Phaharani et al., 2010). The ruminant livestock in Indonesia, which are traditionally carried out by the society, is generally in small-scale category and often face various problems in its development. Consequently, all parties need to contribute more serious attention in this farm to facilitate and to increase farm production, which will be able to improve the welfare of the society. One aspect to note in this condition is to handle the case of f*ailure of passive transfer* after the parturition moment. Birth process must be passed successfully before continuing on the protection of neonate's health naturally against microbial pathogens. The ability for defense on pathogenic agents has to be done through a variety of mechanisms. In this period, neonates

are confronted with various challenges such as nutritional dependence on the dam and the condition of immunity against the pathogenic microbe's threats outside the womb (Thibault and Levasseur, 1991). Transfer of acquired immunity from the mother through the placenta, needed for the growth and development of neonate's organisms. New individual must immediately adapt to the conditions of a new biological environment to maintain its presence in the environment which has a potential infection such as bacteria, viruses and parasites. Neonatal mortality in the first few weeks remains high, where cattle livestock nearly 10% and half of this occurs in approximately 24 hours after birth (Liu et al., 2011), especially in the traditionally maintained farms.

Failure of Passive Transfer (FPT)

When the new born animals have a gammaglobulinemia, they will have a high risk in passive transfer of immunoglobulin as a failure of passive transfer (FPT), and therefore, these animals have difficulty to adapt in their ex-uthero environment (Crisman and Scarrat, 2008). Physical factors fairly extreme in the environments can also deliver new individuals born in critical situations, for example when the surrounding temperature is extreme than the newborn animals cannot tolerate and therefore disrupt the regulatory functions that would impact the immune system in the face of infectious microorganisms pathogens. This situation can affect mortality in new born animals (Rumokoy et al., 2011).

Immunoglobulin Passive Transfer as an Alternative Solution

Buffalos have epitheliochorial placenta type. The epitheliochorial placenta of ruminants does not allow passage of immunoglobulins from dam to foetus. Consequently, ruminant neonates are born in a state of hipoglobulinemia, or even in a state of agamaglobulinemia. For this reason, we need a way to overcome this condition. A good way is to treat with a passive antibody transfer. Passive transfer studies in new born showed there was no difference in IgG serum concentration of the goat after supplementing until 10 g IgG per liter colostrum of horses. In other hand, there is any mortality among experiment animal (Rumokoy et al., 2011). Antibodies in colostrums can prevent infectious diseases by providing passive immune protection (Zeitlin et al., 2000).

Several techniques can be applied to passive transfer of immunoglobulin, when faced with FPT problems, by supplying IgG, either in form of lyophilisate, or in the fresh form.

The immunoglobulin-G passive transfer can be used to treat the immunodeficient of mice against infection of lethal ebola virus infection (Gupta et al., 2001). This antibody can be applied also for preventing disease after exposure to a biological agent which is partially a function of the immunity of the exposed individual (Casadevall, 2002). The passive immunoprotection targeting secreted factor *propionibacterium acnes* as a novel immunotherapeutic (Liu et al., 2011). When applying IgG in colostrums from another parent, the individual recipient will also receive nutrients origin from colostrums of donor. Nutrients of protein mainly dominated in colostrum elements, including the immunoglobulin itself.

Colostrum Milk of Horse for Pathogenic Control in Goat

The equine colostrum contains IgG abundantly, and therefore, it is very important to be used as natural substance for pathogenic control in animal, which it has been tested to the young goats (Rumokoy et al., 2011). The use of equine colostrum for this purpose can be considered for the treatment of failure of passive transfer.

MATERIALS AND METHOD

Animal experiments: Horses local parent, which was used in the first year and the second to assess the potential for the synthesis of biomolecules imunoglobuin-G globular protein with extensive maintenance techniques from Tomohon area. Goat newborn (neonate) during perinatus assesses the acquisition of passive transfer of IgG antibodies against mortality. The cattle used in the period perinatus, i.e. from prepartum to postpartum stages, reared extensively Sea of goat farming village,

Minahasa regency. The IgG analysis tools derived from ID-Biotech (HIgG1 092 811)

This experiment was using a completely randomized design factorial arranged. A factor is the level of immunoglobuline - G (A1 = 0 gL^{-1}, A2 = $5gL^{-1}$A3 = $10gL^{-1}$), and Factor B is an interval h0, h1, h2, and h3. Parent goats gestation period end was chosen to get a local kid, and been born a normal kid. Parent goats have been maintained extensively grounded in surveillance and equal treatment. Goat kids were divided into two groups: those receiving colostrum IgG donor parent and the controls without receiving food or colostrum. The observed number of dead and venous blood sampling at the time *jugulaire* 0 hour and 8 hours after ingestion of colostrums, andthen continued at 16 hours and 24 hours after ingestion. The blood was centrifuged immediately and the plasma tube was inserted in the micro to be analyzed, given data collection of the number of neonates, which died over the treatment. Laboratory analysis was using SRID technique (single radial immunodifusion).

RESULTS AND DISCUSSIONS

Average (ā) IgG blood serum of young goats can be seen in the Figure 1below.

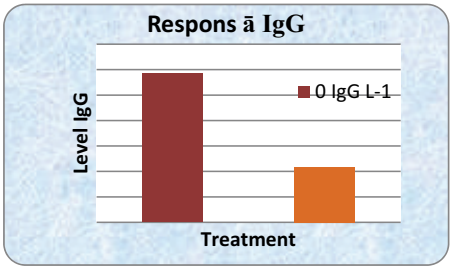
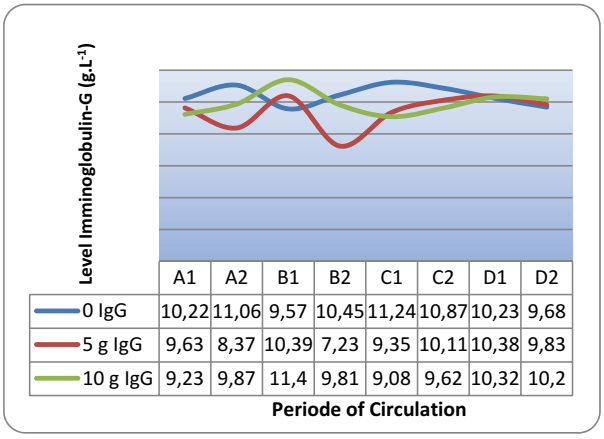


Figure 1. Level of IgG Serum Level of Goat

If the content of IgG plasmatic down less than 10 gL^{-1}, it can lead to death in young goats (Aissata, 1997). Mortality in kids often occurs because the content of goat IgG was low (O'Brien and Sherman, 1993). The low content of IgG plasmatic in ruminant animals can be caused by insufficient colostrum consumption (Levieux, 1984). Figure 2 presents the effect of equine colostrum IgG treatment on IgG serum of goat.



	A1	A2	B1	B2	C1	C2	D1	D2
0 IgG	10,22	11,06	9,57	10,45	11,24	10,87	10,23	9,68
5 g IgG	9,63	8,37	10,39	7,23	9,35	10,11	10,38	9,83
10 g IgG	9,23	9,87	11,4	9,81	9,08	9,62	10,32	10,2

Figure 2. The effect of treatment of 3 level of IgG lyophilized on IgG serum of Goat

The Figure 2 above, shows that time period factor responds to the varying blood serum IgG levels. There were no interactions between the above-mentioned factors (P>0.05). These results reinforce the notion that passive transfer of IgG antibodies to 10 g $IgGL^{-1}$ can tolerate well and did not cause any death in young goats, so that the immunoglobulin protein is very important to control the pathogenic agent *exuthero*. Factors such as the amount of nutrients in the diet and fat PK played a role in antibody levels (Bulla et al., 2004). These

conditions to be stable when young people are not infected with different pathogenic microorganisms (Duarte et al., 2009).

CONCLUSIONS

Treatment of equine IgG colostral from 0 up to 10 g IgG L^{-1} has not raised significantly the level of total serum IgG of experimental animals. The use of horse IgG colostral is in anticipation for the handling of passive transfer of antibodies. The acquisition of passive transfer of IgG antibodies to 10 g IgG L^{-1} can tolerate well and does not cause death in young goats, so that the immunoglobulin protein will be used as a natural biochemical agent in handling failure of passive transfer in young goats.

RECOMMENDATION

Therefore it is recommended to use equine colostrum IgG for the passive transfer of antibodies in the treatment pathogenic agent, so the neonate mortality could be minimized, especially in the farm pattern of traditional maintenance in Indonesia. It is expected that livestock production, which is traditionally handled as practiced in Indonesia, will further increase, and in turn will improve the benefits of its farmer.

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BEE COLONIES EXPLOITATION AT APPLE BLOSSOM POLLINATION IN THE ORCHARDS

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Abstract

It was done an experiment in order to test comparative the three methods (ways) of hives placement on the ground at the apple pollination, in 3 analogous intensive orchards, with four sorts, that are compatible to the pollination, of 10 hectares each, which were located at a distance of 600 m from each other. In all sectors of the orchard, bee colonies have been assigned with the charge of 3 families/ha. In the first sector (I Batch - control) of orchard, hives with bee colonies were located at the edge of the orchard (sector) in the front side of rows. In the second sector (II batch), the hives were located on the technologic road which separated in the centre the sector of orchard, perpendicular to fruit trees rows. The distance between hives was 10 m from each other. In the third sector of the orchard (III batch), the hives were located between the trees rows, in line, at a distance of 100 m from each other and over every 7th row of trees. In each experimental batch were rated the free entomophily pollination results (crossed) and isolated (auto pollination). It was found that, in the Ist batch, frequency of bees visit at tree blossom, has been weaker in the first days of hives placement, but was pretty good in batches II and III, even from first day. With the increase of air temperature, in all batches was registered a growth tendency of bees visits frequency at blossom. The results of the experiment, have shown that free pollination (cross) entomophily (with predominant participation in proportion of 90 - 95% of honey bees) of apple trees in the orchard ensures the flowers fertilization, depending of beehives location on the field, at the level of 22.2 – 47.5%, that is 13.8-22.6 times higher, compared with the isolated pollination - auto pollination (1.5 – 1.6%). Therefore, it has been confirmed, once again, the conclusion of many researchers, that, the participation of honey bees at pollination of apple's culture is an indisputable necessary measure. Uniform and proportional placement of bee hives inside the orchard, between the rows, in line, at a distance of 100 m from each other, and over every 7th row of trees, ensures a significant increase, compared to the control batch(located at the orchards edge), of bees visit frequency to the flowers is 2.4 times more (td = 20.0; P<0.001), of the bees flight intensity (with and without balls) with 24.5 – 53.3% (td = 3.5 – 4.8; P<0.001), of collected pollen quantity - with 46.2% (td = 2.8; P<0.01), of flowers fertilizing degree - with 2.1 times (td = 14.4; P<0.001) and, compared to traditional methods of hives placement (on the technological roads of the orchard), ensured the growth of bees visits frequency on blossom - with 74.4% (td = 12.9; P<0.001), of collected pollen quantity from apple - with 17.5 % (td = 2.4; P<0.05), and of fertilization degree of flowers - with 36.9% (td = 7.0; P<0.001).

Keywords: *exploitation, honey bees, pollination, apple, placement, bee hives*

INTRODUCTION

According to some scientific analysis (Vancea, 2013) in modern global agriculture, in recent years, because of using chemical pesticides and insecticides for wrestling against weeds and pests, there has been a drastic reduction in the number of species and populations of pollinating insects, what did the entomophily pollination in orchards of fruit trees, to be carried out, almost exclusively, by the honeybees, the only insects that you can count on, and that can be controlled by pollination requirements, through various training methods and permutation of bee families to pastoral. Multiple researches in this area (Guladze, 1973; Ivaşcu, 2010; Cîrnu and Cociu, 1973; Curennoi, 1973; Gerster, 2013; Landridge, 1973; Petcov, 1973) demonstrates that the process of pollination on fruit trees is accomplished in proportions of 90% by honeybees. By their number, and power of their exceptional work, through their ability to explore the inflorescences of entomophily plants, on a fairly large area, through ability and their tendency to profit of all the flowers in the area close to the hive, honey bees are the most active pollinating insects. By transporting the pollen from the male floral organ-anther, to receptive surface of female floral organ - stigma, honeybees ensures the cross pollination of fruit trees that leads to the fertilization of

flowers (linking of fruits). Among the species of fruit trees, apple is one of the most popular and valuable sources of nectar and pollen (Coman, 2012). At the same time, tree pollination using bees presents much higher benefits to cultivators than for beekeepers. The researchers from the Institute of Beekeeping Research and Development from Bucharest have reached the conclusion that, by cross pollination, performed by honey bees, are obtained production increase, in the ratio of over 50%. In the meantime, beekeepers get advantages, offered by this fruit species as melliferous source, taking into account, the huge number of flowers on a reduced area and the trees tranche blossoming during several weeks. Other research in this area (Roy, 1973; Sharp, 1973) demonstrated that the cross pollination of apple flowers of different varieties provides a significant increase of harvest by at least 50-60%. For these reasons, raising the effectiveness of fruit tree pollination, using honey bees, has become a permanent and current problem for both - cultivators of fruit trees, as well as for beekeepers. Professional tree cultivators, in many countries realized the importance of entomophily pollination of agricultural crops (Gerster, 2013; Astorre, 1973; Girling, 2014; Vancea, 2013). For example, in Australia, the value of pollination services of agricultural crops constitutes, annually 0.6-1.2 billion US dollars. For every invested dollar in renting of bee colonies for pollination, the benefit of agricultural growers is 185 $. In the United States of America commercial services offered for pollination of agricultural crops were estimated to a number of approximately 14.6 billion dollars per year. The efficiency of pollination, using bees, has been fully realized by the cultivators, particularly at apple and almond, that's why they pay enough the services for bee pollination, rendered by the beekeepers. For example, in the State of California (USA) almond growers are paying up to $ 100 for each colony of bees brought to pollination. At the same time, payment terms depend on completed harvest. In this context, beekeepers are interested in achieving a more effective pollination with a lower number of bee families, thus covering larger areas of orchards and obtaining good harvests. Unfortunately, apple growers in our country (Republic of Moldova), not all realized the need for orchards pollination, with the help of the bees, and payment for the services of pollination is ridiculous (130 lei MD/ha or 40-45 lei MD (3.3-3.5 $) per bee family. In addition, the traditional pollination technique of apple orchards, as well as the proposed one in official editions (Мантоптин, 1990) is not the most efficient, and therefore the need for trees pollination by honey bees is not convincing enough for all orchards cultivators.

In this context, in the present work, we propose a comparative testing of some location techniques of hives with bee families on the field, and the development of some effective proposals for pollination of apple orchards with the help of the bees.

MATERIALS AND METHODS

The work was carried out under the institutional application project: code- 11. 817. 08. 17A "Development of advanced growing technology and diversified exploitation of *Apis mellifera carpatica* bee families". An experiment was carried out in order to test various exploiting techniques of the bee families, at the apple culture pollination in intensive orchards. To do this, during the April 18-23, 2013, was organized the transport of 90 bee families of SRL "Casa Albinei", com. Hulboaca, mun. Chisinau, to the blossomed apple pollination, from orchards of SRL "Codru-ST", Straseni. Within the orchard sectors, there were planted alternated, rows of trees, of four varieties that are compatible to the pollination: Golden, Aidaret, Florena, and Simerenco. The distance between rows was 3.5 m. The distance between the trees in the row was 1.2 m. In this experiment, were tested comparatively, three techniques (ways) of hives location, on the field, at the apple pollination, in 3 similar orchard sectors, 10 ha each, which were over 600 m away from each other. In all orchard sectors, the bee families were distributed, calculated, each 3 families per 1 ha. The first orchard sector (I^{st} batch), has served as control, where the hives with bee families were located according to the old schema (method) - at the edge of the orchard (sector) in front of the rows. In the second orchard sector (II^{nd} batch), the hives with bee families were

located according to the traditional technology, on a technological road, that separated, in the middle, the orchard sector, perpendicularly to the fruit trees rows. The distance between hives, located along the way, was 10 m from each other. The hearth of the hives placement, on the technological road, was toward the hearth of bee hives, located at the edge of the orchard, at a distance of over 600 m, according to the recommendations of the Institute of Zoology of the ASM (Мантоптин et al., 1990).
In the third orchard sector (IIIrd batch), the hives with bee families, were placed in series, between the rows, at a distance of 100 m from each other. Each following number of hives was placed over each 7th row of fruit trees. The third sector of orchard is located at a distance of approximately 600 m from the IInd sector hearth.
In all sectors of the orchards, the beehives with bee families were located at the beginning of the trees full swing flowering period and kept for 6 days, after that being removed.
To speed up the process of bees getting used to the scent of flowers and increasing the flying intensity, in all sectors of hives location, they were fed, daily, throughout all the period, with sugar syrup, mixed with flowers infusion, freshly collected from those trees, in amount of 50 g flowers to 1 litre of syrup. The mixture was administered 50 ml to each frame interval with bee.
In each experimental sector (batch) have been studied:
- the frequency intensity of the bee at 9 representative trees of each experimental orchard batch, where was registered, while 5 minutes, the bees number, visiting the flowers of the tree branch sector with 1000 flowers;
- the quantity and types of pollen, collected by a bee family, during one day of experiment, registering the data of pollen collector, at 30 bee families from each experimental batch of the orchard;
- the flight intensity of the bee family was assessed by the bees number (with, or without pollen balls) arrived to the beehive while 10 minutes;
- the share of entomophily pollination of the trees inflorescence, in the total pollination, for which, in every experimental batch of orchard, was isolated a crown of a the representative tree with an impenetrable net (gauze) for insects;
- the degree of flowers fertilization (fruits binding)-through appreciation, after 18-20 days after flowering, of pollination results and recording the number of fertile and sterile flowers at 1000 inflorescences.
Data obtained, in all experiences, were processed statistically using computer software „STATISTICA - 6" and appreciated their certainty, according to the biometric variation statistics, by the methods of Плохинский Н. А. 1969 (Petcov, 1973).

RESULTS AND DISCUSSIONS

Analysis of the data, obtained in the experiment of apple trees pollination in orchards, demonstrates that, honeybees *Apis mellifera* are attending the flowers quite intensive, starting even in first days of placing the beehives on the orchard's lands (Table 1).

Table 1. The frequency of bees visits at apple tree flowers, on a compact sector of branches with 1000 flowers, *bees/5 minutes*

No of the day	Air tempe-rature, t^{o}C	Ist batch (N=9)	IInd batch (N = 9)			IIIrd batch (N = 9)		
		M ± m	M ± m	d	td	M ± m	d	td
1	17^{o}	10.4 ± 1.0	18.3 ± 2.2	+7.9**	3.2	35.4 ± 1.6	+25.0***	13.2
2	18^{o}	14.3 ± 0.9	18.0 ± 0.8	+3.7**	3.1	33.2 ± 1.8	+18.9***	9.4
3	19^{o}	15.2 ± 1.1	18.2 ± 1.9	+3.0	1.4	31.6 ± 1.6	+16.4***	8.4
4	17^{o}	13.0 ± 1.3	18.1 ± 1.2	+5.1*	2.8	29.3 ± 1.6	+16.3***	7.9
5	19^{o}	16.1 ± 0.8	22.7 ± 1.6	+6.6**	3.6	35.7 ± 1.8	+19.6***	9.9
6	15^{o}	10.9 ± 1.0	15.2 ± 1.6	+4.3*	2.3	26.9 ± 1.5	+16.0***	8.8
Average	17,5^{o}	**13.3 ± 0.5**	**18.4 ± 0.7**	**+5.1*****	**5.9**	**32.1 ± 0.8**	**+18.8*****	**20.0**

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

We have found that, the bees frequency intensity to the flowers, depends on the day of the pollination period, air temperature, as well of the way or technique of the hives placement, at the orchard pollination.
Thus, the bees frequency visiting of the tree flowers, by the first batch is weaker in first

days of bees location on the field, increasing with 46.1% at 3rd day, then dropping with 28.3% in the last (6th) day of pollination. Experimental batches II and III, in which the bee families were located closer to trees from respective orchard sector, the bees frequency visiting of the trees flowers, was high enough, even on the first day of placement of the beehives with bees.

With the increase of air temperature in all experimental batches, has been registered a concomitant rising tendency, in the frequency of bees visit to the apple flowers.

Thus, with increasing of air temperature from 15 – to 19^{0}C, the frequency of bees visits to flowers increases, in Ist batch, from 10.4 to 16.1 bees/5 minutes; or 54.8% ($P < 0.001$) in IInd batch - from 15,2 to 22.7 bees/5 minutes or 49.3% ($P < 0.001$) and in IIIrd batch from 26,9 to 35.7 bees/5 minutes, or 32.7% ($P < 0.001$).

This rise tendency of the flowers bees visits frequency, depending on the air temperature, can be reflected more clearly in the chart from Figure 1.

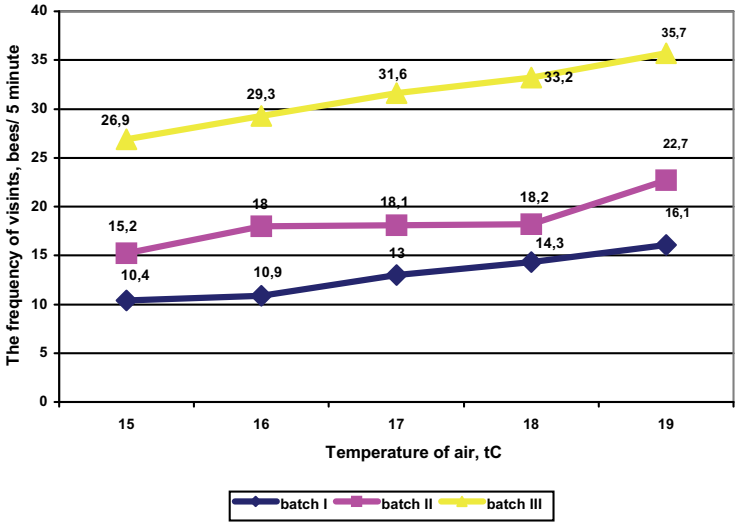

Figure 1. Bees frequency of visiting apple flowers

The results obtained by us, on the bees frequency of visiting apple flowers, are in accordance with the data of Langridge (1973), who mentioned, that, there is clearly a connection between environmental temperature and flight activity of the bees. If would be made a chart, we would notice that, under 13^{0}C – the flying activity is weak, between 13^0 and 16^{0}C – the curve rises suddenly, and between 16^0 and 26^{0}C - maintains at a high level.

At the same time, the greatest influence on the frequency of visits bees to flowers, in our experiment, is the technique (way) to place hives with bee families on the land of fruit trees orchards.

The data in Table 1 shows that, the traditional way of bee hives placement on the edge of the orchard (Ist batch) is overcome, because the bees frequency to the fruit trees from orchard is the lowest. The placement of the bee families on the technological roads (IInd batch) of orchard (according to the recommendations of the Institute of Zoology of the ASM, 1990), the frequency of bee visits to apple flowers is growing, on average, from 13.3 ± 0.5 up to 18.4 ± 0.7 bees/5 minutes, or with 38.3 % (td = 5.9; $P< 0.001$).

The highest frequency of bee visits to tree flowers was recorded in IIIrd experimental batch, in which bee families were placed uniformly between tree rows, at a distance of 100 m of each other, and over every 7 rows. This technique (way) of bee hives placement for pollination, ensure a substantial increase of the bees visit frequency to the apple flowers, compared to the witness batch, with 18.8 bees/5 minutes, or 141.3% (2.41 times; td = 20.0; $P< 0.001$) and, compared to IInd batch -

with 13.7 bees/ 5 minutes, or 74.4% (td = 13.5; P < 0.001).

Based on the analysis of the data obtained in the experiment, we can conclude that, the more uniform and proportional the placement of bee hives between the tree rows is, as higher is the bees visit frequency to the flowers. This is due to the fact that the bees are finding at a smaller distance the source of picking, making less effort in search of food and making more flights in a period of time.

Increasing of the bees visits frequency to the flowers, contributes to the quality improvement of the apple culture pollination. It can be found in the analysis of the pollen amount, collected by the bees and their intensity of flight (Table 2).

Table 2. The collected pollen amount and the bees flight intensity during full swing flowering of the apple culture in intensive orchards

The Indicators	Ist batch (N=30)	IInd batch (N = 30)			IIIrd batch (N = 30)		
	M ± m	M ± m	d	td	M ± m	d	td
The pollen amount totally collected, g/day	173 ± 16	233 ± 11	+60**	3,1	253 ± 23	+80**	2,8
including: apple	131 ± 20	212 ± 14	+81**	3,3	249 ± 6	+118***	5,6
The share of apple pollen in total amount, %	75.7 ± 8.0	91.0 ± 5.3	+15.3	1.6	98.4 ± 2.3	+22.7	2.7
The flying intensity, bees/10 minutes:	x	x	x	x	x	x	x
Without pollen balls	118 ± 3	137 ± 13	+19	1.4	148 ± 8	+30***	3.5
with pollen balls	75 ± 2	101 ± 15	+26	1.7	115 ±8	+40***	4.8
The share of bees with balls towards total, %	65.6 ± 8.8	73.7 ± 8.1	+8.1	0.7	77.7 ± 7.7	+12.1	1.0

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

It was found that, during one day the bees bring to collector 173-253 g of pollen. Most of the pollen collected by bees (75.7 – 98.4%) is of apple. At the same time, bee families from the Ist batch, which were placed on the edge of the orchard, have collected a noticeable amount (131 g, or 24.3%) of polifloral pollen.

The experimental data have shown that the bee hives location of hives at apple pollination, influences all the characters related to the quantity and quality of the pollen collected by the bees, and to the intensity of their flight.

Thus, the smallest amount of pollen, gained from a bee family, on average per day, being at pollination of apple culture, was recorded in Ist batch, where bee hives were located at one side (edge) of the orchard. With a more uniform and proportional placement of bee hives inside the orchard, the pollen amount collected per day from a bee family grows from 173 ± 16 g/day in Ist batch, up to 233 ± 11 g/day in IInd batch, with 60 g/day, or 34.7% (td = 3.1; P< 0.01) .

The biggest pollen amount, gathered by bees in the nest was found in the IIIrd batch, where the hives with bee families were placed in series between the tree rows. Thus, the amount of the accumulated daily pollen, in the nest at apple pollination, at bee families from IIIrd batch exceeded compared to their fellows from Ist batch (control) - with 80 g, or 46.2% (td = 2.8; P < 0.01), and had overcome tendencies toward bee families from IInd batch.

It is important to note that bees from IInd and IIIrd batches ensured the total increase of pollen quantity, collected from the amount of basic culture pollen (apple) submitted to a controlled pollination with the help of the bees.

Thus, the share of apple pollen in the total quantity of collected pollen, bee families from IInd batch had a visible increase tendency, compared to the witness batch, with 15.3 percentage points, or 20.2% (td = 1.6; P = 0.01), and bee families from IIIrd batch exceeded significantly their fellows from the witness batch with 22.4 percentage points, or 30.0% (td = 2.7; P< 0.01). This explains the higher quality pollination in IInd and IIIrd batches, of the basic culture (apple) taken in experiment.

The research results have shown that the bees pollination volume of the apple culture, and its quality is determined also by the intensity of

the bees flight, as of the bees without pollen balls, which bring nectar, as well as of those with balls, which specifically visited the flowers in order to collect pollen.

The data obtained in the experiments demonstrates the fact that, while pollinating the apple in orchards, the IInd batch bee families had a higher intensity flight tendency compared to the control batch, and IIIrd batch bee families, had a higher intensity of flying, compared to those from the witness batch. At the same time, in all experimental groups, the bees flight intensity without pollen balls was higher compared to that of bees with balls.

At the apple pollination, flight intensity of bees without balls in IIIrd batch was higher comparing to the witness batch -30 bees/10 minutes, or 25.4% (td = 3.5; P< 0.001) and, flight intensity of bees with balls of the IIIrd batch was higher compared to the control batch, with 40 bees/10 minutes, or 53.3% (td = 4.8; P < 0.001).

At the same time, it has also been found that IInd and IIIrd batches, in which the hives with bee families were located more uniformly, on the land of pollinated culture, manifested a tendency of share increase of bees with pollen balls, from total number of bees who flew to picking. This shows that the techniques of placement the hives with bee families tested in batches II and III contributes to improve the quantity and quality of pollination and collecting a bigger quantity of trading pollen.

Appreciating, at the end, the result of apple trees pollination with the help of bees and pollination efficiency depending on the technique of hives placement on the land of pollinated culture, we identified the size of the impact of entomophily pollination and its dependency on how are located the hives on the ground (Table 3).

Thus, comparing the number of fertile and sterile flowers, as a result of pollination, as in case of isolated pollination (on branches covered with impenetrable mesh for insect), as well as in case of entomophily free pollination (with access of honeybees and other insects), we found that the fertilization degree of the flowers is determined predominantly by the insect activity and influenced by the system of bee hives placement in orchard.

The isolated pollination (auto pollination), in all sectors and experimental groups, at a research of a sector of a tree with 1000 flowers, were found just 13-18 fertile flowers, the rest being sterile flowers. This means that the flower fertilization degree (GF) at isolated pollination was very low, and accounted for only 1.3 ± 0.3 - 1.8 ± 0.4 %. In the variant of free entomophily pollination (cross), on a surface of 1000 flowers have been registered, according to the batch and sector, from 210 up to 510 fertile flowers, the fertilization degree representing 21.0 ± 1,3 - 51,0 ± 1.6%. Therefore, the impact of entomophily pollination consists in an increase, compared to the isolated pollination, with 16.1-30.0 times of the fertilization degree of apple trees flowers.

The lowest degree of flowers fertilization at entomophilypollination, was recorded in Ist batch, where the hives with bee families were located at the edge of the orchard, according to the traditional plan. With the more uniform and proportional location of bee hives on the field of pollinated culture, the flowers fertilization degree increases substantially.

Thus, the flowers fertilization degree in IInd batch, in all researched sectors, was higher compared to the witness batch, on average with 12.5 percentage points, or 56.3% (td = 10.9; P< 0.001).

The highest degree of flowers fertilization at entomophily pollination was found in the IIIrd batch, where the hives with bee families were located, according to the plan elaborated by us – placement in series uniformly, between rows, at a distance of 100 m from each other and over each 7 rows of the orchard sectors. Thus, the fertilization degree of flowers in this group was higher, so compared to the control group – 25.3 percentage points, or 114.0 % (td = 9; P< 0.001) and compared with group II - with 12.8 percentage points, or 36.9% (td = 7.9; P<0.001).

Table 3. Results of apple flowers pollination, calculated per 1000 inflorescences (12.05.2013)

No. of sector	Indicators	I st batch			II nd batch			III rd batch		
		Pollination type		Free % Isolated	Pollination type		Free % Isolated	Pollination type		Free % Isolated
		isolated	free		Isolated	Free		Isolated	Free	
1	The number of fertile flowers	18	230	1278	15	380	2533	17	510	3000
	The number of sterile flowers	982	770	78.4	985	620	62.9	983	490	49.8
	The degree of fertilization (GF), M + m, %	1.8±0.4	23.0±1.3	1278	1.5±0.4	38.0±1.5	2533	1.7±0.4	51.0±1.6	3000
	The difference (d) GF toward witness	-	-	-	-0.3	+15.0***	-	-0.1	28***	-
	Certainty degree of the difference(td)	-	-	-	0.5	7.5	-	0.2	13.6	-
2	The number of fertile flowers	17	225	1323	15	350	2333	14	485	3464
	The number of sterile flowers	983	775	78.8	985	650	66.0	986	515	52.2
	The degree of fertilization (GF), M + m,%	1.7±0.4	22.5±1.3	1323	1.5±0.4	35.0±1.5	2333	1.4±0.4	48.5±1.6	3464
	The difference (d) GF toward witness	-	-	-	-0.2	+12.5***	-	-0.30	+26***	-
	Certainty degree of the difference(td)	-	-	-	0.3	6.3	-	0.5	12.6	-
3	The number of fertile flowers	13	210	1615	16	310	1937	17	430	2529
	The number of sterile flowers	987	790	80.0	984	690	70.0	983	570	56.0
	The degree of fertilization (GF), M + m,%	1.3±0.4	21.0±1.3	1615	1.6±0.4	31.0±1.5	1937	1.7±0.4	43.0±1.6	2529
	The difference (d) GF toward witness	-	-	-	+0.3	+10.0***	-	-0.4	+22	-
	Certainty degree of the difference(td)	-	-	-	0.5	5.0	-	0.7	10.7	-
To-tal apple	The number of fertile flowers	48	665	1385	46	1040	2261	48	1425	2969
	The number of sterile flowers	2952	2335	79.1	2954	1960	66.3	2952	1575	53.3
	The degree of fertilization (GF), M + m,%	1.6±0.2	22.2±0.7	1385	1.5±0.2	34.7±0.9	2261	1.6±0.2	47.5±1.6	2969
	The difference (d) GF toward witness	-	-	-	-0.1	+12.5***	-	0.0	+25.3***	-
	Certainty degree of the difference(td)	-	-	-	0.4	10.9	-	0.0	14.4	-

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

Generalizing in the end the results of testing various techniques of placement the hives at apple pollination in the orchards, we can conclude in full accordance with communications of researchers Cârnu and Cociu, 1971; Roy, 1970, who have mentioned that dispersed placement of bee hives inside the orchard ensures not only a complete and uniform pollination, but also a larger production, due to the fact that bees moving on small distances do not wear out, realizing higher efficiency.

CONCLUSIONS

1. The free (cross) entomophily (with major participation in approximately 90-95% of honeybees) pollination at apple trees in the orchards ensures the flowers fertilization, depending on the beehives location on the ground, at a level of 22.2 – 47.5%, that is 13.8-22.6 times higher, compared to the isolated pollination-auto pollination (1.5-1.6%). Therefore, the participation of honeybees at pollination of apple's culture is an indisputable necessary measure.

2. The uniform and proportional placement of hives with bee families inside the orchard between the rows, in series, at a distance of 100 m from each other, and across each 7th tree row, ensures a significant increase, compared to the control group (located on the edge of the orchard), of frequency of visit of bees to flowers – 2.4 times (td = 20.0; P <0.001), of the intensity of bees flight (with and without pollen balls) with 24.5-53.3% (td = 3.5-4.8; P < 0.001), of quantity of collected pollen with 46.2% (td = 2.8; P <0.01), of flowers fertilizing degree - with 2.1 times (td = 14.4; P< 0.001) and compared to traditional methods of locating the hives (on technological roads of orchard), ensures the increase of frequency of flowers visiting by the bees- with 74.4 % (td = 12.9; P< 0.001), of apple collected pollen quantity - with 17.5% (td = 1.5; P< 0.05) and of the flowers fertilization degree - with 36.9% (td = 7.0; P < 0.001).

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USE OF ADDITIVE IN BEE FEEDING AT QUEENS' GROWING

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Abstract

For vital processes bee family needs a considerable amount of food - honey and bee bread. In cases when the family food supply is insufficient, the bees must be fed additionally. The purpose of research is to determine the influence of the use of feed additives in bees feeding in queens growth. There was found that the optimum dose of the feed additive Premix Bionorm P (Symbiotic complex) used to feed bee queen is 150 mg/l of sugar syrup. Feeding is performed daily in the calculation of mixing 1 l per a bee family beginning with the introduction of the frame with transferred larvae till the queen-cell capping for 5 days. It was found that the use of the feed additive (symbiotic complex) in the diet ensures larvae adoption in growth of bee queens with 15,3-25,7%, compared with the control groups I and II , the queen-cell mass increased with 60,0-110,0 mg or with 5,7-11%, the length – 0,1-0,2 cm or with 3,7-7,8% , the diameter – 0,03 cm or with 2,5%, the body weight of unmated queens - with 1,94-6,62 mg or with 1,1-3,8% and mated queens with 20,86-26,19 mg or with 9,1-11,7% .

Keywords: *bee's families, feed additive, honey bees, sugar syrup.*

INTRODUCTION

Bees collect nectar and pollen on flowers of plants and process into food - honey and bee bread. Bees feed contains all vital nutrients - proteins, lipids, carbohydrates, minerals, vitamins (Буренин, Котова, 1977).

Bee family needs a considerable amount of food - honey and bee bread for vital processes. In cases the family food supply is insufficient, the bees must be fed additionally.

It is known the method of bees feeding using sugar as honey substitute. For growth of juvenile sugar syrup is used in a concentration of 50% (1 kg of sugar in 1 l of water). At reproduction apiaries in queens growth, bee families were twice a day fed with sugar syrup using small doses (Кривцов et al., 2000; Eremia, 2009).

There were inserted the frames with transferred larvae in bee families after 6 hours of orphaning and there was given sugar syrup 50% to nurse families (Mărghitaş, 2005).

Based on the above research goal is to determine the influence of the use of feed additives in the queen bee diet at their growth.

MATERIALS AND METHODS

To achieve the objectives of the research, the bee families of Carpathian breed from apiary „Albinărie", Straseni district, Republic of Moldova served as an object of the investigations.

To determine the optimal conditions for realization of queens growing method there was studied the influence of the feed additive Premix Bionorm P (symbiotic complex) on stimulating of larvae adoption, bees feeding, increasing of the length, diameter and cells mass, mass of unmated and mated queens. There were formed 5 groups of nurse bee families, including 2 as control groups and 3 experimental that received sugar syrup with feed additive.

Bee nurse families were formed by orphanizing (the queen and all capped brood were removed, depriving it of the opportunity to bring out a new queen in their larva). There were fixed by 30-35 started queen cells on tapping frame and there was transferred by a young larva at the age of 9-12 hours in each by taking it from native family. After 6 hours of bee families orphanizing in the centre of nurse bee families was introduced a frame of transferred brood.

Syrup was prepared as follows for feeding of bee families: the water has warmed up to boiling, then the sugar was added in a ratio 1:1 to 1 liter of water one kg of sugar, the solution was stirred until the sugar was completely dissolved. When the syrup was cooled to 30^0C

there was added a feed additive, which was dissolved in 80-100 ml of water and stirred together.

Nurse bee families in group I (control I) had used the nest honey reserves without supplementary feeding. Nurse bee families in group II (control II) had daily received 1.0 l of pure sugar syrup. Nurse bee families in the experimental group III had received 1.0 l of sugar syrup with 100 mg of feed additive. Nurse bee families in the experimental group IV had received 1.0 l of sugar syrup with 150 mg of feed additive. Nurse bee families in the experimental group V had received 1.0 l of sugar syrup with 200 mg of feed additive.

The experimental families were daily fed from the time of placing of growing frame with transferred larvae to the time of queen cell capping (during 5 days).

To determine the influence of the used feed additives in bee feeding at queens growth there was appreciated the number of transferred adopted queens' larvae, mass, queen cell length and diameter, mass of not mated and mated queens.

The data obtained were processed by means of statistical variations after Mercurieva, 1970; Plohinschii, 1971, using computer programs Microsoft Excel.

RESULTS AND DISCUSSIONS

The results of the research showed that beginning from 16th of July, there were 8-12 combs in the nest and the power was 7-10 spaces between the frames with populated bees. The nurse bee families had received by one frame with 30-35 transferred larvae.

It was found that nurses bee families in the control I and II from transferred larvae (34-35 pcs.) had adopted from 19 to 22 pcs. or from 54.3 to 64.7% (Table 1).

Nurse bee families that received syrup with feed additive, 100-150 mg/l had accepted 21-24 larvae from 30-32 or 65.6 to 80%. The best results had the experimental group IV, that adopted transferred larvae from 15.3 to 25.7% compare to the control groups I and II.

Table 1. Influence of feed additive on the adoption of transferred larvae in queen's growth (16.07.2011)

Group	Number of combs in the nest	Family power, streets	Quantity of used syrup, L	Number of transferred larvae	Adopted larvae	
					number	%
I. Honey (control I)	11	10	-	34	22	64.7
II. Pure sugar syrup (control II)	12	10	1.0	35	19	54.3
III. Sugar syrup + feed additive, 100 mg/l	8	7	1.0	32	21	65.6
IV. Sugar syrup + feed additive, 150 mg/l	12	8	1.0	30	24	80.0
V. Sugar syrup + feed additive, 200 mg/l	9	8	1.0	30	16	53.3

Table 2. Influence of feed additive on weight, length and queen cells diameter (26.07.2011)

Lotul	Nr. of queen cells	Index	X ± Sx	V, %	Limits
I. Honey (control I)	8	Mass, g Length,cm Diametre, cm	1.05 ± 0.040 2.67 ± 0.025* 1.22 ± 0.025	10.82 2.64 5.77	0.9 – 1.22 2.7 – 2.8 1.1 – 1.3
II. Pure sugar syrup (control II)	18	Mass, g Length,cm Diametre, cm	1.0 ± 0.021 2.57 ± 0.036 1.22 ± 0.015	8.99 5.93 5.08	0.89 – 1.2 2.3 – 2.9 1.1 – 1.4
III. Sugar syrup + feed additive, 100 mg/l	21	Mass, g Length,cm Diametre, cm	1.04 ± 0.019 2.57 ± 0.020 1.23 ± 0.014	8.38 3.54 4.10	0.98 – 1.27 2.4 – 2.8 1.2 – 1.3
IV. Sugar syrup + feed additive, 150 mg/l	24	Mass, g Length,cm Diametre, cm	1.11 ± 0.019*** 2.77 ± 0.024*** 1.25 ± 0.008	9.40 4.66 3.40	0.88 – 1.21 2.3 – 2.8 1.2 – 1.3
V. Sugar syrup + feed additive, 200 mg/l	2	Mass, g Length,cm Diametre, cm	0.94 ± 0.045 2.7 ± 0.100 1.2 ± 0.00	6.73 5.24 0.0	0.9 – 0.99 2.6 – 2.8 1.2 – 1.2

Note: The significance of differences between averages is authentic: *** B ≥0.999

At queen cell evaluation on July 26, there was found in the control groups that the weight ranged between 1.0 and 1.05 g, length 2.57-2.67 cm and diameter 1.22 cm (Table 2).

The mass of queen cells obtained in the experimental group IV was from 60.0 to 110.0 mg or from 5.7 to 11% higher than in control groups I and II (*** B ≥ 0.999), the length was 0.1 - 0.2 cm (*** B ≥ 0.999) or from 3.7 to 7.8% and diameter was 0.03 cm or with 2.5% higher.

The body mass of not mated queens on July 29 in the control groups averaged from 175.69 to 180.37 mg, limits ranging from 157 mg to 199 mg. In experimental groups the queens mass was on average of 174.62 to 182.31 mg with a variation between 153 and 202 mg (Table 3).

The best developing had the queens in group IV, that were fed with syrup and feed additive, 150 mg/l with an average of body weight of 182.31 mg, or from 1.94 to 6.62 mg higher than in control groups I and II (* B ≥ 0.95) or from 1.1 to 3.8% .

Table 3. Influence of feed additive (symbiotic complex) on not mated queens body mass (29.07.2011)

Group	Nr. of queens	X ± Sx	V, %	Limits
I. Honey (control I)	8	180.37 ± 4.91	7.70	159 - 199
II. Pure sugar syrup (control II)	16	175.69 ± 2.12	4.82	157 - 186
III. Sugar syrup + feed additive, 100 mg/l	19	174.62 ± 2.52	6.30	153 - 196
IV. Sugar syrup + feed additive, 150 mg/l	23	182.31 ± 2.33*	6.13	159 - 202
V. Sugar syrup + feed additive, 200 mg/l	1	176.0 ± 0.00	0.00	176

The significance of differences between averages: *B ≥ 0.95

The maximum coefficient of variation of body weight of not mated queens was 7.70%. The body weight of mated queens on 5th of August averaged between 223.67 mg (group I) and 249.86 mg (group IV). The queens of group IV, that received feed additive with syrup, 150 mg/l, had higher body mass from 20.86 to 26.19 mg than control groups I and II or from 9.1 to 11, 7%. The biological potential of body weight of not mated queens was 270 mg. The coefficient of variation was from 5.25 to 17.15% (Table 4).

Table 4. Body mass of unmated queens (05.08.2011)

Group	Nr. of queens	X ± Sx	V, %	Limits
I. Honey (control I)	3	229.0 ± 10.408	7.87	209 – 244
II. Pure sugar syrup (control II)	4	223.67 ±19.18	17.15	181 – 270
III. Sugar syrup + feed additive, 100 mg/l	4	244.0 ± 7.29	5.98	216 – 249
IV. Sugar syrup + feed additive, 150 mg/l	7	249.86 ± 6.37	7.03	219 – 270
V. Sugar syrup + feed additive, 200 mg/l	1	243.0 ± 0.00	-	-

Therefore, the use of feed additives in the bee family feeding at queen growth ensures adoption of transferred larvae, increases the mass of queen cells, length and diameter, body weight of not mated and mated queens.

CONCLUSIONS

1. It was found that the optimum dose of the feed additive Premix Bionorm P (symbiotic complex) used to feed bee queens during growth is 150 mg/l of sugar syrup. Feeding is performed daily in the calculation of mixing 1 liter per a bee family, beginning with the introduction of the transferred frame with larvae to the queen cells capping during 5 days.

2. It was revealed that the use of the feed additive (symbiotic complex) in bee feeding of growing queen ensures the adoption of transferred larvae in queens growth from 15.3 to 25.7%, compared with the control groups I and II, increases the queen cells mass 60.0–110.0 mg, that is higher with 5.7-11%, the length - 0.1-0.2 cm or higher with 3.7-7.8%, diameter – 0,03 cm or 2,5%, body mass of not mated queen - 1.94-6.62 mg or higher with 1.1 to 3.8%, and body mass of mated queen was 20.86 to 26.19 mg or higher with 9.1-11.7%.

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MORBIDITY INCIDENCE IN A CATTLE FARM EXPLOITED IN STABULATION CONDITIONS

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Abstract

This paper describes the health status of a herd of 480 dairy cattle from a private farm from Botosani County for a period of three years (2005-2007). The exploitation system of cattle was the one of loose housing with individual spaces of rest. There were presented the morbidity share (proportion), disease categories (gynaecological and obstetrical diseases, medical maladies, ruminal-reticular indigestions, leg anomalies, surgical problems) that affected the animals within the farm during this period, the incidences of these diseases, as well as the causes of their occurrence (some hygienic weaknesses in shelters and some imperfections of animal feed), all these issues being interpreted in ecopathogenic context (hereditary predisposition of animals, environment and technological factors). There were indicated some prophylactic measures that must be promoted in this farm.

Keywords: *cattle, bovine pathology, animal welfare*

INTRODUCTION

Cattle have a social, economical, health, environmental and ecological importance of the first order in the economies of many countries (Reijs et al., 2013). An issue of great importance for increasing the productivity of cattle is the application of appropriate selection and breeding technologies to ensure optimal conditions to animals for their feeding, care, maintenance and reproduction to avoid the losses through illnesses and mortalities. All these aspects relating to animal husbandry are incorporated in a current enough concept designated *animal welfare*. Considering this value judgment, the human welfare depends, in the greatest extent, of animal welfare. Welfare concept still lacks a precise definition, but all the experts who have studied the animal welfare agree that this notion includes health, production comfort and protection of animals (Orăşanu et al., 2011).

Failure to ensure the animal welfare conditions to a conflict among the metabolic characteristics of animal body and its existential environment which means the triggering of some diseases from entire pathological spectrum, the morbid entity type being depending on the nature of this conflict (Paraschivescu et al., 2011).

Another tricky issue is that the grazing has long been a traditional practice in cattle. Current trends in the bovine sector have led to a decline in cattle feeding by grazing. As a result, this agricultural activity was focused increasingly on cattle breeding in stabulation system which modified (if not downright altered), in some extent, the relationship between the animal body and its environmental conditions (Ioniţă at al., 2011; Reijs et al., 2013).

Consequently, the objective of this study is to manage the health status in a cattle farm reared in stabulation conditions and to evaluate the influence of some factors in this microclimate by their exteriorization in various morbid entities.

MATERIALS AND METHODS

Researches were performed in a private farm of cattle breeding for milk production, called SC PrisLact S.R.L. of Botosani County, during 2005-2007, on a herd of dairy cows composed of 330 individuals of Holstein breed and 150 animals of Romanian Black Spotted breed. The

breeding system of the animals was the intensive one, of loose housing type with individual rest spaces for Holstein cows, and for those of Romanian Black Spotted breed the system was a mixed one (loose housing and grazing in fenced plots); conditions of feeding, housing and zoo-veterinary treatments of animals were ensured.
Health status of the animals and its evolution was monitored. The diseases that have affected some animals were diagnosed by procedures of veterinary medicine and were monitored from clinical and paraclinical point of view.

RESULTS AND DISCUSSIONS

The health of the cattle was monitored for three years. During this time interval, 81 animals were affected by different diseases, representing 16.87% of the overall population. 399 cows (83.13%) did not suffer from any disease (fig. 1).

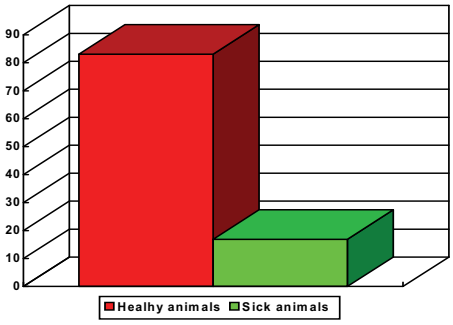

Figure 1. Health status from a private cattle farm

In order for the research results to be as accurate we recorded in the register of consultations and treatments all pathological disorders classified into five categories of diseases:
- *gynaecological and obstetrical diseases* (genital infections, dystocia, uterine prolapse, genital tumours);
- *medical maladies* (disturbances of metabolic profile);
- *ruminal-reticular indigestions (*simple biochemical indigestions, acute ruminal meteorism, ruminal acidosis, traumatic reticular peritonitis, indigestions by the sudden change of forage ration, indigestions by overloading the rumen, reticular ruminal pareses);
- *leg anomalies (*pododermatitis, lamina);
- *surgical problems* (traumatisms related to stabulation system, caesareans).
Thus, in the overall population of dairy cows studied, the highest incidence of illnesses is represented by gynaecological and obstetrical diseases (5%). The disease group of a medical nature (4.37%) is situated immediately on the second place concerning the morbidity frequency. The leg anomalies are less common (2.5%) and the surgical problems (1.67%) record the lowest frequency in population. The ruminal-reticular indigestions (3.33%) occupy a median position in the clinical panel of animals (fig. 2).

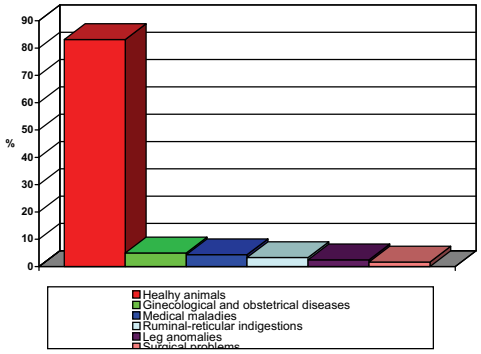

Figure 2. Incidences of morbid entities per total cattle population

By doing a strict reporting only to the group of animals that have suffered by various illnesses in this period, the clinical panel of morbid entities shows as follows: gynaecological and obstetrical sicknesses 29.63%, metabolic disorders 25.93%, ruminal-reticular ailments 19.75%, leg troubles 14.82% and surgical ailments 9.87% (fig. 3).

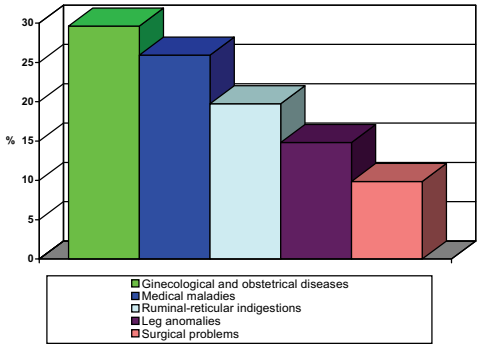

Figure 3. Incidences of morbid entities per total ill cattle

The high incidence of gynaecology and obstetrics affections is closely related to the puerperal period. This period is characterized, in terms of metabolic profile, as a state of maximum physiological strain, when the animal body is sensitive to even action of the lowest stressful factors, leading to various clinical metabolic dysfunctions. On the other hand, also the ruminal-reticular indigestions within the general morbidity represent an important factor in the aetiology of the other disorders that are specific to maximum metabolic strain after calving, such as the medical diseases, but especially the various puerperal disorders of gynaecology and obstetrics, like genital infections, foetal annex retention and ovarianopathies. These maladies had the highest incidences in this period and they can, in their turn, to constitute a trigger factor of the ruminal-reticular indigestions. Also the existence of other medical conditions can often lead to simple biochemical reticular indigestion, ruminal acidosis, ruminal-reticular paresis and ruminal alkalosis.

The investigations carried out revealed that the improvement of animal health and consequently of human health, like the first consumer of products derived from animals, requires multiple concomitant demarches, including of course, the study of animals and their functions, but just as well a better understanding of the environment in which animals live, both as separate individuals and industrial holdings.

Health surveillance of cattle requires the implementation of some measures such as hygiene shelters, correct management, efficient ventilation, efficient programs of deworming and vaccination, stress deceasing, all being corroborated with an adequate diet of feeding. These actions must be implemented according to the specific physiological statuses of animals: weaning, calving, beginning and at the peak of lactation etc. Also, the animal diet should be adjusted according to their maintenance status.

Knowing and determination of the environmental risk factors have a great importance and represent perhaps the most valuable activity in promoting and maintaining the health of animals and human, environment being able to influence the animal health by:

- physical factors (climate, air, water, soil, noise, pollution, radiation);
- biological factors (food, microorganisms, nutritional and microbiological quality of feedingstuffs);
- socio-behavioral factors (stress).

In this context, a new concept called *ecopathology* has emerged representing the living field of the healthy body in environmental and technological conditions consistent with its metabolic needs (Bacic et al., 2006; Ioniţă at al., 2011).

Therefore, our intention is to continue and deepen these studies. We take into account, primarily, to estimate the share of each morbid entity within the large disease groups; for example the percentage distribution of simple biochemical indigestions, acute ruminal meteorism, ruminal acidosis, traumatic reticular peritonitis, indigestions by the sudden change of forage ration, indigestions by overloading the rumen, reticular ruminal pareses, framed in the morbidity ailment group generically called the ruminal-reticular indigestions. Also, the dynamics of these diseases would be not devoid of interest (as distinct entities or as malady groups) over time, their annual (years 2005, 2006, 2007) and seasonal (winter, spring, summer, autumn) evolution. All these investigations will take into account the compatibility of animal body metabolism with the exploitation conditions of farm animals.

Discerning as accurate as possible and complete the factors that can harm the health of animals (lactating cows in our case) is one of major terms of preventing and combating their diseases.

CONCLUSIONS

The morbidity frequency in a private farm of dairy cows reared in stabulation conditions during three years is moderate.

The cattle herd was affected in the highest degree by gynaecological and obstetrical diseases and then by metabolic dysfunctions; the ruminal-reticular indigestions occupied a median position in the clinical panel; the surgical sicknesses and especially the leg ailments recorded the lowest frequencies.

The occurrence of morbid entities in the cattle farm is caused by some hygienic deficiencies in

shelters and feeding imperfections of animals.

The causes of morbidity in cattle studied must be considered in a multifactorial manner by association of genetic factors (predisposition to certain diseases) with environmental and technological factors (in particular the stabulation conditions).

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STUDY REGARDING EVOLUTION, CURRENT STATE AND PERSPECTIVES IN SHEEP BREEDING IN ROMANIA

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Abstract

After the integration of our country within the EU have occurred major changes in the sheep breeding sector. The purpose of this paper is to investigate the evolution and situation of sheep breeding sector in Romania in relation with the sheep livestock, the number and size of sheep exploitations, sheep breed structure, improving of genetic potential of livestock and sheep productions. To achieve these objectives we have studied the official statistical data, we calculated the percentage difference between the reference years and we interpreted data obtained. The results showed that the sheep livestock has increasing with 20.8% in 2012 compared with 2002 and with 15.1% compared with 2006. In terms of number and size of sheep exploitations, the results showed that in the year 2012 there were 271,266 exploitations of sheep of which 63.9% are individual exploitations that have an average number of 4.2 heads sheep per unit. However, compared with the existing situation in 2002, the number of exploitations is 2.4 times lower in the year 2010, especially based on decreasing of small exploitations respectively those which are framed in class less than 10 heads. Compared to the total number of sheep from our country, namely 8.83 million heads in 2012, the percentage of the number of sheep included in official control is very low, respectively 3.3%. The study shows also that meat production and milk production in particular have increased significantly over the period considered, while the wool production increased slightly.

Keywords: *sheep, livestock, farms, production.*

INTRODUCTION

Sheep breeding is a basic occupation in almost all rural areas of Romania and especially in the hills and mountains areas. This work dates from the beginning of his training and then continued, under various extensive or intensive forms. The development of this sector was due in large part to the conditions of soil and climate of our country, Romania being from this point of view one of the countries with the most appropriate conditions for sheep breeding (Taftă et al., 1997).

After the integration of our country within the EU have occurred major changes in many areas of activity and including in the sheep breeding sector (Răducuţă et al., 2008; Răducuţă and Ghiţă, 2009).

Transition to market economy and changes in land ownership determined changes in sheep breeding. This requires a new approach, both technically and economically based on the market demand and compliance with veterinary requirements from this field.

In present the sheep livestock have decreased considerably compared to those existing in 1990 and the predominant direction in sheep breeding is mixed respectively for milk and meat. This fact is due to a better price for milk products and for lamb meat compared with wool.

The purpose of this paper is to investigate the evolution and situation of sheep breeding sector in Romania in relation with the sheep livestock, the number and size of sheep exploitations, sheep breed structure, improving of genetic potential of livestock and sheep productions.

MATERIAL AND METHOD

The analyze of sheep breeding evolution was made between 2002 and 2012 period. To achieve the research objectives we have studied the official statistical data provided by different institutions (Eurostat, Ministry of Agriculture

and Rural Development from Romania - MARD, National Institute of Statistics - NIS and National Agency for Amelioration and Reproduction in Animal Science - NAARAS), we calculated the percentage difference between the reference years and we interpreted data obtained. Finally, were issued the conclusions and recommendations arising from this study.

RESULTS AND DISCUSSIONS

The results of this study showed that the current sheep number in Romania amounts to 8.83 million heads in 2012, registering an increase of 20.8% compared to the existing one in 2002 and with 15.1% compared with 2006 (Table 1). But if we compare the number of sheep in 2012 to the year 1989 we see that it decreased by 45.5% (Eurostat, 2014).

Table 1. Dynamics of sheep livestock (thousand heads)

Specification	2002	2006	2012	2012/ 2002 (%)
Sheep number	7,312	7,678	8,834	+20.8

Almost the entire sheep livestock existing in the country in present are in private ownership (99.9%), with the exception of that existing in research units.

In terms of number and size of sheep exploitations, the results showed that in the year 2012 there were 271,266 exploitations with an average number of 30.9 heads per unit (Table 2).

Table 2. The structure and size of sheep exploitations in Romania in the year 2010

Specification	Number of exploitations	% from total	Average number (heads/unit)
1-9 heads	173,305	63.9	4.2
10-19 heads	44,027	16.2	12.2
20-49 heads	19,115	7.1	27.6
50-99 heads	14,383	5.3	66.8
100-199 heads	10,800	3.9	133.4
200-499 heads	7,464	2.8	292.9
over 500 heads	2,172	0.8	923.7
Total	271,266	100	30.9

From all 271,266 exploitations 63.9% are individual exploitations that have an average number of 4.2 heads sheep per unit and which hold these animals exclusively for self-consumption of family and only 7.5% of total exploitations have more than 100 heads, as average size of sheep farms existing in the EU.

From the table 2 data it can be seen also that in 2010 there are 2,172 farms with a capacity over 500 heads (of approx. 3 times more than in 2002), which although representing only 0.8% of total holdings, they have about 24% of the total number of sheep in our country (MARD, 2014; NIS, 2014).

However, compared with the existing situation in 2002 and 2007, the number of exploitations is with 2.4 times and respectively 2.0 times lower in the year 2010, especially based on decreasing of small exploitations, respectively those which are framed in class less than 10 heads (Table 3).

Table 3. The total number of sheep exploitations and average size of farm in the year 2002, 2007 and 2010

Specification	Number of exploitations	Average number (heads/unit)
2002 year	640,011	11.4
2007 year	533,094	15.9
2010 year	271,266	30.9

In 2002-2007 period the total number of sheep exploitations has decreased and especially those with size less than 10 heads while the average number of animals per farm has increased. These are positive things registered in the past 10 years in the Romanian sheep sector which are in compliance with the trend existing in developed countries, respectively the concentration of the animals per farm and increasing their genetic potential (Diaconescu and Nicolae, 2013).

As regards the sheep breed structure, there are six breed classes in Romania (year 2012), which hold, in order, the following percentages from the sheep livestock: Tsurcana (55.5%), Tsigai (22.1%), Merinos (10.9), Karakul (5.6%), Crossbreeds (5.7%) and Other breeds (0.2%). In the last class are breeds which were imported in the recent years for improving the morpho-productive parameters of our local breeds such as Lacaune, Friesian, Texel, Suffolk, Bluefaced Leicester, Ile de France, etc. (Table 4).

From this situation it is pointed out that the Tsurcana has decreased from 65.0% (year 2002) to 55.5% (year 2012) of the total sheep number in Romania, the difference being taken

mainly by the Crossbreeds which have a superior production compared with Tsurcana. From this point of view it is notable that the structure of sheep breed has changed in the right direction (NAARAS, 2014).

Table 4. The sheep breed structure in Romania

2002 year		2012 year	
Breed	%	Breed	%
Tsurcana	65.0	Tsurcana	55.5
Tsigai	25.5	Tsigai	22.1
Merinos breeds	6.5	Merinos breeds	10.9
Karakul and other breeds	3.0	Karakul	5.6
-	-	Crossbreeds	5.7
-	-	Other breeds (imported)	0.2

Genetic improvement of animals is achieved through selection and crossbreeding methods on the base of official control of livestock performance and through the controlling of animal reproduction process.

After 1990 year some economic, technical and social changes, led to the sheep breeding modifications, such as changing exploitation direction, cancelling herd improvement activities through the disappearance of populations with genetic role, reducing of farm size, increasing of operating expenses, very low prices for the obtained products, which have limited the application of technical activities on animal breeding, improvement of genetic potential of breeds and the production of breeding material.

In Romania, improvement of genetic potential of livestock and conservation of genetic resources are activities funded from the public budget. After 1990, however, the funds allocated for these activities were inadequate, and besides this it is noted the fact that for the sheep species was developed an improvement breeding program in 2003 (ie after 13 years).

Presently, official control of production in sheep and goats is technically coordinated by the National Agency for Amelioration and Reproduction in Animal Science and executed by accredited associations. In 2012 existed 42 accredited associations for official contol of productions in ovine and caprine animals and 10 associations accredited to conduct genealogical register for these species.

In the control system, "origin and productivity" - OP, during 2004-2012 the total number of sheep controlled increased from 26,565 to 106,268 head and in the control system, "own performances "- PP, the total number of sheep controlled increased from 79,114 to 182,387 head (Table 5). As compared to 2008, the total number of sheep controlled in 2012 fell by about 27%, the decrease is very significant among the control of animals in the "own performances " system – PP (NAARAS, 2014).

Table 5. Dynamics of sheep livestock taken in official control during 2004 – 2012

Specification	2004	2008	2012
Total number	105,679	39,842	288,655
System of Origin and Productivity - OP	26,565	53,169	106,268
System of Own Performances - PP	79,114	344,673	182,387

Compared to the total number of sheep from our country, namely 8.83 million heads in 2012, the percentage of the number of sheep included in official control is very low ie 3.3%, which means that there is a lot of work to significantly improve the genetic potential of our sheep livestock and the increase of the quality and quantity of yields obtained from this species nationally.

In the structure of the sheep breeds included in the official control are different breeds in function of share of economic character. From the table 6 data it can be seen that in 2012 local breeds account for 96.39% of the sheep included in the official control of the production, imported breeds 1.87% and crossbreeds 1.74% (NAARAS, 2014).

Table 6. Share of sheep breeds contained in the official control of productions in 2012

Specification	Number of exploitations	Average number (heads/unit)
Local breeds	278,232	96.39
Imported breeds	5,408	1.87
Crossbreeds	5,015	1.74
Total	288,655	100

The maintenance system of animals is extensively, based on the maintenance of sheep to pasture in warmer seasons (spring, summer, autumn) and maintenance in shelters in cold season (winter) or semi-intensive system. The extensive system is more economical, by

exploiting of cheap fodder (grass on pastures and hay from meadows) and the exploitation of local breeds (Tsurcana, Tsigai), but less productive in terms of yields obtained (Răducuţă, 2012).
The maintenance system of animals is extensively or semi-intensive, based on the maintenance of sheep to pasture in warmer seasons (spring, summer, autumn) and maintenance in shelters in cold season (winter). The extensive system is more economical, by exploiting of cheap fodder (grass on pastures and hay from meadows) and the exploitation of local breeds (Tsurcana, Tsigai), but less productive in terms of yields obtained.
The intensive system is rarely met in our sheep farms, because needs big investment in biological material, machinery, equipment, instalations and feed additives (Marin and Drăgotoiu, 2002).
Regarding the evolution of main productions of sheep in our country, we can observe that all registered an increasing in 2002-2012 period and especially milk production (Table 7). However it should be noted that this production growth is not due to the increase of yield per sheep but it is caused by the increasing of sheep number.

Table 7. Dynamics of sheep productions (thousand tonnes)

Specification	2002	2012	2012/ 2002 (%)
Wool production	16.7	18.6	11.4
Meat production	51	69	35.3
Milk production	345	651	88.7

In perspective, the sheep livestock will remain relatively constant or will continue to record a slight growth and the concentration of animals in middle-sized farms will increase, with increasing yields per animal.
Sheep sector in Romania needs to be restructured to improve its competitiveness. A transparent information on the cost of production, price of products and profit is a necessary step towards reducing imbalances in the food chain. To preserve and develop the interest of consumers, particularly to young people, for food products obtained at this species is necessary strengthening and improving promotion of these products.

CONCLUSIONS

The current sheep number in Romania amounts to 8.83 million heads in 2012, registering an increase of 20.8% compared to the existing one in 2002 and with 15.1% compared with 2006.
In terms of number and size of sheep exploitations, the results showed that in the year 2012 there were 271,266 exploitations with an average number of 30.9 heads per unit.
As regards the sheep breed structure, there are now six breed classes in Romania, respectively: Tsurcana (55.5%), Tsigai (22.1%), Merinos (10.9), Karakul (5.6%), Crossbreeds (5.7%) and Other breeds (0.2%).
The percentage of the number of sheep included in official control is very low, respectively 3.3%. All productions registered an increasing in 2002-2012 period, but this fact is due to the increasing of sheep livestock.
Sheep sector in Romania needs to be restructured to improve its competitiveness. A transparent information on the cost of production, price of products and profit is a necessary step towards reducing imbalances in the food chain.

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STUDY ON EVOLUTION OF PRODUCTION PARAMETERS TO THE QUAIL YOUTH OF BALOTEŞTI POPULATION BETWEEN 0-6 WEEKS OF GROWTH BY DAILY LIGHT DURATION

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Abstract

In order to determine the influence of the daily duration of illumination on growth parameters as weight gain and feed valorization in young quail of Baloteşti population, an experiment was organized by a total of 600 chickens divided into two groups of 300 animals each. A lot has been exposed to light 24 hours a day during 0-6 weeks of growth and the other to 24 hours light per day during the first 3 weeks and 14 hours per day in the last 3 weeks of growth. Overall, growth parameters were higher in chicks subjected to a daily 24 hours light per day, while the mortality rate was reduced by about 50 % and the electricity consumption for lighting by 20.83%in the other group.

Keywords: quail, youth, growth, lighting duration.

INTRODUCTION

Light, as duration, intensity and intensity and variation of duration and intensity influences determinately the growth and reproduction of wild birds, but also that of the domestic ones. Quail, raised of less time by humans compared to other species of birds and has a very short growth duration is more in control by light. Most farmers raise young quail under continuous light, so to locate and benefit better of feeding and watering places, although it seems that they learn to focus in the early days of life and the wild chickens grow in the spring to a light period of the day of 14-15 hours. Thus, Woodard et al., 1973; Alexandru, 2001; Văcaru - Opriş et al., 2002; Van et al, 2004 recommended illumination of 24 hours of 24 hours per day throughout the youth raising period. Coban O. (2008) used 24 hours light per day in the first week of chicks life, 21 hours the second week and 14 hours per day in the third week to the end of the growth of young quails. Popescu - Micloșanu (2007) recommends the first weeks 24 hours light /day with 30 minutes of darkness for resting at start. Further, light can be kept constant (24 hours a day) or the duration is reduced quickly or gradually, leading to 10 to 15 hours / day.

MATERIALS AND METHODS

The experiment was conducted in Lucian T. Ioniță Individual Enterprise society Bucharest, farm located in the Gheorghița commune, Ungureni village, Prahova County, on a total of 600 quail chicks of the Baloteşti population that was divided at hatching into two groups housed in two separate rooms.

The control group was subjected to 24 hours of light per day for the entire growth period of 6 weeks, while the other experimental group, to 24 hours light per day for the first 3 weeks and to 14 hours /day during the last three weeks of growth. The light intensity was the same in both analyzed groups.

Also, chicks from both groups were maintained on permanent litter at ground during 0-3 weeks of age and in adult quail batteries for between 4-6 weeks of growth. The feeding chickens of both groups was performed with quail chicks mixed feed type starter during 0-3 weeks and growing feed type during 4-6 weeks of growth.

Other environmental conditions of the experiment were within the limits of literature. The measurements were made individually by simple random sampling and processing was performed using Microsoft Excel 2010.

RESULTS AND DISCUSSIONS

The results are shown below in summarized main groups of parameters on weight gain, food consumption and mortality as registered in the study.

1. The average weight and weekly average weight gain in quail chicks of the control group versus the experimental group during 0-6 weeks of growth

Table 1 presents the weight gain evolution of chickens. As expected, benefiting of the same growing conditions during 0-3 weeks, average body weight evolution was about the same in both studied groups, the difference between them being insignificant. Thus, at the age of 3 weeks chickens in the control group had an average weight of 110.65 ± 1.76 g, while the experimental group 108.33 ± 1.95 g.

Table 1. Changes in body weight and weekly weight gain of quail chicks in the control group compared with the experimental growth during 0-6 weeks

Age	Average live weight (g)		Average weekly weight gain rate (g)	
	Control group	Experimental group	Control group	Experimental group
Day1	8.44 ± 0.80	8.35 ± 0.88	x	x
1 week	30.85 ± 0.95	29.55 ± 1.58	22.41	21.20
2 weeks	54.75 ± 1.45	55.75 ± 1.75	23.90	26.20
3 weeks	110.65 ± 1.76	108.33 ± 1.95	55.90	52.58
4 weeks	147.75 ± 2.78 aaa	135.45 ± 2.15 aaa	36.90 ± 1.15 ddd	27.12 ± 1.95 ddd
5 weeks	175.75 ± 2.85 bbb	155.55 ± 2.45 bbb	28.00 ± 1.75 eee	20.10 ± 1.65 eee
6 weeks	200.50 ± 2.95 ccc	167.55 ± 2.32 ccc	24.75 ± 1.55 fff	12.00 ± 0.85 fff
Weeks I-III	x	x	34.07	33.33
Weeks IV-VI	x	x	29.88	19.74
Weeks I-VI	x	x	31.98	26.53

Note: between values marked with the same letter are significant differences

At the age of 4 weeks average live weight was 147.75 ± 2.78 g / head in the control chickens group and 135.45 ± 2.15 g in the experimental group, the difference of 8.3 % between them being very significant and at 5 weeks of 175.75 ± 2.85 g / head in control chickens and 155.55 ± 2.45 g in the experimental group, 11.5 % difference between them being very significant. At 6 weeks the weight was 200.50 ± 2.95 g / head in control chickens and 167.55 ± 2.32 g in the experimental group; the difference between them, of 16.4 % was very significant.

The average weekly weight gain recorded around the same evolution during 0-3 weeks of growth in both groups analyzed, the differences being insignificant.

In the fourth week of life the control chicks saw an average weight gain of 36.90 ± 1.15 g/capita, while the chickens in the experimental group registered a gain of 27.12 ± 1.95 g/head, the difference between them being highly significant. In the week V in control chickens the gain was 28.00 ± 1.75 g/capita, while in the experimental group of 20.10 ± 1.65 g/head, the difference being very significant. In the sixth week of life the control chicks saw an average gain of 24.75 ± 1.55 g/capita, while those in the experimental group of 12.00 ± 0.85 g; the difference was very significant.

The average weight gain during the period of IV - VI weeks was of 29.88 g/week in control chickens, who benefited from the possibility of continuous feeding and watering during the 24 hours illumination and 19.74 g, by 33.93% less in those of experimental group, with low light duration by 41.7%, in the same period of growth. Average gain between I- VI weeks was 31.98 g/week in control chickens and 26.53 g, by 17.04% lower in the experimental group.

2. Average daily consumption of mixed feed and specific consumption of the quail chicks in the control group versus the experimental group

At chickens in the control group there was an average consumption of 4.70 ± 0.55 g combined feed / head / day in the first week of growth (Table 2), of 9.55 ± 0.85 g/head/day in the second week, 12.50 ± 1.35 g in the third, to 15.55 ± 1.75 g in the fourth week, of 20.35 ± 2.25 g in the fifth and 24.58 ± 2.12 g in the sixth week of growth.

The chickens in the experimental group recorded an average consumption of 4.85 ± 0.82 g combined feed / head / day in the first week of growth, 9.83 ± 0.95 g in the second, 12.11 ± 1.45 g in the third, to 10.45 ± 1.55 g in the fourth week, of 14.43 ± 2.30 g in the fifth and 18.54 ± 2.22 g / head / day in the sixth week of growth.

Table 2. Combined feed consumption and specific consumption evolution in quail chickens

Week	Average consumption of mixed feed (g / head / day)		Specific consumption of feed (g / g gain)	
	Control group	Experimental group	Control group	Experimental group
I	4.70 ± 0.55	4.85 ± 0.82	1.47	1.60
II	9.55 ± 0.85	9.83 ± 0.95	2.78	2.63
III	12.50 ± 1.35	12.11 ± 1.45	1.57	1.61
IV	15.55 ± 1.75aaa	10.45 ± 1.55aaa	2.95 ± 0.46ddd	2.69 ± 0.87ddd
V	20.35 ± 2.25bbb	14.43 ± 2.30bbb	5.08 ± 1.86eee	5.03 ± 1.76eee
VI	24.58 ± 2.12ccc	18.54 ± 2.22ccc	6.95 ± 2.10fff	10.81 ± 2.06fff
Weeks I-III	8.92	8.93	1.94	1.95
WeeksIV-VI	20.16	14.47	3.32	6.18
Weeks I-VI	14.54	9.30	3.47 ± 0.45	4.06 ± 0.33
TOTAL, g	610.26	491.47	x	x

Note: between values marked with the same letter are significant differences

The differences between the average combined feed consumption in the two groups were not significant during 0-3 weeks of growth and were very significant in 4-6 weeks, when the control chickens consumed on average 14.54 g/head/day, by 36% more than those in the experimental group.

The total combined feed in the control group during 1-6 weeks was 610.26 g/head/period, while in the experimental group it was of 491.47 g/head, with 19.50% less compared with the control group.

The differences between feed valorization of combined feed in the two groups were not significant during 0-3 weeks of growth and very significant in 4-6 weeks, when the control chickens consumed on average 3.32 g feed/g weight gain, by 46.3% less than the experimental group. Average specific consumption in the control group during 1-6 weeks was 3.47 ± 0.45 g/head/week, while in the experimental group it was of 4.06 ± 0.33 g/head, with 14.50% more compared with the control group.

3. Evolution of mortality in quail chicks in the experimental group compared with controls during 0-6 weeks of growth

In both groups the highest mortality was recorded in the first week of life (5% in the control group and 3.33 % in the experimental), when the very small quail chicks learn to orient in the environment. In the week 5th and 6th of life, although in the experimental group the lighting was reduced to 14 hours a day, there was no mortality in chickens, which have already accustomed to locate food and water and seems to benefit from the period of 10 hours darkness to rest.

If the control group during 4-6 weeks of growth recorded a total mortality rate of 7.33%, similar to the 1-3 weeks of growth (8.67%), in the experimental group during 4-6 weeks of growth percentage was significantly lower than in 1-3 weeks period (0.67% vs. 7.33%).

On the whole, in the period 1-6 weeks of growth, control chickens had an average mortality of 2.67%, while in the experimental group a rate of 1.33%, the difference between the two groups being highly significant.

The rate of total mortality during 1-6 weeks of growth in the control group chickens was 16%, while those in the experimental group recorded a total of 8%.

Table 3. Mortality of the quail chicks in the experimental group compared with control during 0-6 weeks of growth (%)

Growthweek	Control group	Experimental group
1week	5 %	3.33 %
2weeks	1.67 %	2 %
3weeks	2 %	2 %
4weeks	2.33 %	0.67 %
5weeks	2.67 %	0 %
6weeks	2.33 %	0 %
Average 1-3 weeks	2.89 %	2.44 %
Average 4-6 weeks	2.44 %	0.22 %
Average 1-6 weeks	2.67 %	1.33 %
Total 1-3 săptămâni	8.67 %	7.33 %
Total 4-6 săptămâni	7.33 %	0.67 %
Total 1-6 weeks	16 %	8 %

The total duration of illumination of the house in the 6 weeks of growth was 1008 hours in the control group and 798 hours in the experimental, which led to a reduction in electricity consumption required for illumination by 20.83 %.

CONCLUSIONS

From the research presented we can say that the application, in the growth period of 1-6 weeks of 24 hours light per day, resulted in superior growth parameters in the young quails of control group as respects in the body weight that averaged 200.50 ± 2.95 g/capita, up by 16.4% compared to the experimental group and the specific consumption of 3.47 ± 0.45 g/g gain, decreased by 14.50% compared to the experimental group.

Reducing the length of the light at 14 hours a day during the last three weeks of growth has led to a significant reduction of mortality rate to the experimental group, which was of 8%, compared to 16% in the control group, as well as of the energy consumption for the housing lighting, by 20.83%.

It remains to be further studied the effect of reducing the duration of illumination in young quail upon the future adult birds, exploited either to the production of eggs for consumption, or to the production of hatching eggs. Also, given the positive effect in reducing mortality in quail chickens, it is necessary to further study witch is the optimal age at which it can be applied the reduction of the illumination and also witch is the optimal duration of daily illumination to which can be reduced the light from the maximum of 24 hours per day.

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ANIMAL SCIENCE LEXICON STRUCTURE

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Abstract

Our work will follow the major terminological lines requested by the new theories in the field. However for the beginning, we'll follow the General Terminology Theory and we'll make an overview of the general, specialized and technical lexicons, every terminologist has to work with. We'll also exploit the difference between word and term, because we consider it to be an essential aspect not only for the interpreter and translator but also for the specialist. As a second research line, we'll make an overview of the terminological entry due to its major importance in the treatment of any specialized text or speech. We know well that every terminological entry includes information on the concept related to a specialized field but also on related terms. Thus, the terminologist will deal with the fields related to the term (main term, grammar value, etymology, status, syntagms, synonyms) and on the concept (domain, sub-domain, definition, concept representation, explanatory context and relations among concepts). In order to better understand the treated terms we'll also try to create a structure of the animal science lexicon, guiding ourselves on the syntactic aspect and taking into account the conceptual aspect for a future work.

Keywords*: animal science, terminological entry, specialized lexicon, technical lexicon, concept*

INTRODUCTION

Giving a definition of the lexicon has proven to be a very difficult task for any person, even if we speak of linguists, terminologists or specialists, as we deal with a lot of definitions given by important linguists depending on their theoretical perspective. Though, we have chosen two definitions which show our perspective on the lexicon.

''The lexicon is really an appendix of the grammar, a list of basic irregularities'' (Bloomfield, 1933). This definition seems to precede what Rey (1977) states in his famous work ''Le Lexique: images et modèles. Du dictionnaire à la lexicologie, concerning the lexicon. He sees it like <<objet historique et anthropologique, énorme et confus>>. Then, we may reach the conclusion that we cope with huge irregularities rendered by a linguistic community; but the truth is that any human being is endowed with the capacity of creating a lexicon of one's own. And for this reason we consider to be useful to develop this topic in a scientific work.

Mentioning the scientific and technical lexicons in our work demands to give a proper definition for each one.

Thus, scientific lexicon represents the lexicon used by any specialist, not taking into account one's training. It serves to express common notions for all scientific fields. The scientific lexicon terms express common notions for all science fields. Consequently, we don't find complete and precise information like in the technical lexicon.

However, scientific lexicon represents a connection among different areas of activity, being a communication centre.

The technical lexicon represents the inventory of words used by specialists, in a well-established field when putting into practice specific knowledge. We have also to mention that it is the technical lexicon the one which comprises the linguistic treasure of nomenclatures. Using the model offered by the authors of «L'Introduction à l'étude des langues de spécialité» (Miclau et al., 1982), we can suggest the folowing examples in order to better understand the definitions above:

Value - scientific lexicon term;

Nutritive value - half-scientific term;

Feeding value of forage – technical lexicon term

We have to mention the fact that scientific lexicon presents a general comprehension

value, while the specialized terms are well defined and specified giving the possibility to the specialist and to the terminologist to categorize them within technical lexicon. Thus, the term *milking flow* belongs to the technical lexicon.
Thus, in the following examples: *suckling pig, milk surpluses, soured milk, fat-corrected milk, coarse wool*, we remark that the main terms belong to the general lexicon , but the syntagms make part of the technical lexicon.

Word vs term
We have to emphasize the fact that the central notion in terminology is the term notion. But in order to understand better, we have to see which the difference between word and term is. For this reason, we state that the term is a word, consequently term is a co-hyponym of the word notion, but we have to bear in mind that each term is a word but each word isn't a term. Consequently, the words are categorized depending upon syntax, more exactly on the speech parts: noun, verb, adjective, adverb, preposition, conjunction, article, pronoun, etc., but only a lexical word (noun, verb, adjective or adverb) can be a term. We may apply the same categorization in case of terms too but bearing in mind that a term can't be expressed by means of article, pronouns, etc.
It is generally accepted that words are polysemantic. Every word knows a variety of meanings, meanings we find in each dictionary but <<les termes, en théorie, sont en soi univoques et monosémiques […] La polysémie de la langue commune constitue en terminologie une homonymie>> (Cabré, 1998).
According to Béjoint and Thoiron (2000), the difference between term and word resides in the meaning plan: the word depends on the linguistic environment, meanwhile the term is connected to the pragmatic environment. Thus, we can assert that the term represents the precise form of the word, more exactly, its application.
Maria Teresa Cabré considers the word and term like two independant units, more exactly <<les termes sont [..] des unités composées de la forme (dénomination) et du contenu (le concept) qui coïncident avec les mots seulement en apparence>> (Cabré, 2000). Thus the difference word/term will be better represented by the semiotic triangles, the classic one and the terminology one:

Figure 1. Saussure's semiotic triangle

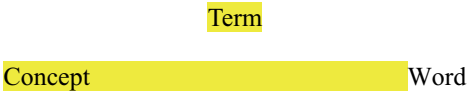

Figure 2. Terminology semiotic triangle

By comparing the two triangles, we can assert that the words reference content has no limits, fact which allows the polysemy development. We also meet the term defined as a word subject to restrictions. More exactly, we make the difference between label-term which belongs to a nomenclature and the speech terms.
III. Animal science syntactic lexicon structure
After having seen the difference between word and term we consider that it is necessary to make a classification of the lexicon we work with. This lexicon, as our title has already suggested, is the one belonging to the field of Animal Science. Thus, firstly, we'll try to make a classification following the nomenclatures existing in the animal science field.

- **Denominations and animal classes:** upgraded animal, domestic animal, game animal, store animal, grazing animal,

draught animal, goat, pigs, poultry, cattle, dairy cattle, nurse cow, dairy cow.

- **Animal products denominations:** cheese, hard cheese, soft cheese, pressed cheese, aged cheese, goat cheeese, mild cheese, storage cheese, factory cheese, soured milk, liquid-milk, fat-corrected milk, sterilized milk, powdered milk, cow milk;
- **Animal diseases denominations**: salmonellosis, pest, avitaminosis,, Gumbaro disease, Marek disease tuberculosis, pneumonia, mycoplasmosis, coryza, etc.
- **Machines, devices, equipments denominations in animal science field:** constant level drinker, (poultry) nipple drinker, pump drinker, smoke house, watering device, feeding device, compressor etc.

Analysing these terms, we acknowledge that the basic forms belong to the general lexicon like: milk, cattle, cow but however making part of a syntagm they acquire the term status.
Syntactically speaking, we can make the following classification:

- **Nouns**: poultry, animal, cattle, cow, goat, device, cheese, milk, factory, disease, etc.
- **Verbs:** to milk, to feed, to water, to process, to rear, etc.
- **Adjectives**: watering, feeding, powdered, fat-corrected, mild, hard, etc.

Terminological entry
Making a presentation of terminological entries means to explore varied fields which constitute real exploring domains both for specialist as for terminologist. In fact, the basic term will form the starting point for any terminological entry. It is essential to know that terminological entry comprises information on concepts of the specialized fields and associated terms. Thus, the terminologist should treat the fields connected to the term (basic term, grammatical value, etymology, status, synonyms, syntagms) and on the concept (field, sub-field, definition, concept representation, explanatory or associative context, concepts relations). Besides the information we have already mentioned, we may find additional information in the technical note for the concept and in the linguistic note for the term.
The terminological entry we have decided to present here show how the words expand themselves and turn from words into terms. We'll see the way how a word belonging to the general lexicon "MILK" receives technical values by means of the different semantic information rendered by the term in the technical lexicon.

Basic Term	Milk
Grammatical value	Noun
Domain	Animal Science
Sub-domain	Food industry
Definition	Milk is a white liquid produced by the mammary glands of mammals. It is the primary source of nutrition for young mammals before they are able to digest other types of food.
Definition source	http://www.thefreedictionary.com/Cow+milk
Generic	Milk
Specific	Cow's milk, goat's milk, ewe's milk, mare milk, yak'etc
Meronym	Soured milk, liquid milk, pasteurized milk, sterilized milk, fat-corrected milk, powdered milk etc.
Technical note	Milk is a nutritive beverage obtained from various animals and consumed by humans. Most milk is obtained from dairy cows, although milk from goats, water buffalo, and reindeer is also used in various parts of the world. In the United States, and in many industrialized countries, raw cow's milk is processed before it is consumed. During processing the fat content of the milk is adjusted, various vitamins are added, and potentially harmful bacteria are killed. In addition to being consumed as a

	beverage, milk is also used to make butter, cream, yogurt, cheese, and a variety of other products.
Source	http://www.answers.com/topic/milk#ixzz2uXMNrriI
Characteristics	Composition – proteins, lipids, lactose, minerals, vitamins, enzymes, etc. - Factors affecting composition – species breed, feed, stage of lactation - Physical and chemical properties – density, freezing point, color, pH, acidity, flavor - Bacteria - Pathogenic Bacteria - Somatic Cells and Microorganisms - lactobacilli, history, significance in cultured products
Source	http://www4.ncsu.edu/~adpierce/u03_characteristics_milk.pdf
Milk products	Cheese, hard cheese, soft cheese, yogurt, butter, etc.
Destination	Like animal or human food.
Linguistic note	Common noun, simple form, masculin, singular.

CONCLUSIONS

We can state that the difference between words and terms is essential in any terminological treatment of any text or speech because as we have already seen in the terminological entry the technical and linguistic notes offer various information, useful both for the terminologist as for the specialist.

Concerning the animal science lexicon, we can assert that we deal with a very rich field, even if from the terminological point of view, there is still a lot of work to do, because works on animal science terminological field are very rare.

Another aspect, worth to be mentioned is the structure of the lexicon which is however simple, nouns, adjectives and verbs occupying the first places.

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THE EFFECT OF ASCORBIC ACID ON BODY WEIGHT LOSS OF BALI CATTLE DURING TRANSPORTATION

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Abstract

Transportation caused stress and had impact on the body weight loss of Bali cattle after arriving at the destination. Body weight loss due to the stress of transport can be suppressed and reduced by administration of ascorbic acid. The purpose of this study was to determine the dose of ascorbic acid how best to give effect to body weight loss of Bali cattle during transportation. The experiment used randomized block design (RBD) consisted of 4 treatment dose levels of ascorbic acid (0 mg/kg body weight, 50 mg/kg body weight, 100 mg/kg body weight and 150 mg/kg body weight) were repeated 6 times, it took 24 head of Bali cattle. The difference between the treatment effect was tested using the Dunnett test. The results showed ascorbic acid significantly (P<0.05) reduced body weight of Bali cattle loss during transportation. Treatment with ascorbic acid dose of 100 mg/kg body weight gave the lowest loss of 4.083 kg (2.431%) at the time of transportation.

Keywords*: ascorbic acid, body weight loss, Bali cattle, transportation*

INTRODUCTION

Due to the geography of Indonesia is an archipelago, the Bali cattle shipments from NTT to other islands, particularly to the island of Java requires transportation by land or sea. In its delivery to the island of Java requires a relatively long travel time, because the first 8 hours of cattle transported by road from the city of Kupang heading to the Port, and for 53 hours transported by ship from the port to Surabaya.

Transportation causes stress livestock and its impacts on energy depletion and dehydration or lack of body fluids that cause loss of body weight of cattle upon arrival at destination. In turn farmers at a disadvantage, because it is usually the buyer is only willing to pay to the merchant after the cattle gets to their destination.

Transportation causes cattle to lose weight between 3-11 percent for the 18-24 hours of long transportation (Knowles and Warris, 2000 cited by Suryadi, 2011). In the current condition of transportation facilities, loss of cattle body weight during transport to around 5.5 % in Java and outside Java, 10.5 % (Ilham and Yusdja, 2010). Travel for 12, 24, 48 and 96 hours caused loss of body weight 6, 8, 12 and 14 % (Wythes, 1982 cited by Suryadi, 2011). The cattle that were transported 5-6 hours lost 2 to 6.3% of body weight before. Transportation 24 hours by road, live weight of cattle lost about 8% (Mayes et al., 1979, Lambooy and Hulsegge, 1988; Tarrant et al., 1992 cited by Suryadi et al., 2011).

The research conducted by Penu et al. (2008) to 218 heads of cattle was gained an average loss rate of body weight that was transported from NTT to Jakarta at 12.60% of the body weight when purchased from a breeder. The loss rate was still higher compared with the results of Ilham et al. (2004), who reported a loss rate of body weight of cattle delivered different islands from Jakarta to Mataram in 11-12%.

Each year approximately 60,000 sales occur selling with an average weight of 250 kg and the selling price per kg of live weight Rp. 15.000. The amount of Bali cattle loss were transported to the island of Java from Mataram could reach 11-12% (Penu et al., 2008) up to 1 head of cattle will loss of 27.5 kg or will suffer a loss of Rp . 412.500 per cattle, therefore, each year achieve fantastic losses amounting to Rp. 24.75 billion. Therefore, in an effort to reduce of losing transported cattle body weight can be done by providing useful feed additive that keep the immune system during transport, one of which is the administration of ascorbic acid.

Ascorbic acid has attracted the attention of scientists in recent years because of its ability to cope with stress (high environmental temperature, disease and transport) in various animal species. Ascorbic acid is a natural antioxidant that can specifically stimulate GABA (gamma amino butyric acid), a type of receptor that serves to modulate the communication between cells in the brain. Recent research has shown that GABA will cease to function in the brain when ascorbic acid is not available in the body or in the brain (Calero et al., 2011). Asala et al. (2010) showed a decrease of antioxidants in the body caused by stress. Ascorbic acid is a vitamin that gives potential antioxidant function for the body, relatively inexpensive and virtually no toxic effects and easily metabolized in the body.

At the time of stress due to transport livestock, the hormone cortisol is secreted into the blood vessels, thus generally giving effect to the excretion of urine and faeces. This leads to loss body weight in cattle during transport. However, the administration of ascorbic acid, which is the stress hormone cortisol can be inhibited by GABA, so that defecation and urination can be reduced. The positive emotional response from acidifying ascorbic acid will run through the body and is received by the brain stem, then transmitted to one of the major parts of the thalamus of the brain. Then, contact the thalamus hippocampus to secrete GABA which served as a control emotional response, and inhibits acetylcholine, serotonis and other neurotransmitters that produce cortisol secretion (Calero et al., 2011).

MATERIALS AND METHODS

The animals used in this study were 24 male Bali cattle with a range of 2-2.5 years of age. Before transported, cattle were grouped first for 1 week based on randomization.

The ascorbic acid was administered orally to Bali cattle before they were transported. The ascorbic acid in the form of white powder with the chemical formula $C_6H_8O_6$ or by systematic IUPAC is 2-oxo-L-threo-hexono-1, 4-lactone-2, 3-enediol. Before it was given to cattle, ascorbic acid dissolved in water (H_2O).

Livestock transported in an open truck capacity 2 units of 12 cows per unit and were placed randomly as grouping. Journey performed for 8 hours, starting at 08:00 am the day until around 16:00 pm.

The tools used in this study were: Digital scale with a capacity of 1 kg to 0.5 g precision scale to weigh the dose of ascorbic acid. The fruit scales electric capacity of 600 kg/0.2 kg. OCS-XZ-GGE. This tool was used for weighing animal observed.

The preparation before the transport was grouping cattle. Livestock grouped 1 (one) week prior to transport. Furthermore weighing cattle before transporting, conducted to determine the weight before transporting.

Ascorbic acid administration procedures in this study were:

a. Before fed to livestock, ascorbic acid was weighed according to the first dose of body weight each cow;
b. Ascorbic acid which has been weighed and then diluted with water to 20 ml;
c. Ascorbic acid solution incorporated into the "Oral Bottle";
d. Oral ascorbic acid administration to cattle made 45 minutes prior to the transport.

The transport path traversed was Politani Kupang – Merbaun - South Coast - Politani Kupang, with the distance of 250 km and a long time 8 hours. During the journey the cattle were not fed and watered.

Transporting procedures performed in this study were:

a. Provision of ascorbic acid did before transporting;
b. One truck transporting 12 head unit which consisted of each treatment as a group;
c. After all the animals transported on trucks, then performed the journey for 8 hours. Time off only for 15 minutes at 12:00 to eat and drink drivers.

The collection and recording of data were done after 8 hours of transport was completed, and all the animals have been unloaded from the truck. The data collected in this period were body weight before, after transportation and body weight loss.

The parameters observed in this study were body weight (kg) and body weight loss (%).

The research conducted experiments using a randomized block design (RBD) with four treatments and replications of each treatment done 6 times. The treatment according to the dose of ascorbic acid of research results of Minka and Ayo (2010), namely:
R0 = Control
R1 = Ascorbic acid 50 mg/kg body weight
R2 = Ascorbic acid 100 mg/kg body weight
R3 = Ascorbic acid 150 mg/kg body weight

RESULTS AND DISCUSSIONS

The effect of ascorbic acid administration on the body weight loss of Bali cattle during transport can be seen in Table 1. From Table 1 it appears that the results of the study showed the presence of loss of body weight in each treatment after transporting, in line with the dose of ascorbic acid used. The loss was the highest body weight of 10.75 kg and was obtained at R0 treatment (control) and the lowest was 4.00 kg in R3 (dose 150 mg/kg body weight). The increasing doses of ascorbic acid were given, the lower loss percentages of body weight or body weight. The amount of weight percentages of each treatment to the control (R0) was equal to 49.6% at R1, R2 62.05% and at 64.29% in R3.

Table 1. Administration Effect of Ascorbic Acid on Average and Body Weight Loss

Doses	Analysis	Variables		
		Body Weight (kg)		
		Before Transport		After Transport
R0	Mean (kg)	201.42		190.67
	Std Deviation	30.92		24.78
	Changes		-10.75	
	BB Loss (%)		5.34	
R1	Mean (kg)	186.75		181.34
	Std Deviation	21.25		21.12
	Changes		-5.42	
	% of R0 (%)		49.60	
	BB Loss (%)		2.90	
R2	Mean (kg)	167.92		163.83
	Std Deviation	33.44		31.89
	Changes		-4.08	
	% of R0 (%)		62.05	
	BB Loss (%)		2.43	
R3	Mean (kg)	153.00		149.00
	Std Deviation	34.90		35.12
	Changes		-4.00	
	% of R0 (%)		64.29	
	BB Loss (%)		2.61	
	Probability (P)		P<0.05	

From this study, it can be proven, that the transportation was an activity that causes stress on livestock, due to the increase in physical activity suddenly, which were generally the transport process in areas such as NTT done the traditional way and with minimal facilities, so usually cattle experienced coercion and restraint during transportation. The higher the stress the animal received the higher the production of free radicals that can lead to increase generation of free radicals and ROS (Reactive Oxygen Species) in the body (Adenkola and Come, 2010), but with the administration of ascorbic acid appears the preparation of a lower body weight or loss that occurs can be suppressed.

To determine the difference in the effect of ascorbic acid dose on cattle body weight loss during transport, performed statistical tests to test variability. Results of analysis of variance showed that the dose of ascorbic acid ($P<0.05$) reduced body weight loss cattle during transport. To find the difference between the treatment effect followed by Dunnet's test, and the results are presented in Table 2.

Tabel 2. Dunnett Test Results Rate Differences Between Body Weight Loss of Control and Treatment

The Difference with Control Treatment	Loss (kg)	Std. Error	Significance
R1-R0	5.3333	2.3497	0.094
R2-R0	6.6667	2.3497	0.032
R3-R0	6.7500	2.3497	0.030

Notes: * Indicates significant difference ($P<0.05$)

Different results of body weight loss on each treatment with the control in Table 2 above shows that the administration of ascorbic acid at a dose of 50 mg/kg body weight of cattle (R1) did not make a difference loss to the control treatment (R0), but the administration of ascorbic acid with a dose of 100 mg/kg body weight (R2) and 150 mg/kg body weight (R3) showed differences in different loss of body weight significantly ($P<0.05$) greater than control (R0). It means that a decrease in body weight or body weight loss R2 and R3 markedly lower than the control (R0).

The difference between the effect of treatment at doses of ascorbic acid low of 50 mg/kg body weight, yet showed the presence of excess body weight loss during transportation means. Difference in loss for new transport body weight were evident ($P<0.05$) in R_2 and R_3. The increasing doses, leads to changes and

differences in the loss of cattle body weight. The difference was due to the ability of ascorbic acid to stimulate amino butyric acid (GABA), which is located in the brain. GABA is one of the major inhibitory neurotransmitter of the central nervous system, serves to inhibit the release of the hormone cortisol as a cause of stress. The cortisol is released into the blood causing an increase in body homeostasis by means of defecation and urination so the cattle's body weight loss during transport. In line with the higher doses of ascorbic acid, the higher the ability amino butryric acid (GABA) stimulate to inhibit the release of cortisol into the blood. Thus the administration of ascorbic acid with the higher dose also more effective in reducing the release of the hormone cortisol, so the impact of body weight loss due to transportation can be reduced (Minka and Ayo, 2010). To clarify how the loss of body weight each treatment is shown in Figure 1. The illustration shows that the higher doses of ascorbic acid the lower the loss of cattle body weight during transport.

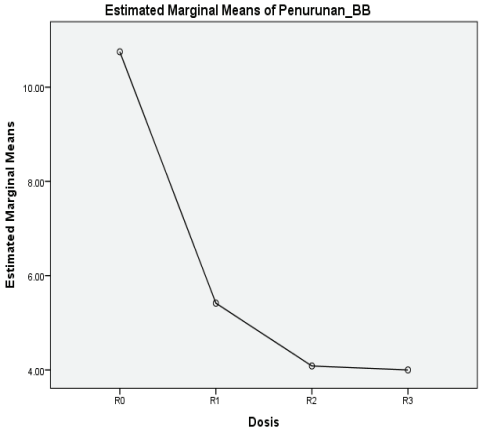

Figure 1. Graph of the effect of ascorbic acid on body weight loss

In normal conditions, the needs of ascorbic acid in ruminants have been met and no need for additional supplements. However, under stressful conditions, the status of ascorbic acid in the body becomes less (Ozimek and Kennelly, 2010). Therefore, the necessary conditions of stress livestock is the intake of ascorbic acid in order to meet the needs of the body against the effects of stress, so it appears the role of ascorbic acid in reducing body weight loss due to transportation.

CONCLUSIONS

Based on the results of the discussion, the following conclusions can be drawn:

- Provision of ascorbic acid up to 150 mg/kg of body weight lowered loss weight of Bali cattle during transport;
- Provision of ascorbic acid at a dose of 100 mg/kg body weight gave the lowest loss of body weight in Bali cattle transport.

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INFLUENCE OF APPLYING BROILER WELFARE LAWS ON UNIT COST

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Abstract

European Union and especially some national governments have gradually increased production costs by political decisions of introducing new rules concerning food safety, protection of environment and animal welfare. This paper has been performed to assess economical consequences of applying broiler welfare laws on unit cost by kg live weight. The study was performed during four years with production data from six top poultry farms from Romania before (V_1) and after the introduction ob broiler welfare rules (V_2 and V_3).
Percentage of savings and losses was calculated for each variant based on unit cost which was found before welfare rules came into action based on expenses categories in same economical conditions to emphasize economical consequences of applying broiler welfare rules. Analyzing these percentages has revealed that unit cost is 1.81% higher in variant V_2 than in variant V_1 and 3.43% higher in variant V_3 compared to base variant. Highest losses by expense category have been noted for lightning (48.22% - V_2 and 105.50% - V_3) followed by fuel, amortisements and labour force expanses. Lowest losses are in other expenses category with values between 4.86% in variant V_2 and 16.16% in variant V_3. These losses are due to more lamps used for lightning. Besides these losses welfare rules also brings savings in unit costs between 1.12% for biological material and feeds – V_2 and 37.50% for ventilation energy – V_3.

Key words*: evolution, milk production, NW Region, Romania, trends.*

INTRODUCTION

Concern for animal welfare aspects has appeared in many countries and especially in the strong developed ones due to introduction and extension of the intensive animal production system to supply food needed for an ever growing human population.
Importance of animal welfare for parts of human society is having ethical, social, political and economical motivations. Social motivation is actually triggering the others by making consumers aware that their welfare and health are dependent on quality of material factors of the environment in which they are living and on the safety of food they are eating in close relationship welfare level of animals from which their food is coming (Custura et al., 2010).
European Union and especially some national governments in the North-west of European Union have gradually increased production costs by political decisions of introducing new rules concerning food safety, protection of environment and animal welfare.
This paper was perform to assess economical consequences of applying broiler welfare legislation (Directive 43/2007/CE and Edicts MADR 239 and 264/2012 – Measure 215), on unit cost by kg live weight.

MATERIALS AND METHODS

The study was performed during four years with production data from six top poultry farms from Romania before and after the introduction ob broiler welfare rules. Resulting data were recorded and statistically processed by known classical procedures and differences significance was tested by multiple Student test.
Influence of these rules on unit cost in poultry meat was studied by simulating two working conditions. The first involved the minimal requirement (V_2) - Directive 43 (average density 35 kg live weight/sqm and light intensity of 20 lux/sqm) and the other involved the higher requirement (V_3) – Measure 215 (average density 32 Kg live weight/sqm and light intensity of 30 lux/sqm) and results were

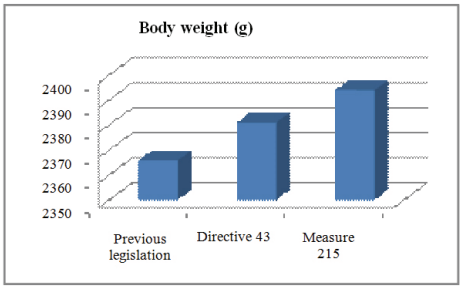
compared with those obtained before these rules came into force (V_1) - (average density 44 kg live weight/sqm and light intensity of 10 lux/sqm).
Unit costs by kg live weight were find based on structure, consumption and cost of used feeds and consumption and cost of other resources and final production performances for each working condition and income lost and supplementary expenses were calculated based on these costs.

RESULTS AND DISCUSSIONS

Cost is value expression for consumption of income yielding factors. Expense became cost by consumption so cost is consecutive to consumption. Reducing production costs is a priority so detailed analyses of cost forming expenses and studying their efficiency and studying relationship between production cost and yield are compulsory.
Analyze of final production parameters is of major significance as their size has an influence on final production costs. Results from the six poultry farms during four years are showing that values are different by variant for each studied parameter (Table 1, Figure 1).
Body weight is between 2365.89 ± 174.34 g in V_1 and 2393.93 ± 176.62 g in V_3; specific consumption is between 1.76 ± 0.14 kg in V_3 and 1.79 ± 0.12 kg, la V_1; mortality is between 3.18 ± 1.22 % V_3 and 4.45 ± 0.12 % in V_1.

Table 1. Broiler production performances based on welfare conditions

Specification	U. M.	Previous legislation		Directive 43		Measure 215	
		Average	StDev	Average	StDev	Average	StDev
Average live weight	g	2365.89	*174.34*	2381.01	*126.65*	2393.93	*176.62*
Average daily gain	g	55.38	*4.15*	55.74	*3.01*	56.03	*4.21*
Cumulative mortality	%	4.45	*0.97*	3.95	*0.8*	3.18	*1.22*
Specific intake	g	1.79	*0.12*	1.77	*0.14*	1.76	*0.14*
BPI	points	300.69		307.64		313.47	



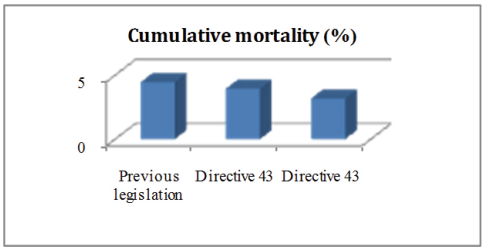


Figure 1. Final production performances

Production index (BPI) was used at the end of the experimental period for a more complex quantification of results. This index of assessing broiler production efficiency based on broiler's age at slaughtering, livability, live weight and feed efficiency has values between 300.69 points in V_1 and 313.47 points in V_3.
In Table 2 are shown results obtained by testing differences for analyzed parameters. Analyze of these data reveals no significant differences between working variants.

Table 2. Significance of differences between average production performances (Student test values)

Specification	Previous legislation / Directive 43	Previous legislation / Measure 215	Directive 43 / Measure 215
Average live weight	0.1719^{NS}	0.2708^{NS}	0.1388^{NS}
Average daily gain	0.1720^{NS}	0.2693^{NS}	0.1373^{NS}
Cumulative mortality	0.9741^{NS}	1.9959^{NS}	1.2928^{NS}
Specific intake	0.4899^{NS}	0.3985^{NS}	0.8831^{NS}

Table 3. Influence of biological material on unit cost

Specification	Previous legislation	Directive 43	Measure 215
Meat production/house (tons)	44	35	32
Chicks delivered (heads)	422.67	419.98	417.82
Cumulative mortality (%)	4.55	3.95	3.18
Chicks placed (heads)	441.47	436.56	431.10
Unit price (lei/heads)	1.52	1.52	1.52
Unit value (lei/ton)	671.03	663.57	655.27
Savings (lei/ton)	-	8.34	17.62

Unit cost means cost by product or by utile effect. Production unit costs for product „live meat productions" found in these researches comprise both direct and indirect production expenses. When unit cost is concerned fixed cost becomes also variable by decreasing when products amount increases and increasing when products amount decreases.

Therefore influence of direct and indirect expenses on cost at product „live meat productions" was found based on average prices of used raw materials taking legislation into account (Van et al., 2003). Main direct expenses influenced by welfare conditions are shown in Tables 3, 4 and 5. Analyzing these data reveals that biological material has a positive influence on unit costs of between 8.34 lei/ton in variant V_2 and 17.62 lei/ton in variant V_3 especially because of a better variability recorded at lower densities. Lightning has a negative influence on unit cost of between 38.48 lei/ton in variant V_2 and 84.19 lei/ton in variant V_3 due to both hour consumption and supplementary daily consumption. Also lamp number is having a negative influence due to supplementary used number and values between 2.37 lei/ton in variant V_2 and 7.88 lei/ton in variant V_3.

Table 4. Influence of lightening on unit cost

Specification	Previous legislation	Directive 43	Measure 215
Meat production/house (tons)	44	35	32
Consumption/hour/house (kHz)	2.04	4.02	6.00
Lightning difference (kHz)	-	1.98	3.96
Supplementary consumption/day (kHz)	-	35.64	71.28
Supplementary consumption house/cycle (kHz)	-	1496.88	2993.76
Unit price (lei/kw)	-	0.9	0.9
Value of supplementary electrical energy	-	1347.19	2694.38
Supplementary unit cost (lei/ton)	-	38.48	84.19

Percentage of savings and losses was calculated for each variant based on unit cost which was found before welfare rules came into action based on expenses categories in same economical conditions to emphasize economical consequences of applying broiler welfare rules (Table 6). Analyzing these percentages has revealed that unit cost is 1.81% higher in variant V_2 than in variant V_1 and 3.43% higher in variant V_3 compared to base variant. Highest losses by expense category have been noted for lightning (48.22% - V_2 and 105.50% - V_3) followed by fuel, amortisements and labor force expanses. Lowest losses are in other expenses category with values between 4.86% in variant V_2 and 16.16% in variant V_3. These losses are due to more lamps used for lightning. Besides these losses welfare rules also brings savings in unit costs between 1.12% for biological material and feeds – V_2 and 37.50% for ventilation energy – V_3.

Table 5. Influence of lamps number on unit cost

Specification	Previous legislation	Directive 43	Measure 215
Meat production/house (tons)	44	35	32
Lamps number / house (pieces)	34	67	100
Working period (hours/lamp)	279	549	820
Number of supplementary lamps (pieces)	-	270	541
Unit price (lei/piece)	2	2	2
Value of supplementary lamps (lei/house)	-	540	1640
Supplementary unit cost (lei/ton)	-	2,37	7,88

Table 6. Influence of welfare rules on unit cost

No.	Specification	Previously	Legislation			
			Directive 43		Measure 215	
		%	Savings	Losses	Savings	Losses
			%	%	%	%
A	Direct expenses	100	-	1.87	-	3.55
I	Material expenses	100	-	1.47	-	2.98
1	Biological material	100	1.12	-	2.40	-
2	Feeds	100	1.12	-	1.70	-
3	Medicines	100	-	-	-	-
4	Energy. fuel. water	100	-	11.59	-	24.91
4.1.	Fuel	100	-	25.70	-	37.49
4.2.	Feeding energy	100	25.86	-	35.55	-
4.3.	Ventilation energy	100	25.71	-	37.50	-
4.4.	Lightning energy	100	-	48.22	-	105.50
5	Amortisements	100	-	25.71	-	37.49
6	Other expenses	100	-	4.86	-	16.16
II	Working force expenses	100	-	25.70	-	37.48
B	Indirect expenses	100	-	-	-	-
C	Total production expenses	100	-	1.81	-	3.43

CONCLUSIONS

Following conclusions were drawn from studies performed in this paper:

- final production performances are different by working variant and they

are usually better in variant with lower density and higher lightning intensity;

- in variant V_2 unit cost is 1.81% higher than in variant V_1 and in variant V_3 unit cost is 3.43% higher compared to base variant;
- highest losses by expense category have been noted for lightning (48.22% - V_2 and 105.50% - V_3);
- lowest losses are in other expenses category with values between 4.86% in variant V_2 and 16.16% in variant V_3;
- highest savings in unit cost are noted for ventilation energy (37.50% - V_3).

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STUDY REGARDING THE PRODUCTION PERFORMANCE OF MONTBELIARDE DAIRY COWS IN THE SOUTHERN AREA OF ROMANIA

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Abstract

Generally cattle produce 95% of the total milk from the market, over 33% of meat production, 90% of all raw hides and 75% of the livestock manure. Romania can ensure forage for seven millions cattle. At present, there are 2.162.013 cattle, of which 1.231.857 dairy cows. Based on this situation requires a review of breeding strategies and the distribution of species and breeds in different geographical areas. The preservation of natural resources as a support for biological, social and economic life is the key for sustainable development of society. In last years it has imported and other breeds of cattle. Montbéliarde breed has adapted very well to the climatic conditions and plant resources in our country. In France, the country of origin, it is considered a milk breed with the average milk yield about 7500 kg / normal lactation (305 days),3.8 to 3.9% fat and 3.45% protein. The total number of Montbeliarde dairy cows is at present in Romania over 2000, of which 676 animals in official control of production. The current study was conducted in three farms in the south of Romania. In these farms animals have been brought from France, heifers in pregnancy months IV-VII. After the end of the first lactation, production data were recorded and statistically analyzed. The statistical analysis was performed on a number of 379 primiparous cows. It was noted that the main parameters of production showed variations between farms. Thus, the duration of lactation had values between 314-330 days, with implications on the quantity of milk and the reproduction activity. Regarding the average quantity of milk per cow values were recorded between 5646 and 6320 kg milk and fat percentage of 4.14, respectively 3.80%. The production values are influenced by many factors (conditions of feeding, milking, sheltering) and they influence the total quantity of milk for processing, and quality.

Keywords: *breed, dairy cows, fat, milk*

INTRODUCTION

The world passes through a phase of economic crisis and also it provides the emergence of a food crisis. Based on this situation requires a review of animal breeding strategies and the distribution of species and breeds in different geographical areas. From the point of economic perspective, the Romanian agriculture makes a contribution of 6% to gross domestic product (GDP). Out of the 23.8 million hectares which represents the total area of Romania, the agricultural land is 14.7 million hectares (61.7%), of which 9.38 million hectares is arable land. Romania has very good agricultural potential and ranks 5th as arable surface after France, Spain, Germany and Poland.

Cattle in Europe are constituted by two species, namely cattle and buffalos the first is the most important and widespread. Thus, it is found in all European countries, the ratio being 38.24 / 1.00 cattle / buffalos. Regarding cattle farming, Romania currently has 2.162.013 cattle, dairy cows are 123.1857 heads, which means 57%.

At the beginning of 2013, in Romania 93% of cows are in the farms are family type. During the last few decades this species has fluctuated numerous, but downsizing was very important. In Romania the cows in official control of milk production represents 2,1% of the total herd queen .Regarding the structure of cattle breeds, Romanian Spotted breed is owned first position with 30.2%, Romanian Black Spotted 23%, Brown breed 16.7%, Pinzgau breed 0.7% and other breeds 0.6%.

In dairy cattle growth strategy is outlined two fundamental objectives: growing number and improve the quality of the herd queen, increasing and improving the milk

production. For efficient use of raw milk must invest in upgrading work so that to obtain controlled dairy products with high quality and safety.
Cattle ensure workforce stability in rural and mountainous, safe source for trade, better use of feed obtained from natural pastures.
In recent decades, in Romania have brought specialized cattle breeds from different countries. These specialized breeds for milk or meat are brought in Romania, because we need milk and meat on the internal and European market. Montbeliarde breed is a breed imported which meet the needs of farmers to obtain income from multiple sources. The milk quality and quantity are high, the technical indicators of fattening young male are higher than indigenous breeds and disease resistance and adaptability to conditions of farms in Romania are good.

MATERIALS AND METHODS

In Romania there are currently about 2,000 Montbeliarde breed cows. From this herd 676 head of dairy cows are in official control of milk production. This research was conducted in three farms with different capacities in South-Romania. The animals studied are imported from France as heifers with gestation ranging between IV - VII months. They have calved on farms in Romania, which purchased animals. The study was conducted after completion of the first lactation.
The farm no. 1 is located in Prahova county and has a herd of 580 heads brought from France of which 232 dairy cows. The farm is currently in the process of certification as organic farm.
The farm no. 2 is located in the Teleorman county, in the most southern part of Romania, near Zimnicea city. This farm has a herd of 1,000 head of two breeds Prime Holstein and Montbeliarde. The study was conducted on a total of 125 heads Montbeliarde breed cows which have completed first lactation.
The farm No. 3 is located near Bucharest in Ilfov county and belongs to the University of Agronomical Sciences and Veterinary Medicine Bucharest. Animals have been imported from France in 2010. Total number of animals on the farm is 59 heads, of which 22 heads of dairy cows.
The data necessary for the study resulted from consultation records of livestock farms studied. The production data resulted from periodicals official controls production.
In terms of quality milk we conducted analyzes on milk fat and protein content using EcoMilk portable device. It was created a database, the date were statistically processed by known methods and were compared with those from the literature, in especially those in the country of origin.

Figure 1. Picture of Montbeliarde cows in Romania

RESULTS AND DISCUSSION

The duration of total lactation is the time between the day of calving and cow's weaning. The duration of lactation is particularly important indicator that influences productive performance in the direction of milk production and reproduction activity. The factors that have significant influence on the total lactation period are: the order of lactation, the genetic structure of the population, age of first calving and calving season, individual variability and conditions for exploiting.
In dairy cows of Montbeliarde breed from southern Romania it observed that duration of

the first lactation had average 324.4 days, with a low coefficient of variation (7.21%).

From Table no. 1, that the longest duration of lactation was recorded in the farm no. 1, situated in the Teleorman county (330.12 days). Primiparous cows from the farm no. 2 had an average value of 329.5 days, with 35 days longer than their mothers in France (Vidu, 2011).

The high value o of the first lactation at primiparous cows indicates a good precociousness of dairy cows lactation and good persistence.

By applying the test of significance between the averages of three farms there are not differences ($p < 0.05$).

To cows of Montbeliarde breed imported from France in Moldova area average value of total lactation period was 309.60 days (Buceag et al., 2013).

The comparative analysis with other breeds in Romania showed that Romanian Spotted breed had an average between 332 days (Georgescu et al., 1988) and 341.87 days (Reman, 2004). For Brown breed duration of total lactation was between 317-357 days (Alexoi, 1983). For Romanian Black Spotted breed Murat (1995) determined an average of 357.9 days.

The milk quantity of total lactation is the most important parameter of milk production that underpins economic hierarchy of animals, genetic improvement and is the main result of using modern technologies. The analysis of 379 lactations completed was obtained an average of 5977 kg milk with high variability (23.11%). The highest milk production was recorded in farm no. 2 -6320 kg, but dairy cows are very heterogeneous (32.9%) in the genetic aspect.

On the farm no.1 average production was 6086, 8 kg milk and on the farm no.3 quantity of milk was 5524 kg. The results on the farm no.1 are similar to those found by Bugeac et all (2010) in farms in Moldova in Montbeliarde cows imported from France. At these dairy cows average production was 6036, 12 kg, with a 47% greater heterogeneity. Vidu (2011) calculated for Montbeliarde cows in the farm of University of Agronomic Sciences Bucharest an average value of 6921.47 kg milk at equivalent of maturity. The statistical data recorded after performing official control of production have shown that the national averages for milk production is 7947 kg milk Romanian Black Spotted breed and 6151 kg milk Romanian Spotted breed. The average value recorded at Montbeliarde cows in southern Romania placed this breed to the position third in the performance structure.

Table 1 The average values of production parameters at the primiparous of Montbeliarde breed in southern Romania

The farm	n	The statistical parameter	The parameter			
			The duration of total lactation (days)	The quantity of milk (kg milk per total lactation)	Fat (%)	Protein (%)
1	2	3	4	5	6	7
1	2	3	4	5	6	7
Far no.1	232	$X \pm s_X$	314±4.81a	6320±78.16a$_1$	3.86±0.02a_2	3.42±0.01a_3
		V%	4.78	32.9	16.11	17.05
Farm no.2	125	$X \pm s_X$	330.12±3.62 b	6086.8±262.11b_1	3.80±0.02b_2	3.55±0.04b_3
		V%	6.19	18.6	7.11	8.12
Farm no.3	22	$X \pm s_X$	329.5±3.63c	5524±191.12c_1	4.18±0.09c_2	3.61±0.04c_3
		V%	10.5	21.2	3.21	4.16
Total	**379**	**$X \pm s_X$**	**324.4±3.91**	**5977±182.16**	**3.95±0.08**	**3.53±0.05**
		V%	**7.21**	**23.11**	**9.81**	**9.98**

The level of significance: a,b,c,a_1,b_1, a_2,b_2,c_2, a_3,b_3,c_3 ($p<0.05$); b_1,c_1, b_2, c_2 ($p<0.01$)

The milk quality. Milk fat and protein content represents important criteria in the selection, the share of over 80%, depending on the country. The importance of the fat and protein content consists of the following: it provides a certain biological value of milk (1 gram of fat provides for human body 9 calories and a content of 20 amino acids plus the macro-and oligoelements); the milk protein have a role plastic, the main component of cell and genetic role, the support material of heredity; the content odoriferous substances in fat provides of the milk flavor and aroma volatile special; fat influences the efficiency in obtaining dairy fat; the content of casein at protein (over 80%) affects the yield of the cheese (Reman, 2004). The Table 1 can see that the percentage of fat varied between 3.80% and 4.18% with an average 3.95% fat. It is noted that in farm no. 3 recorded a value of 4.18%, with low variability. The average content of protein is 3.53%, with average limits between 3.42% and 3.6%.

The phenotypic correlations between milk production characters. The knowledge of the correlations between milk production characters is very important to establish of objectives and improvement method. Correlations between characters are given by the intensity the relationships between genes or blocks of genes with pleiotropic action. Analysis of two or more characters show that between them there is interdependence due to the direction of phenotypic expression. The study correlated character of particular interest for improving methodology because it provides the possibility of establishing the causes which causes correlation and to assess the extent to which improving a character will cause simultaneous changes in other characters (Reman, 2004).

The correlation between the quantity of milk and duration of lactation. The average value is 0.46, which represents a mid-correlation, but very significant statistical (table 2).

The correlation between the quantity of milk: fat percentage, protein percentage. Between quantity of milk and fat content (-0.031), and the milk quantity and protein percentage (-0.044), the correlation is not significant and negative, which means that the selection of one of the two characters is accompanied by unfavorable response to another but little. For these couples of characters at Romanian Spotted breed other authors have found the following: Petre (1988) quantity of milk: - fat% 0.08 ... -0.10, Alexoiu (1983) -0.21. It is noted that the value found by us is the lowest. To the Brown breed, Alexoiu (1988) established the weakly positive correlation between the quantity of milk and fat percentage respectively 0.01. Between milk quantity and fat content at Romanian Black Spotted breed were established values between -0.15 and -0.315 (Murat, 1995).

Table 2 Correlation analysis between the characters of milk production for total lactation at Montbeliarde cows in southern Romania

Specification	The duration of total lactation (days)	The quantity of milk (kg milk per total lactation)	Fat (%)	Protein (%)
The duration of total lactation (days)	1			
The quantity of milk (kg milk per total lactation)	0.460±0.011 ***	1		
Fat (%)	0.037±0.020 NS	-0.031±0.03 NS	1	
Protein (%)	-0.130±0.04 ***	-0.044±0.03 NS	0.480±0.016 ***	1

CONCLUSIONS

Montbeliarde breed was imported in the last decades in Romania. This breed has adapted very well to the climatic conditions of our country and gives good productions. From our research that has been done in three farms in southern Romania the production obtained from cows that have completed the first lactation were similar to those obtained in France. We consider that Montbeliarde breed can be raised successfully in many farms in Romania, in the submontane, hill and plain areas.

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TECHNOLOGIES OF THE AGRO FOOD PRODUCTS PROCESSING

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COLD SUPPLY CHAIN MANAGEMENT IN PROCESSING OF FOOD AND AGRICULTURAL PRODUCTS

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Abstract

The quality and safety of food and most agricultural products during storage time in cold stores and distribution is highly dependent on management and monitoring of time-temperature history concept. Control of temperature should be taken into account throughout the cold chain, from farm or factory to consumers (i.e. farm-to-fork) , to ensure food safety and hygiene and to maintain product quality. Microbiological and biochemical changes take place in food stuffs, and therefore the end quality depends on the temperature and moisture history of products. In order to control the end quality, it is therefore necessary to trace and control the temperature. The Radio frequency identity (RFID) and the Temperature monitoring tags are showing a future in completing the cold chain of perishable products (such as dairy, meat, seafood, fruit and vegetables). Time-Temperature indicators or integrators labels (TTIs) are another traceability tools that could be used as an intelligent shelf life decision system for quality optimization of the food and agricultural products during chill chain. In this paper some important tools for traceability and monitoring of safety and quality of main agricultural products in cold supply chain were reviewed.

Keywords: *Cold chain, Time-Temperature indicators, RFID tags, agricultural products*

INTRODUCTION

Refrigerated foods are one of the fastest growing sectors of the food industries. Continued success relies upon effective management of the 'cold chain', a term used to describe the series of interdependent operations in the production, distribution, storage and retailing of chilled and frozen foods. The control of the cold chain is very important to preserve the safety and quality of cooled and refrigerated foods. A typical cold chain is illustrated in Figure 1.

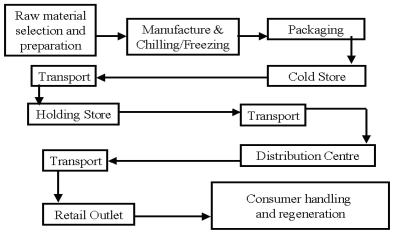

Figure 1. A typical cold chain

Chilling involves reducing food temperatures to below ambient temperatures, but above –1°C. This results in effective short-term preservation of food materials by retarding many of the microbial, physical, chemical and biochemical reactions associated with food spoilage and deterioration. However, chilled foods are perishable and they deteriorate progressively throughout their life. The safe and the high quality chilled foods require minimal contamination during manufacture (including cross-contamination), rapid chilling and low temperatures during storage, handling, distribution, retail display and consumer storage (Prusi, 1990). Freezing preserves the storage life of foods by making them more inert and slowing down the detrimental reactions that promote food spoilage and limit quality shelf life. However, it should be recognised that a number of physical and biochemical reactions can still occur and many of these will be accentuated when recommended conditions of handling, production and storage are not maintained. The production of safe frozen foods requires the same attention to good

manufacturing practices (GMP) and HACCP principles as the chilled or fresh foods (Bogh-Sorensen and Olsson, 1990).
A good temperature control is being achieved throughout the cold food chains as a result of improved equipment design, quality control and food safety. Transfer points, e.g. chiller/freezer to cold store, factory to distribution vehicle, retail the cabinets to consumers' refrigerators, are well known problem areas. Monitoring the cold chain requires detailed information on food product temperatures. Temperature monitoring includes both measurement and recording.

Materials and Methods

The quality and safety of food and most agricultural products during storage time in cold stores and distribution is highly dependent on management and monitoring of time-temperature history concept.
In this paper some important tools for traceability and monitoring of safety and quality of main agricultural products in cold supply chain were reviewed.

Results and Discussions

Traditional temperature monitoring
The traditionally temperature management has been applied using thermometers installed in trucks and warehouses and recently some businesses have employed compact temperature loggers for temperature management. When thermometers installed in warehouses and trucks are used for the management, periodical temperature checks are required. In the case of management systems that also use temperature loggers, each logger should be connected physically to a PC and the data collection becomes a manual operation. Conventionally management has been done by sampling individual packages on a per-warehouse basis. However, temperatures at the entrances and deep inside warehouses tend to vary greatly because there is a wide variation in the temperature at the entrance due to the opening and closing of the doors. Therefore, the thermometers installed in the warehouses or trucks are sometimes incapable of recording the correct temperatures of the products. The traceability of the entire chain is important, but the market also needs a simple risk management technique for use in cause analysis as a prior step to fault tracing. For instance, temperature management is applied not only in the food industry but it is also needed in various aspects of various businesses handling products that have a temperature management requirement.
For overcoming these problems, some new techniques should be applied which are real-time and wireless. In the following, some novel methods will be reviewed.

Novel time-temperature monitoring techniques
The wireless temperature sensor allows managers to monitor cold chain and temperature fluctuations over time during shipment and storage of temperature sensitive goods such as seafoods and perishable agricultural products. This system offers information on the entire food chain, from farm to fork, within minutes, compared with hours or days required in current traceability systems. Information is also collected and stored on ingredient and packaging movements, transformation and quality from the source to retailer's shelf around the world.

Radio Frequency Identity (RFID) Tags
RFID recorder is perfect for temperature mapping and cold chain management of seafood and agricultural products. RFID is an area of automatic identification that is gaining momentum and is considered by some to emerge as one of the most pervasive computing technologies in history. In its simplest form, RFID is a similar concept to bar coding. It is seen as a means of enhancing data processes and is complementary to existing technologies. It is a proven technology that has been in use since the 1970. Since 1998 there has been a revolution in new materials and processes that have driven costs for memory chips, batteries and circuitry down dramatically. This has lead to the Radio Frequency Identification (RFID) or so called Smart Label revolution (Das and Harroup, 2000) with predictions that such tags will replace the UPC code in two to three years. There are several companies developing two way RFID temperature sensor tags that record

temperature which can be downloading at various receiver ports along the way for abuse analysis. Several others are taking the temperature monitoring one further step i.e. integration as is done in the chemical tag using the microbial kinetic parameters in an on board memory chip. The advantage is that such tags can integrate over all three stages of growth and can have an exact good/no good indicator (light) to eliminate sorting. In addition these tags will store the whole temperature time sequence of exposure thus allowing the processor to determine where abuse occurred. With RFID, by downloading of the data at various points in the chain, product close to being unsafe can be removed before the last stage, thus ensuring a safe food supply.

A more complex description is an electromagnetic proximity identification and data transaction system. Using ‘‘RFID tags’’ on objects or assets, and ‘‘readers’’ to gather the tag information, RFID represents an improvement over bar codes in terms of non-optical proximity communication, information density, and two-way communication ability. Operational RFID systems involve tags and readers interacting with objects and database systems to provide an information and/or operational function.

RFID is used for a wide variety of applications ranging from the familiar building access control proximity cards to supply chain tracking, toll collection, vehicle parking access control, retail stock management, tracking library books, theft prevention, vehicle immobilizer systems and railway rolling stock identification and movement tracking.

A major constraint on the widespread use of RFID technologies is the cost of the tags. The most widely used tags are Electronic Article Surveillance (EAS, class 0) tags, which cost between 1 and 6 US cents each. Over 6 billion of them are used annually. Passive tags (class 1) with some data storage cost between 5 and 10 US cents each in large quantities (several million). High value items, cartons and pallets are being tagged (class 2–4) and here costs may be up to US$100 per tag. At current prices it is not economic to incorporate tags into every retail item. Prices will fall as manufacturing technologies improve. In the last 50 years only one billion RFID passive tags (other than EAS tags) and 500 million active tags have been sold. While the use of RFID technologies is predicted to grow significantly, it may take several years to get to the point where the majority of retail items are tagged.

Time-Temperature Indicators and Integrators (TTIs)

An integrated approach could be used to enable traceability of the cold chain of fresh, chilled meat and fish products by means of tailor-made Time-Temperature Indicators (TTIs). The indicators will be tailored to the shelf life and optimum storage conditions of the products. The use of both chemical and RFID, time temperature integrator tags (TTI) placed on food packages to essentially integrate the time-temperature history and indicate actual shelf life left will be evaluated with respect to cold chain management. Such a tag would be used to make a conservative estimate of time to detect for cold chain management. Thus the time to end of shelf life based on safety criteria would be solved by labelling with the expiration date along with a statement such as "use by the date indicated unless the tag turns red".

An solution to the problem of spoilage and waste is to have a device (TTI) on the pallet, case or individual package that integrates the time temperature exposure in the cold chain with the same temperature response as the spoilage rate of the food or the growth rate of the pathogenic organism. Thus, if properly designed, the TTI would indicate visually to distributors, retailers and consumers depending on the type of tag used, the extent of degradation that has occurred. This TTI tag would need to show a sharp color change just before or at end of shelf life (expiration date) when the spoilage level or pathogen number reaches some critical value that could lead to a consumer or regulatory risk, e.g. the time to be able to detect, TTD, a pathogen in a serving of food. To be on the conservative side, this indicator change should occur at some time (hours, days) before the actual risk is present.

Time-temperature integrators (TTI) are small, physical devices that are placed on the food package to measure the temperature history of a product and indicate a definitive change at the end of shelf-life through “integration” of the

time temperature exposure, e.g. "Use food by July 30, 2004 unless dot turns red"(Rice, 1989; Anon, 1989; Sherlock and Labuza, 1992). TTIs are reliable indicators of end of shelf-life for food products if they have similar temperature sensitivities (Ea) as for the food deterioration mechanism (Taoukis et al., 1991). The devices can used on individual consumer packages, so they establish a control system because not all products will receive uniform handling, distribution and time-temperature effects (Labuza et. al., 1992). As a result, TTIs can increase the effectiveness of quality control in distribution, stock rotation practices of perishable foods in grocery stores, and efficiency in measuring freshness by the consumer as we noted earlier. (Sherlock, 1991; Wells and Singh, 1988a, 1988b). Taoukis showed that for the most part, the commercially available TTIs are both reliable and applicable for use in combination with open dating of refrigerated foods including RTE products. Malcata (1990), in addition, showed that although the tags respond more quickly to temperature abuse then the actual food because they are on the surface of the package, thus the response is on the conservative side of safety, i.e. the tag shows an endpoint before the food is spoiled.

Computational Fluid Dynamics (CFD)

Computational fluid dynamics (CFD), which has been around for many years, is a powerful numerical technique for solving industrial fluid flow problems. CFD calculation involves the use of a computational grid where the governing equations describing fluid flow—the continuity equation and the set of the Navier–Stokes equations, and any additional conservation equations such as energy balance are solved across each grid cell by means of an iterative procedure in order to predict the profiles of velocity, temperature, shear, pressure, and other parameters.

CFD offers a powerful design and analysis tool to the agri-food engineer. In the agri-food industry, many applications involve fluid flow and heat and mass transfer (Sun, 2002).

Most of the early CFD applications in the food industry focused on refrigeration facilities. Research in refrigeration facilities is still continuing. Moureh, Menia and Flick present simulation results of air-flow in a typical refrigerated truck, with experimental verification using laser-Doppler velocimetry. This work aims to improve and optimise the air-distribution systems in refrigerated vehicles for uniform temperature distribution within the loading. Strict temperature control is required throughout the cold-chain (Norton, 2006).

Simulation of large refrigeration facilities using CFD is always a problem due to the limitation in computer capacity. Mirade, Kondjoyan and Daudin adopt a four-step strategy by separating the computation of air velocity field, heat and mass transfer coefficients and product chilling process. This strategy is applied to a large pork chiller containing 290 carcasses. This part is concluded with a paper by Moureh, Laguerre, Flick and Commere on the modelling of the temperature history of a heat-sensitive food product during its shipping and storage in pallets with and without an insulating cover.

CONCLUSIONS

As seen in this review, both chemical and electronic RFID-TTI time-temperature integrators can integrate this abuse and relate it to shelf life expiration. To create these tags there is a need for collection of data on time to detect (TTD) and growth kinetics for each pathogen on each type of food. This data is sorely lacking especially TTD. But once collected, we can set an expiration date based on some level of risk, i.e. the time to detect the pathogen or some higher regulatory action level. This information can then be used to design a time-temperature integrator device, TTI, that chemically or electronically integrates the stage the pathogen or the level its toxin is at and indicate distinctly the end point set as the "expiration date". Thus, the device can be used as a HACCP monitor to evaluate in real time the extent to which temperature abuse during distribution and holding at retail and in the home affects the safety of the product.

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EFFECTS OF VEGETAL OILS ON THE QUALITY CHARACTERISTICS OF BEEF MEAT PRODUCTS WITH LOW ANIMAL FAT

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Abstract

Meat industry tries to modify his approach concerning the composition of its products for health purposes, to respect nutritional principles in processed foods. Many researchers propose partial substitution of animal fats with vegetable oils in food; these oils are rich in essential fatty acids, mono-and polyunsaturated. But it was observed that this substitution has affected sensory quality of compositions of meat in which the fat was replaced by vegetable oils. Study analysed the modification of the chemical compositions of the beef meatballs with low fat and the correlation with sensory analyse. Was effectuated rheological characterisation for all samples, were measured the storage modulus (G') and loss modulus (G"), indicating the viscoelastic character of the beef mincemeat with vegetal oils. This study tries to establish the acceptability of the meat products with partial replacing of the animal fat with some vegetal oils, in fact sunflower oil, canola oil and hemp oil. Finally, sensory analyses suggest that the use of beef meat products with these oils can be well accepted by the consumers because they are healthier.

Keywords: *beef mincemeat, canola oil, hemp oil, inulin, rheological characteristics.*

INTRODUCTION

The importance of the food consists in the nutritional compounds, proteins, lipids, carbohydrates which have important biological role and energy value. But the eating of high processed products, very concentrated in some of these compounds produces disagreements and increasing of obesity and cardiovascular diseases motivating the study of the causes of these disagreements.

Processed meat products contain high amounts of fat, related to chronic diseases such as obesity and cardiovascular illness. It was suggest by health organizations to reduce the intake of total dietary fat, particularly saturated fatty acids and cholesterol, to prevent cardiovascular heart diseases (NCEP, 1988; Moon et al., 2009).

Studies have shown that vegetable oils in meat products improves the nutritional quality by reducing caloric and cholesterol contents (Liu, et al., 1991; Paneras and Bloukas, 1994).

But reducing fat levels in meat products can lead to an unacceptable product texture, flavor and appearance (Miles, 1996).

In a same way, fats in meat products play important technological role in stabilizing meat emulsions, can reduce cooking loss and improve water holding capacity, provide juiciness and hardness (Karema A et al., 2011). It has considerable effects on the binding, rheological and structural properties of the meat product (Karema A et al., 2011).

In some studies it was demonstrated that replacing animal fat with soy products or carbohydrate is successful in textural and sensory properties of low-fat products (Moon et al., 2009)

Some researches reported that replacement of animal fat with vegetal oils may have a positive effect on consumer health, they are free of cholesterol and have a higher ratio of unsaturated fatty acids (Karema et al., 2011).

The reduction of animal fat in meat products and the substitution of animal fat with vegetable oils and dietary fibers could result in healthier products (Choi et al., 2009; Sanjeewa et al., 2010).

Olive oil has used in fermented sausages (Bloukas et al., 1997;Kayaardi et al., 2003; Koutsopoulos et al., 2008) and beef patties (Hur et al., 2008). Has been tasted partial replacement of pork back fat with olive oil in frankfurter sausages and low-fat products by (Jiménez-Colmenero et al., 2007; López-López

et al., 2009) who reported an increased MUFA contents without significantly altering the n-6/n-3 ratio.
Dietary fibers (wheat bran, soy protein) have been used by (Moon et al., 2009) to emulsified meat low fat products, to support and ensure binding.
It was used canola oil, low in saturated fat and containing both omega-6 and omega-3 fatty acids in a very good ratio, 2:1. it was used. Many health professional organizations, including the Academy of Nutrition and Dietetics and American Heart Association, confirm that the consummation of canola oil it also reduces low-density lipoprotein and overall cholesterol levels, and represent a significant source of the essential omega-3 fatty acid. It was associated this with the reducing of cause and cardiovascular mortality by (Ryszard Rezler et al., 2007; O'Brien, 2008).
Our research is followed to obtain low fat beef mincemeat with vegetable oil and dietary fibers added, inulin, the proportions of oil going up to the total replacement of pork fat.
Compositions were characterized by physico-chemical and sensory analyze.
It was study the effect of the addition of vegetable fats on the structure and consistency of meat compositions, how the addition of oils affects the taste and appearance of these products and influences the acceptability of the consumers. The products have been designed for the catering sector.

MATERIALS AND METHODS

Material

The experiment consisted of 4 samples for each of three oils and one control. Was used pork fat and fresh beef meat, without bone and fat. Minced beef meat and pork fat were chopped at sieve of 5 mm. Pork fat was gradually replaced with oil from 30 to 100% of quantity (Table 1). Salt and inulin was incorporated, and the composition of meat was mixed for 15 minutes and stored at 2÷4°C for 12 hours. After, in the samples 1 to 4 it was mixed the corresponding quantity of oil, separately for each three types of oil, sunflower oil, canola oil and walnut oil.

Table 1. Recipe used in the experiments (%)

Specification	Control	Sample 1	Sample 2	Sample 3	Sample 4
Beef mincemeat	50	50	50	50	50
Animal fat	30	20	10	5	-
Oil	-	10	20	25	30
Inulin	-	4	4	4	4
Waterfor hydration and ice	20	20	20	20	20

The samples were subsequently analyzed for physical-chemical and rheological properties.
Oils used to replace the animal fat in meat paste were purchased from commercial or directly from the manufacturer. Inulin fibers were purchased from SC Enzymes & Derivates SA, Neamt.

Methods

Physical-chemical properties were determined for row material, beef meat and pork fat and for each composition with oil.

- water content: conforming AOAC method (1995);
- total content of nitrogen: according to SR ISO 9037 (2007) standard (for samples digestion and distillation was utilized by Kjeldahl Velp Scientifica UDK 127 System); protein degree of hydrolysis was estimated by the determination of non-protein nitrogen according to AOAC (1990) method and aminic nitrogen according to the method described by Vata et al. (2000);
- fat content: according to the AOAC (1984) method utilizing Fat Extractor SER 148;
- ash content: conforming AOAC 972.15;
- pH was determined according to A.O.A.C. method (1984) with a Hanna digital pH-meter;
- cooking loss: were calculated with the formula: P= [Mi-Mf] x 100/Mi, where Mi= initial weight of the sample (raw meat); Mf= final weight of the sample (after thermal treatment).

Determination of rheological characteristics
Rheological measurements were performed in triplicate, using a voltage controlled rheometer (AR 2000, TA Instruments, New Castle, DE) attached to computer control software (Rheology Advantage Data Analysis Program, TA, New Castle, DE). The temperature was monitored by using a Peltier temperature

control system. Rheological measurements were made using plate geometry of 40 mm, with an angle of 2° and a gap of 1000 μm. The samples used were approximately 2.5 g of mincemeat for each test. It was placed on the bottom rheometer plate and low viscosity silicone was added around the plate edges to prevent dehydration. Measurements were made at constant frequencies (between 1 to 10 Hz) and different temperature (between 20 to 71,6^{o}C) in double samples. Changes in the storage (G') and loss (G") modulus and the phase or deformation angle (δ) were recorded. For each test, the sample was maintained for 5 minutes to balance the temperature.
All the results were statistics analyzed.

RESULTS AND DISCUSSIONS

Physical-chemical properties

Compositions for uncooked and cooked products with oils are presented in Tables 1, 2, 3 and the sample was defined like that: Control- beef mincemeat and pork fat; Sample 1 – 30% of pork fat was replaced with oil; Sample 2 - 60% of pork fat was replaced with oil; Sample 3 - 85% of pork fat was replaced with oil; Sample 4 - 100% of pork fat was replaced with oil. The samples where it was used sunflower oil are presented in Table 2. It can observe significant differences in moisture, fat, and ash contents of the samples with sunflower oil face to control, protein contents were not significantly different. In fact, moisture contents of the samples with oil are higher than the control because in the samples was added more water, to hydrate the inulin.
This fact was observed by (Choi et al., 2007; Choi et al., 2008; Choi et al., 2009), when they added vegetable oil and dietary fibers to meat emulsions. Fibers have increased the moisture content of meat products and have produced higher water retention and improved emulsion stability.
Fat content was decreasing with the increasing of the replacement of pork fat with oil, with 20-35% in the samples with sunflower oil compared to control. These results agree with (Luruena-Martinez et al., 2004) who reported similar characteristics for low-fat frankfurters in which it was replaced the pork fat with olive oil. Ash content was higher with 10-15% for all samples containing sunflower oil face the control, in accordance with (Choi et al., 2008; Choi et al., 2009), who reported that the ash content is significantly increased in the low-fat meat products with the addition of rice bran fiber. Ayo et al. (2007) reported that ash content significantly increased with the addition of walnut to low-fat meat products. Others reported no significant difference in ash contents when vegetable oil replaced pork fat (Paneras et al., 1994).
In Table 3 are presented compositions with canola oil, for uncooked and cooked samples. The compounds of samples had significant differences in moisture, fat, and ash contents face to control, protein contents are the same. But it exist differences between the samples with sunflower oil and the samples with canola oil. It was observed that the moisture content is less and lipid content is higher for the products with canola oil. It can observe that the differences of moisture between the samples with sunflower oil and canola oil are 3.84-7.46%. Lipid content in the samples with canola oil is progressive decreasing but not so much than in the samples with sunflower. In the same time, viscosity of the mincemeat was better, probable because of high density and physico-chemical characteristics of canola oil. The compositions with hemp oil in a place of pork fat are presented in Table 4. There are no significant differences in the content of moisture of samples face the products with sunflower and canola oil. Lipid content is smaller than the sample with canola oil with 6.25-9.81%. Viscosity of mincemeat was better than the samples with sunflower but less than the samples with canola oil. pH results for the mincemeat are presented in the Figure 1. It can appreciate that the value for uncooked mincemeat present a progressive increase with small value, no more than 0.1-0.26 unit of pH, between 5.8-6.14. For cooked product results was higher with 0.4-0.8 unit, between 6.6-6.72, it was increasing at the samples 1 and 2, after it was decreasing to the end, as reported (Choi et al., 2009).

Table 2. Composition of beef mince where the pork fat was replaced with sunflower oil (%)

Uncooked					
Compounds	Control	Sample 1	Sample 2	Sample 3	Sample 4
Moisture	64.16±0.81	67.51±1.01	69.12±0.62	70.51±0.39	70.46±0.80
Proteins	20.40± 0.52	20.18± 0.33	19.41± 0.12	19.40± 0.17	19.57± 0.21
Lipids	13.22± 0.11	10.06± 0.23	9.58± 0.15	10.11± 0.41	8.52± 0.13
Ash	1.18± 0.03	1.22± 0.07	1.32± 0.08	1.29± 0.01	1.43± 0.06
Cooked					
Compounds	Control	Sample 1	Sample 2	Sample 3	Sample 4
Moisture	49.94±0.34	53.35±0.51	54.03±0.60	52.15±0.32	55.41±0.43
Proteins	28.23±0.35	25.76±0.74	25.78±0.88	26.98±0.81	25.16±0.64
Lipids	18.27±0.52	18.02±0.25	19.01±0.54	19.63±0.74	18.22±0.12
Ash	1.75±0.02	1.72±0.00	1.57±0.02	1.63±0.03	1.75±0.06

Table 3. Composition of beef mince where the pork fat was replaced with canola oil (%)

Uncooked					
Compounds	Control	Sample 1	Sample 2	Sample 3	Sample 4
Moisture	63.75±0.22	67.58±0.54	68.69±0.18	69.17±0.42	68.25±0.53
Proteins	20.59± 0.33	19.63± 0.47	20.18± 0.08	20.95± 0.53	21.44±0.24
Lipids	13.42± 0.18	11.76± 0.40	10.29± 0.07	9.46± 0.25	9.89± 0.09
Ash	1.23± 0.02	1.31± 0.05	1.40± 0.01	1.38± 0.00	1.34± 0.04
Cooked					
Compounds	Control	Sample 1	Sample 2	Sample 3	Sample 4
Moisture	48.56±0.21	49.37±0.18	51.62±0.42	53.60±0.56	53.28±0.48
Proteins	29.35±0.23	29.31±0.41	28.76±0.27	28.43±0.13	28.03±0.35
Lipids	19.31±0.16	18.94±0.09	17.93±0.33	16.01±0.28	16.14±0.06
Ash	1.84±0.00	1.83±0.07	1.62±0.04	1.71±0.01	1.78±0.12

Table 4. Composition of beef mince where the pork fat was replaced with hemp oil (%)

Uncooked					
Compounds	Control	Sample 1	Sample 2	Sample 3	Sample 4
Moisture	64.28±0.45	66.87±0.21	68.25±0.64	68.73±0.17	70.12±0.42
Proteins	20.03± 0.29	20.49± 0.18	19.57± 0.32	19.86± 0.33	20.38±0.14
Lipids	13.54± 0.21	10.85± 0.34	10.08± 0.45	8.86± 0.65	8.92± 0.45
Ash	1.35± 0.01	1.39± 0.02	1.37± 0.07	1.50± 0.12	1.37± 0.05
Cooked					
Compounds	Control	Sample 1	Sample 2	Sample 3	Sample 4
Moisture	49.36±0.37	50.23±0.54	52.94±0.46	52.73±0.27	53.69±0.43
Proteins	28.76±0.12	29.06±0.27	28.66±0.38	28.95±0.45	28.26±0.13
Lipids	19.12±0.48	19.57±0.13	19.03±0.27	18.81±0.41	15.14±0.24
Ash	1.78±0.06	1.75±0.03	1.80±0.15	1.62±0.09	1.73±0.18

Control- beef mincemeat and pork fat; Sample 1-30% of pork fat was replaced with oil; Sample 2 -60% of pork fat was replaced with oil; Sample 3 -85% of pork fat was replaced with oil; Sample 4 - 100% of pork fat was replaced with oil.

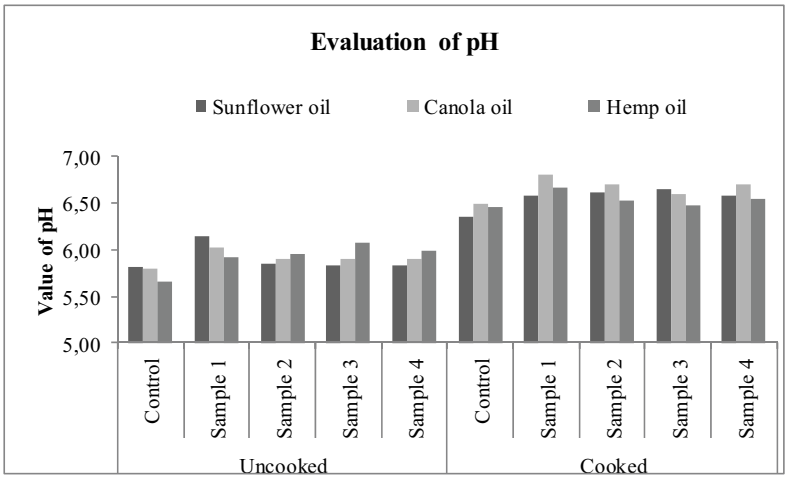

Figure 1. Evaluation of pH value for uncooked and cooked samples

Cooking loss values (Figure 2) have increased, but not linear, as the amount of pork fat is replaced with oil. For the samples with sunflower, cooking loss increase progressive, from 21.45% at the control to 23.71% at the sample 4, where all the pork fat was replaced with oil. The small cooking loss was obtained at the samples with canola oil, between 20.43-20.67%. These results were concordant with the good viscosity obtained for mincemeat with canola oil and the results reported by Choi et al. (2009), who find that the samples with canola and soybean oil had contained the lowest total expressible fluid at cooking.

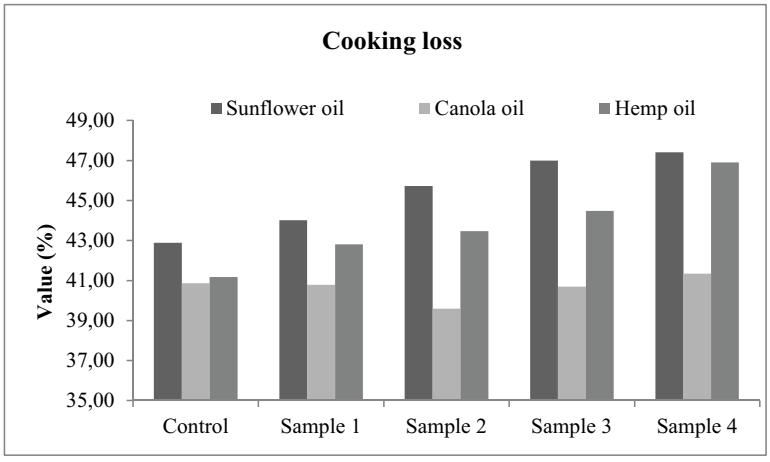

Figure 2. Evaluation of cooking loss of the samples

Dynamic rheology tests

Frequency sweep test for mincemeat and dynamic frequency tests were carried out within the limits of viscoelastic range to determine the frequency dependence of storage and loss modulus (G' and G").

Storage modulus (G') and loss modulus (G") was analyze for beef mincemeat with different percentage of replacement of pork fat with each oil, sunflower oil, canola oil and hemp oil.

It can observe that the rheological response was difference for each sample, in correlation with the composition and the type of oil.

With the increasing of temperature, rheological properties were affected by the structural changing, elastic module were increasing,

observation reported by (Marchetti et al., 2012).

It was obtained an inflection point at 43°C, where the proteins began to coagulate and mincemeat with oil went from the state of viscous fluid at the state of elastic gel with soft texture.

Increasing of temperature over 43°C have produced an increase of consistency of all samples, the last scan give close values of the storage modulus (G'). Possible explanation consist in denser structure of the mincemeat obtained by addition of salt and inulin and the storage for 12 hours at 4°C, period who have permitted to format a protein network in the meat paste. After this point of inflexion from 43°C, consistency of mincemeat decreased progressively with increasing temperature.

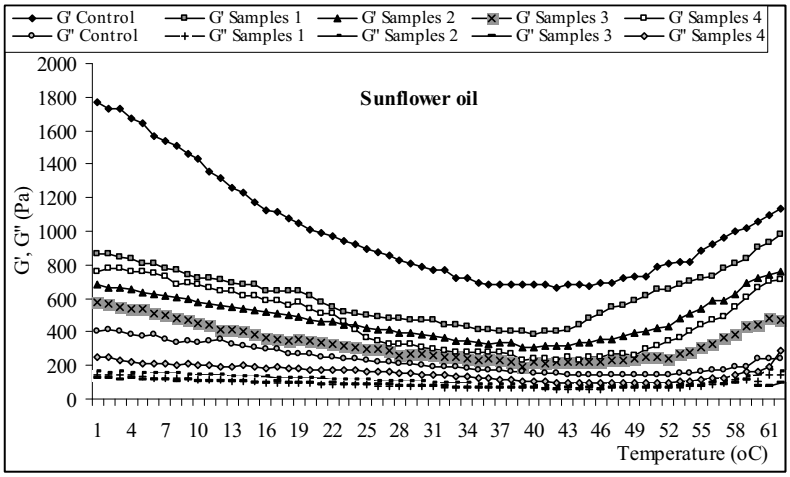

Figure 3. Rheological properties for the samples with sunflower oil. Storage (G') and los modulus (G") depending by the temperature

In Figure 3, for the samples with sunflower oil it can observe that the storage modulus (G') has proved that control had the best rheological properties. For the samples with oils, value of storage modulus (G') was lower face the control and their behavior was quite similar. In order, the higher rheological properties were for the samples 1 (30% replacement of the pork fat), samples 4 (100% replacement of the pork fat), samples 2 (60% replacement of the pork fat) and, finally, samples 3 (85% replacement of the pork fat).

Loss modulus (G") confirms that the beef mincemeat with sunflower oil has a good viscosity and can be used in cooked products with low animal fat.

In the Figure 4 it can observe the difference face samples with canola oil. Mincemeat with canola oil it have the largest value of the modulus G', samples with total replacement of pork fat with oil have a higher rate of consistency compared to other samples.

Conclusion is that replacing of the fat with canola oil favored the formation of a protein network which improved elasticity of the gel. Result is confirmed by Senouci et al. (1988), who found that the fat replacing with some vegetable oils permit to form a protein matrix extended in the emulsion, who produce an increase in the viscoelastic parameters.

Storage modulus (G') values are higher than mincemeat samples with sunflower oil with approximate 12% and the elasticity of product is smaller, probably because of its higher viscosity and composition, who contribute to increase the consistency of mincemeat in the conditions we compared with others oils used in the experiment.

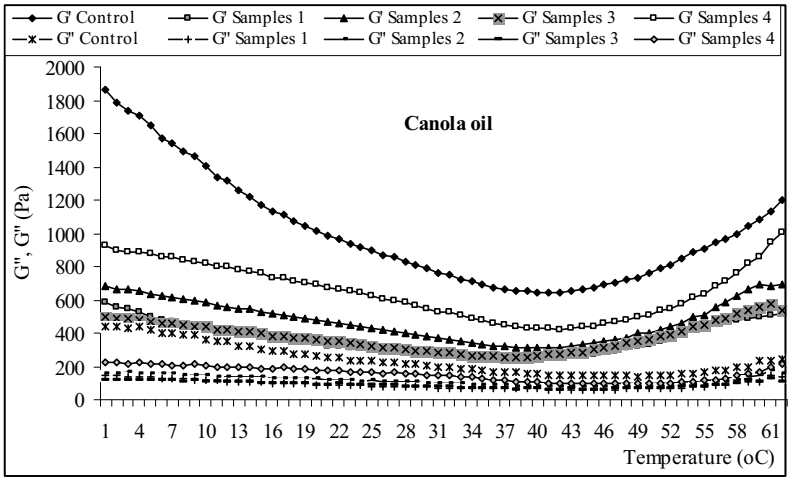

Figure 4. Rheological properties for the samples with canola oil.
Storage (G') and los modulus (G") depending by the temperature

Figure 5 shows that the samples with hemp oil have a different behavior face the samples with sunflower and canola oil, the order of the results confirm that total replacement of pork fat with oil permit mincemeat with good rheological characteristics, better than samples 1 to 3. Samples with oils allow incorporation of more water and have a lower consistency, so G' value is lower.

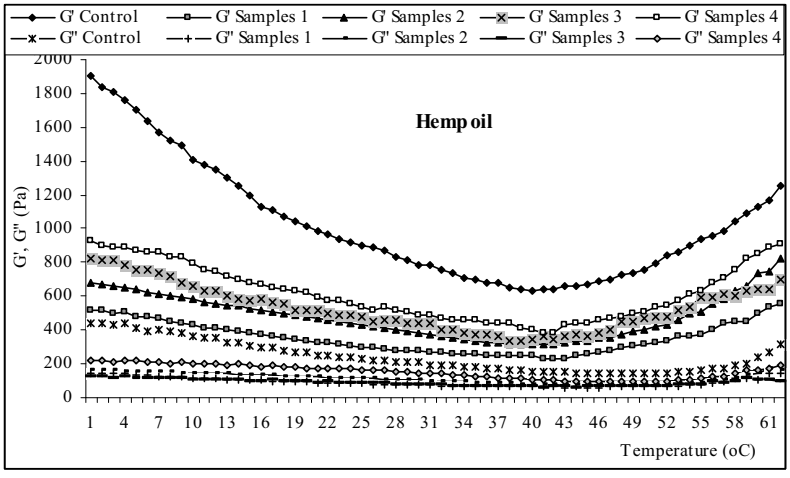

Figure 5. Rheological properties for the samples with hemp oil.
Storage (G') and los modulus (G") depending by the temperature

Storage modulus (G') value were higher for all samples than the values of G", this indicates a predominance of elastic properties compared with the viscous. Experiments confirms that a good gel is characterized by a proportional change of modulus G' and G" in the case of frequency sweep in a wide range, reported by Park et al. (2008).

Mincemeat behavior is consistent with mechanical spectra obtained for polysaccharide gels, where G' is always much higher (about 10 times) than G" and is practically independent of the applied frequency range, conclusion reported by Fernández-Ginés et al. (2005).

With increasing of storage modulus (G') it is increased loss modulus (G") at increasing of frequency oscillations, possibly due to

structural changes in the systems. At scanning at the temperature between 20 to 71.6°C, loss modulus (G") had a similar pattern to that of the storage modulus (G') but in lower values.
It can estimate that the analysis of storage modulus (G') and loss modulus (G") depend on frequency applied on the mincemeat and show, at addition of oil, it decreases the consistence of mincemeat.

CONCLUSIONS

The study permitted to analyze some meat compositions with beef meat, in which was partially or totally replaced the pork fat.
The results show that the addition of vegetal oils can be useful for preventing lipid oxidation of cooked products.
Added inulin can help to increase the percentage of replacement of the pork fat in the meat products.
Reduction of pork fat content in percentage of 30-100% has determined a softening of the mincemeat and an increasing of the cooking loss with 12-14%.
Acceptability of products decreased with increasing of amount of vegetal oil used, and after 50% of the replacement the consumers don't agree the taste of cooked products.
It can conclude that the products obtained are acceptable in terms of technology, because pork fat replacement with vegetable oils produces lower consistency of mincemeat face the control. Adding inulin with oils it was improved the texture and consistency has favored the formation of a protein network, forming a gel with increased elasticity.
The study showed that vegetable oils used to replace pork fat in some mincemeat are an alternative to improve the nutritional value of meat for catering sector. We believe that this researches contribute to a new approach in this new culinary technology, that will reflect an improved of nutritional value of products, beneficial to health.

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EFFECT OF USE OF DATE PROCESSING BY-PRODUCT ON SOME PHYSICO-CHEMICAL AND SENSORY PROPERTIES OF SAUSAGE

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Abstract

Five concentrations (0, 1.25, 2.5, 3.75 and 5%) of date processing by-product powder as fat replacer and antioxidant were added to sausages. Chemical, physicochemical, lipid oxidation, residual nitrite level and sensory evaluation were done. The moisture, dietary fibre and protein content were significantly increased. Fat content and colour coordinates lightness (L) and redness (a*) were significantly reduced but yellowness (b*) was not severely affected by the date processing by-product content. Moreover, the addition of date processing by-product to sausages represented an improvement in their nutritional properties, possibly due to the presence of active bio compounds which induce a significant decrease in residual nitrite level and lipid oxidation. Also, in terms of overall acceptability, panellists preferred samples with added 5% date processing by-product.*

Keywords*: sausage, date processing by-product, fat replacer, antioxidant*

INTRODUCTION

Nowadays consumers are more concerned with their health and its relation with diet, demanding healthier foods. However, meat products are associated with a high content of fat, cholesterol, salt, nitrite or lipid oxidation products, which are related with several illnesses, such as cardiovascular diseases, cancer, hypertension and obesity (Martin-Sanchez et al., 2011). Therefore, production of low fat and healthy meat products is very important.

The date palm (*Phoenix dactilifera L.*) is an important member of the family *Arecaceae* (Palmae) (Sanchez-Zapata et al., 2011). Iran with an expected production of 1100000 ton of dates in 2013 is the second major producer after Egypt. In view of this, several date processing industries are developing new processes to produce many products like date juice, date syrup, date dip and date vinegar from various date varieties. Unfortunately, this progress of production is accompanied by a substantial increase of date losses during conditioning of the dates. These by-products that are rich in phytochemicals such as dietary fiber, phenolics and natural antioxidant, are used in animal feeding (Sanchez-Zapata et al., 2011).

Dietary fiber may also be used for its functional and technological properties. Thus, fiber rich by-products may be incorporated into food products as inexpensive and non-caloric bulking agents, as enhancers of water and oil retention and to improve emulsion or oxidative stabilities (Aguedo et al., 2012).

Incorporation of date by-products could be an easy and economical strategy to develop healthier meat products and with improved technological properties, but also to increased the eco-efficiency in the date palm and meat industry (Martin-Sanchez et al., 2011).

The objective of this study was to develop low-fat sausages whit antioxidant properties.

MATERIALS AND METHODS

Date processing by-product preparation

The date processing by-product obtained from date syrup production plant. The seeds were ground by hammer mill, separately. Seeds and pastes were dried by cabinet dryer (Armfield, England) at the air flow rate and temperature of 1.1 m/s and 50°C, respectively in 10-12 h.

Dehydrated date by-product were ground in a domestic mill, passed through a 60 mesh sieve to a particle size of 0.250 mm. This powder was kept in three layer polyethylene pouches at 4°C.

Sausage manufacture

Lean beef meat and fat were ground separately in a grinder (BEEHIVE DEBONER, USA) through a 4 mm plate and according to Pearson square were mixed to achieved desired fat level. The main ingredients in formulated sausages were shown in Table 1.

At first, meat and fat were transferred to the cutter (Iran steel, Iran). During the comminution in a bowl cutter the nonmeat ingredients were added (null flour, wheat starch, modified potato starch, gluten, casein, spices, salt, phosphate, sugar, isolated soy, garlic, capsicum, egg white, nitrite and ascorbic acid).

After homogenization, the mixture stuffed into polyamide casing and cooked in a water bath (77°C, 55 min). Next, the sausages were chilled with cold water. After reaching chilling temperature, the products were transferred to the lab. All analyses were carried out in triplicate for each formulation.

Proximate composition

Table 1. The main ingredients of sausages

4	3	2	1	control	Treatment / Ingredient (%)
55 12%fat	55 14%fat	55 16%fat	55 18%fat	55 20%fat	Beef meat
3	5	7	9	11	oil
18.95	18.2	17.45	16.7	15.95	ice
5	3.75	2.5	1.25	0	Date processing by-product

Moisture, ash, protein and dietary fiber were determined by AOAC methods (AOAC, 1997). Moisture (g water/100 g sample) was determined by drying a 3-g sample at 105°C to constant weight. Ashing was performed on a 2-3g sample after combustion in a muffle furnace at 550°C for 8 h (g ash/100 g sample). The protein (g protein/100 g sample) was analyzed according to the Kjeldahl method, using a factor of 6.25 for the conversion of nitrogen to crude protein. The fat (g fat/100 g sample) was calculated by weight loss by extraction for 8 h with n-hexane in a Soxhlet apparatus. Total dietary fibers (TDF) were determined using an enzymatic method.

Physicochemical analysis

The pH was measured in a suspension resulting from blending a 10 g sample with 10 ml de-ionized water for 2 min using a pH-meter (HANNA, Romania) (Sanchez-Zapata et al., 2011).

Color was studied in the CIELAB color space using a hunter lab (colorflex, USA). The CIELAB coordinates studied were lightness (L*), co-ordinate red/green (a*) and co-ordinate yellow/blue (b*).

Residual nitrite

Residual nitrite level (mg $NaNo_2$/kg sample) was determined according Iranian National Standard 923.

TBA values

Lipid oxidation was assessed by the 2-thiobarbituric acid (TBA) method of Choi et al. (2010).

Sensory evaluation

A hedonic sensory test was performed on cooked sausages. The sensory panel consist of ten trained panelists evaluated six attributes of sausages (taste, odor, texture, color, appearance and palatability). Each attribute was evaluated on a scale from 1 to 5. The samples were served on a white paper plate, labeled with a three-digit number, and served in random order.

Statistical analysis

All tests were carried out in triplicate. Statistical analyses were carried out using MSTAT-C in a randomized complete block design. Analysis of variance (ANOVA) was used to determine significant differences ($P<0.01$) between sausages with different date processing by-product. Comparison between means was performed using Duncan's multiple range tests.

RESULTS AND DISCUSSIONS

Chemical composition of sausages

Results of chemical composition of sausages are presented in Table 2. Date processing by-product addition and reduction of fat increased (P<0.01) moisture, protein and TDF content and decreased (P< 0.01) fat content but ash content was not significantly affected (P> 0.01).

The increase in water level in all formulations along with addition of date processing by-product level increased moisture content. The protein increase could be due to the high protein content of date processing by-product because the amount of meat and binders as sources of protein in all samples was fixed. Also, the increase in TDF content was higher (P<0.01) when high amount of date processing by-product was added because this by-product is a rich source of dietary fiber. The differences (P<0.01) observed in fat content between treatments could be explained by the reduction of fat level in all formulations.

Table 2. Chemical composition (g/100 g sample) of sausages

	Protein	fat	Moisture	Ash	TDF
control	12.61±0.11b	18.99±0.3a	60.6±0.2e	2.1±0.1a	0.22±0.08d
1.25%date powder	12.90±0b	16.45±1.05b	62.35±0.95d	2.05±0.05a	0.50±0.11d
2.5% date powder	12.97±0.37b	14±0c	63.55±0.25c	1.8±0.3b	0.77±0.11c
3.75% date powder	13.57±0.27a	11.95±0.25d	64.9±0.6b	2.05±0.05a	1.62±0.25b
5% date powder	13.5±0.5a	10.3±0.4e	67.4±0.7a	2.0±0a	2.14±0.28a

Values with different letters in the same column are significantly different (P<0.01).

Residual nitrite

Results of residual nitrite are shown in Table 3. The incorporation of date processing by-product at amount higher than 1.25% to sausages produced a significant decrease (P<0.01) in residual nitrite level, which could be due to the reactions of nitrite with the active bio-compounds present in the date processing by-product. This decrease in residual nitrite level is healthy because it reduces the possibility of nitrosamine formation: a risk related to the consumption of meat products with nitrite in their formation (Fernandez-Gines et al., 2004).

Lipid oxidation

The effect of the different level of date processing by-product and reduction of fat on lipid oxidation of sausages is shown in Table 3. Lipid oxidation was studied to evaluate the effects of adding date processing by-product, rich in phenolic compounds (Martin-Sanchez et al., 2011).

The date processing by-product caused a significant reduction (P<0.01) in TBA values for all treatments. These results must be due to the phenolic compounds present in the date processing by-product. Moreover, fat reduction in formulation of sausages could also reduce the lipid oxidation (Martin-Sanchez et al., 2011).

pH and color parameters of sausages

pH and color parameters of sausages are shown in Table 4. The pH value declined (P<0.01) with increasing percentages of date processing by-product in the formulation. This pH decrease would be related with the date processing by-product pH (5.81), lower than that of the control sausage (6.25), probably due to the presence of organic acids in the fruit (Martin-Sanchez et al., 2011).

L* and a* values decreased (P<0.01) when date processing by-product was added. May be in this case, the higher contribution of Maillard reactions in sausages with date processing by-product take a role to play in L* value, causing the darkening of the product (Sanchez-Zapata et al., 2011). Also, reduction of fat resulted in a darker product (Seraroglu and Ozsumer, 2003).

Table 3. Residual nitrite level and TBA values

	Residual nitrite (mg/kg)	TBA (mgMA/kg)
Control	48.9±6.23a	2.68±0.16a
1.25%DP	49.35±3.45a	2.18±0.11b
2.5% DP	36.8±4.51b	1.85±0.23b
3.75% DP	23.56±4.63c	1.28±0.1c
5% DP	21.9±3.74c	0.8±0.08d

Values with different letters in the same column are significantly different (P<0.01).

The a* behavior is similar to moisture content behavior, so the samples with the highest moisture content correspond with the samples

with the lowest a* values. In fact, water has a dilution effect for a* values (Fernandez-Gines et al., 2004). b* did not differ (P 0.01) between control and treated samples. This indicates that date processing by-product did not have any positive or negative effects on b* values of sausages. The reason why date processing by-product did not affect b* values of sausages could be that yellow compounds presents in date processing by-product would have been masked by meat emulsion (Fernandez-Gines et al., 2004).

Sensory evaluation

Results from sensory evaluation are presented in Table 5. Addition of date processing by-product and fat reduction caused an increased (P<0.01) in all evaluated parameters. According to other researches reduction of fat or increase in moisture content may be reduce meat products quality but in this case incorporation of date processing by-product reversed this effect.

Table 4. pH and color parameters sausages

	pH	L*(Lightness)	a*(Redness)	b*(Yellowness)
Control	6.25±0.7a	66.68±0.17a	9.46±0.03a	14.76±0.06a
1.25% DP	6.14±0.016b	64.41±0.03b	8.16±0.01c	14.94±0.01a
2.5% DP	6.13±0.03b	59.01±0.02c	7.86±0.03d	14.92±0.07a
3.75% DP	5.98±0.02c	57.59±0.06d	8.12±0.01c	13.8±0.01a
5% DP	5.93±0.06c	55.26±0.22e	8. 5±0.01b	14.16±0.01a

Values with different letters in the same column are significantly different (P<0.01).

Table5. Results of sensory evaluation of sausages

	Appearance	Palatability	Color	Texture	Odor	Taste
Control	2±0c	2.33±0.57b	2±0b	2±0b	2±0c	3±0c
1.25%DP	2.66±0.57b	3±1ab	2.66±0.57b	2.66±0.57ab	2.66±0.57bc	3.33±0.57 bc
2.5%DP	3.66±0.57a	3.66±0.57a	3.66±0.57a	3±1ab	3±0ab	2.66±0.57c
3.75%DP	4±0a	3.66±0.57a	4±0a	3.33±0.57a	3.66±0.57a	4±0ab
5%DP	4±0a	3.66±0.57a	4±0a	3.66±0.57a	3.66±0.57a	4.33±0.57a

Values with different letters in the same column are significantly different (P<0.01).

CONCLUSIONS

The results presented that date processing by-product has been successfully added (until 5%) to the sausages. The addition of date processing by-product to sausages and fat reduction decreased their pH and fat content but increased TDF, moisture and protein content in formulated sausages. Moreover, data processing by-product decreased residual nitrite and lipid oxidation, both aspects contributes to increase the nutritive value of the products. In addition, sausages with date processing by-product were greatly accepted by trained panelists. Therefore, date processing by-product showed potential as a good source of dietary fiber and antioxidant which can be used as functional ingredient for meat products.

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INFLUENCE OF ENZYMATIC TREATMENT AND MIXING ON HARDNESS AND COOKING LOSSES FOR PORK MUSCLE

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Abstract

The production of meat products witnessed great development in recent years, a necessary condition in the context of dynamic change of the population lifestyle. On the other hand, many existing products in the market are not optimal in terms of innocuousness, multitude of additives used making consumers reluctant to be and to turn our attention to eating fresh meat. To meet the requirements, therefore meat should provide outstanding sensory properties, of which a significant relevance it is the tenderness and the juiciness. It is known that meat toughness and its ability to retain water are correlated with the phases of post-slaughter meat and biochemically changes that occurring these phases. This study aims to follow the addition of proteolytic enzymes and mixing operation influence on the development of pig muscle hardness and cooking losses, starting at 2 hours post-slaughter, over a period of 14 days. Following experiments it was found that injection of enzymes, and the association of enzymatic treatment mixing generated to reduce muscle tissue hardness, assessed by determining the cutting forces, and reducing the losses associated with heat treatment. Thus, the samples without heating treatment, the cutting force has the following evolution: on the first day post-slaughter of 13.19 kgf for the control sample and injected with enzyme sample, 10.61 kgf for injected and mixed sample; at three days post-slaughter 20.88 kgf for control sample, 16.83 kgf for injected sample and 15.49 kgf for injected and mixed sample; at 14 day post-slaughter to 10.42 kgf for control sample, 6.85 kgf for enzymatically treated sample, and 5.66 kgf for injected and mixed sample. When the meats was heat-treated, we registered similar developments but with lower values. Thermal losses increased with the evolution to the maximum rigidity, then fell to maturation, the biggest losses occurring to the controls samples and lowest in injected and mixed samples.

Keywords: *cutting force, muscle hardness, papain*

INTRODUCTION

The production of meat preparations experienced a great development in recent years, being a necessary condition in the context of dynamic change of lifestyle of the population.

On the other hand, many existing products in the market are not optimal also in terms of safety, the multitude of additives used making the consumers to be reluctant and to direct their attention to the consumption of fresh meat, which is associated with a „clean" product. To meet the demands, the meat should therefore submit outstanding sensory properties, of which the major importance are tenderness and juicy.

The study aims to assess progress of meat quality under the action of enzymes and mixing operation, resultingby determination of meat cutting force and of the valuation of losses from pasteurization, starting from 2 hours post-slaughter, for 14 days.

The cutting force and the meat ability to retain water are influenced by a number of factors such as: the post-slaughter stage, pH, the ratio muscle tissue – connective tissue-fatty tissue, water content, the boiling temperature or additives used etc (Banu et al., 1997).

Myofibrillarprotein are mostly responsible for the textural properties of the meat products (Asgharet al., 1985; Yasuiet al., 1980), while Miyaguchi et al. (2000), studying the thermal and functional properties of the sarcoplasmatic proteins of pork meat, have found that sarcoplasmatic proteins have reduced properties to retain the water, to form gels and little influence on the texture.

On the other hand, the pH of the meat also affects gelification. At the isoelectric point, the proteins have an electrical charge almost nil

and present reduced properties of water retention. This leads to the formation of low consistency gels or even prevents the formation of gel (Smith, 2001). Trout et al. (1986) considers that polyphosphates and pH are responsible for 80% of water binding capacity.
The collagen, associated with the hardness of the meat, is made up of three polypeptide chains stabilized by intramolecular and intermolecular bonds. During the process of aging of animals, the more covalent bonds within and among the molecules of collagen are formed, which helps the meathardness (Asgharet al., 1985; Kijowski, 2001).
Although pig muscle tissue shows no hardness as evident as in the case of beef meat, enzymes may be used to obtain meat with more pleasant texture.
Apart from the aspects of texture and boiling, there can be considered other issues related to the consume of enzyme treated meat.

MATERIALS AND METHODS

The research material was meat pork after slaughter, derived from adult animals slaughtered in the slaughterhouse Romsuintest Peris S.A.
From the chosen meat of the coarse connective tissue and fat have been formed three groups: the control group represented by pork pulp, a consignment consisting of pork pulpinjected with papain, and a third group was composed of pork pulp that has been subjected to injection with papain and also mixed. Thus prepared, the samples packaged under aerobic conditions in polythene bags, were stored in refrigerated conditions (from 0...2°C) and their evolution was monitored over a period of 14 days.
Injection and blending of meat were made under laboratory conditions. The enzyme preparation was added in the amount of 10 mg/100 g meat, in the form of solution using a syringe, and mixing was done in series of 10 min alternated with breaks of one hour, for 8 hours.
For the determination of meat rigidity, appreciated by the cutting force, from each group were made parallelipipedic samples of 2 × 2 cm in section and about 20 g, which have been cut at the texturemeter TA-XT Plus. To highlight the differences between the three analyzed groups, for each sample were made minimum 5 cuts.
For every group of meat, daily treatment has been carried out, samples being placed in sealed containers and subjected to pasteurization at a temperature of 70...75°C for 10 minutes in the heat center of the product. After heat treatment, the samples were tempered at 2^0C and weighted, settling losses, which were expressed in the form of juice and fat.

RESULTS AND DISCUSSIONS

The evolution ofuntreated meat hardness

In order to assess the evolution of cutting forces for the groups: control, injected and injected-mixed (Figure 1) there were sampled daily for 2 weeks.
On the first day, cutting force recorded an average of 13.19 kgf for control, 13.11 kgf for injected sample, respectively 10.611 kgf for injected and mixed sample. These values have started to grow up in day three, when it reached an average of cutting force of 20.88 kgf in the control, 16.83 kgf for injected sample, respectively 15.49 kgf for the injected and mixed sample, value associated with the moment when the meat has reachedthe point of maximum rigidity.

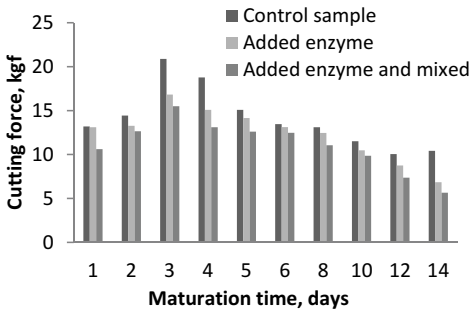

Figure 1. Cutting force variation during maturation

In this period, the pH correlation was inversely proportional to the meat cutting force, respectively immediately after slaughter was registered pH of 7.14 coming up on the third day to drop to 5.55 (considered the ultimate pH of the meat, at which point also theglycolysis ceased).

After the period of rigidity, with the entry of meat in the maturation phase, cutting forces started to decrease gradually, as the last day to register averages of 10.42 kgf for control sample, 6.85 kgf for injected sample, respectively 5.66 kgf for injected and mixed sample.

Throughout the research, between the three groups of meat there was a continuous correlation of cutting force, so that the highest values were recorded in the control sample, followed by enzyme-treated sample values, while the lowest values were recorded in case of injected and mixed sample.

The cutting forces decrease during maturation, correlated directly proportional to meattenderizing, is due to proteolytic enzymes of muscle tissue from cathepsin group, but also to enzymes produced by saprophytic microflora, which act on the myofibrillar and connective tissue proteins generating a decrease of its hardness. The addition of papain enhanced the proteolysis and helped the process of maturation, favoring the tenderness compared to uninjected samples.

In the case of the third group, injected and mixed, besides the meat tenderizing action generated by enzymatic treatment, the mixing process has led, on the one hand a better distribution of enzymes in the muscle tissue and has facilitated their action, and on the other hand there was a mechanical destruction of fibres that have become softer and more exposed to the action of enzymes by destroying the protective membranes.

The evolution of pasteurized meat hardness

In each of the groups analyzed (control, enzyme-treated and enzyme-treated - mixed) were made samples which have been subject to the heat treatment at 70...72°C and after for each sample was determined the cutting force, in conditions similar to those of raw meat.

The development of cutting force values recorded in the case of pasteurized samples for the three groups is shown in Figure 2.

Throughout the study, for all the three analysed groups the cutting forces values of heat-treated samples were lower than those of untreated samples, but keeping the same trends in evolution. This can be explained by protein denaturation (between 60 and 80^0C sarcoplasmatic and myofibrillar proteins are completely denaturated), gelification of collagen. At 60^0C begins the thick filaments coagulation (myosine), disintegration of thin filaments of actin and the loss of the line M, at 70^0C massive disintegrate the thin filaments and continue the coagulation of the thick ones.

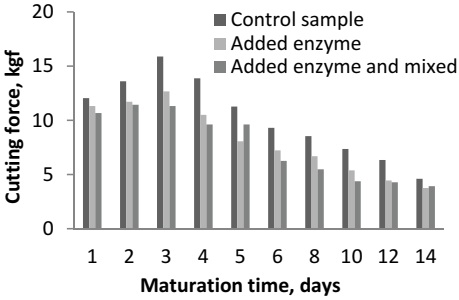

Figure 2. Cutting force variation for boiled meat

Cutting forces ranged from 10.04 kgf control sample, 11.32 kgfenzyme treated sample, respectively 10.67 kgf enzyme treated-mixed sample in the first day, to 15.88 kgf control sample, 12.67 kgf enzyme treated sample, respectively 11.32 kgf enzyme treated-mixed sample in the third day and up to 4.60 kgf control sample, 3.75 kgf, enzyme treated sample, respectively 3.92 kgf enzyme treated-mixed sample in the last day.

Cooking losses

Losses to heat treatment at atmospheric pressure are dependent mainly on the type of meat, the temperature and duration of heating, which cause denaturations in the proteins level.

Analyzing the loss from thermal treatment (Figure 3), results that they vary in direct proportional to the values of the cutting forces, both analyzed parameters being influenced by the post-slaughtertransformations of the meat. Thus, in the first three days, losses have an increasing evolution because the meat enters in muscle stiffness, the pH decrease to 5,4, then evolution is descending on the rest of the analysis period, simultaneously with the gradual increase of pH.

The temperature is one of the most important factors in the formation of gels, for that is the driving force that determines the unfolding of proteins (Totosaus et al., 2002).

The thermal denaturation of a protein is usually accompanied by several conformational transitions in structure (Lesiów et al., 2001).

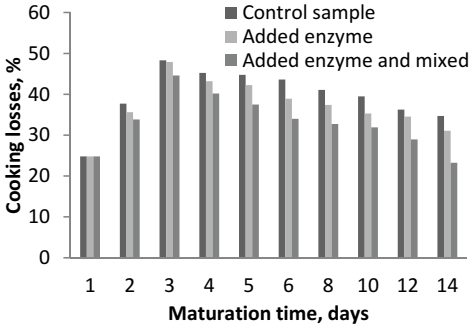

Figure 3. Cooking losses depending on the treatment applied and maturation time

At present, it is generally accepted that the myosin molecule undergoes two major transitions during heating: the first is the denaturationand the second is the formation of aggregates (Burke et al., 1973; Samejima et al. 1981). It is essential that the aggregation rate remains lower than the denaturation to get quality gels (Totosaus et al., 2002).

Several studies have reported an increase of hydrophobic character during the first part of the warming, followed by a decrease in the second stage of gelification (Wang et al., 1994). Wang and Smith suggested that the low temperatures (low heating rate) have favored the aggregation process, while high temperatures (rapid heating rate) have weakened the intramolecularbonds and the cross bonds between myosine gels.

Losses from heat treatment ranged from 24.78% on the first day, to 48.31% for control sample, 47.9% for the enzyme-treated sample, respectively 44,6% for the enzyme-treated – mixed sample in the third day and up to 34.68% for control sample, 31.07% for the enzyme-treated sample, respectively 23.23% for the enzyme-treated – mixed sample in the last day.

CONCLUSIONS

Muscle hardness and losses at thermal treatment are closely related to the post-slaughter stages of the meat, the temperature and duration of heat treatment.

Adding ofenzymes helps proteolysis and thus the hardness and capacity of water retention. The use of enzymatic preparations in the meat maturation flesh presents the advantage that helps shorten maturation period, improves digestibility, reduce the culinary cooking time, and the nutritional value of the meat is improving.

The association of enzymatic treatment with mixing was also effective in improving the analyzed properties.

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EFFICIENCY OF DIFFERENT TYPE OF TENDERIZATION FOR IMPROVING TECHNOLOGICAL PROPERTIES OF BOVINE *BICEPS FEMORIS* MUSCLE

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Abstract
Meat quality is defined as a combination of sensory characteristics and technological aspects, such as color, water-holding capacity, cooking losses, and texture. Tenderness and juiciness, has been considered as the most critical characteristics because it influences repeat purchases by consumers. Since natural aging is a long-term process, artificial aging is recommended to be widely used in the meat industry and catering. Thus, in the present study was investigated the effect of different type of tenderization including chemical methods (injection with 0.4 M $CaCl_2$), enzymatic methods (injection with papain extracted from papaya) and marinating in wine marinade on technological properties of bovine Biceps femoris muscle. The control samples were represented by raw meat stored in the same conditions as analyzed samples. After injecting with $CaCl_2$, papain and marinating, meat pieces were vacuum packed and stored at refrigeration temperature 4°C for 7 days. In this experiment, the influence of tenderization applied to beef meats was evaluated by monitoring pH evolution, storage losses, cooking losses, changes in meat texture. During storage and artificial tenderization of the samples vacuum packed, were noted for all indicators followed variations indicating the proteolysis process development, which signifies an improvement in meat tenderness.

Keywords: *beef tenderization, calcium chloride, papain, marinating*

INTRODUCTION

Consumer acceptance of meat is strongly influenced by the eating quality. Meat quality can be defined as a combination of diverse properties of fresh and processed meat. These properties contain both sensory characteristics and technological aspects, such as color, water-holding capacity, cooking losses, and texture (Walsh et al.; 2010; Kargiotou et al., 2011). Of the sensory characteristics, eating quality, which consists of flavor, tenderness and juiciness, has been regarded as the most critical characteristics because it influences repeat purchases by consumers. Consumers have identified tenderness as the most important beef sensory attribute (Miller et al., 1995). Red meat industry needs to produce high quality meat of consistent tenderness to increase consumer confidence and encourage further purchase of meat products (Boleman et al., 1997; Han et al., 2009; Jayasooriya et al., 2007). Tenderness differs among bovine muscles from various anatomical locations largely because of differences in the structural components, which influence tenderness namely the myofibrillar and connective tissue proteins (Von Seggern et al., 2005). In general, muscles from the forequarter are less tender than those from the loin and these are classified as low value cuts. Therefore, there is considerable interest in developing strategies to improve palatability, in order to add value to these muscles (Molina et al., 2005). Papain (Schenkova et al., 2007) and calcium chloride (Ilian et al., 2004; Koohmaraie et al., 1998) have been the most studied and are probably the most effective tenderizing agents. However, papain has a tendency to over-tenderize the meat surface, leading to undesirable ''mushy" meat (Ashie et al., 2002), leading to a limited use as a commercial meat tenderizer. Although the infusion of $CaCl_2$ solution can improve meat tenderness (Koohmaraie et al., 1998), calcium ions reduce the color stability of fresh meat and decrease the product shelf life (Bekhit et al., 2005). Marinating is an effective means of enhancing the quality of meats. Marinating is the process of soaking or injecting meat with a solution containing ingredients such as vinegar,

lemon juice, wine, soy sauce, brine, essential oils, salts, tenderizers, herbs, spices and organic acids to flavor and tenderize meat products (Bjorkroth, 2005; Fernandez-Lopez et al., 2005; Pathania et al., 2010).

The objective of the present study was to investigate the influence of different methods of tenderization (enzymatic, chemical and marination) on technological properties of bovine *Biceps femoris* muscle vacuum packed and stored at refrigerated temperature.

MATERIALS AND METHODS

Materials

The raw material, utilized in research program, was represented by the beef thigh from adult cows. The meat was purchased in refrigerated state from a local slaughterhouse at maximum 24 hours post-slaughter. Salt was of food-suitable purity, being a largely used additive in meat industry, papain and bromelain were purchased from Lay Condiments, Bucharest (Papain Chilko P, Bromelin EC 3.4.4.24). Garlic (*Allium sativum)* have been purchased from Quatre épices Company (Bucharest, Romania), lime-tree honey was purchased from S.C. Apisalecom S.R.L. (Bacau, Romania) and dry red wine, minimum 12% vol. alcohol content, from S.C. Viovin Prodserv S.R.L. (Odobesti, Romania).

Sample preparation

The adult beef meat separated from conjunctive tissue and fat was cut into pieces of the same size in length and thickness (1.5 – 2.0 cm) weighing approximately 150 g, cut along the muscular fibers. The meat pieces were then divided into four groups and were used for a certain treatment. For each treatment series were constituted, consisting of:

- Sample 1 – pieces of meat injected with 10% brine with papain addition to a concentration of 1,5 mg/100 g;
- Sample 2 – pieces of meat injected with 10% $CaCl_2$ to a concentration of 0.4 M;
- Sample 3 - pieces of meat marinated in dry red wine (300 ml/kg), honey (40 g/kg), garlic (9 g/kg), pepper (2 g/kg) and salt (5%);
- Sample 4 – control samples, pieces of meat without applying any treatment.

The injection was performed manually with a syringe, so that the entire brine quantity could be uniformly pumped into the whole muscular mass. The eliminated brine was reinjected. For marination treatment, meat slices were placed into polypropylene boxes. A 300 ml volume of the marinade per one kg of meat was then added to cover all the meat pieces, followed by agitation by hand to ensure an even distribution of the solid components of the marinades. All boxes were over-wrapped with a polyethylene cover and held at 4^0C for 48 hours. After approximately 24 hours the meat pieces and were turned over, to ensure uniform marination. Following marination, the meat samples were removed from the trays and the excess liquid was allowed to drain off. After tenderization treatments samples were vacuum packaged and stored at 4^0C for 7 days. Vacuum packaging was performed using a vacuum packaging machines, VACSY System, produced by the company Zepter International using a package type, with the following characteristics: permeability to O_2 (at 23^0C and 0% RH) <30 cm^3/m^2•24h•atm and CO_2 permeability (at 23^0C and 0% RH) = 150-200 cm^3/m^2•24h•atm.

Chemical analysis

Tenderness degree was determined according to the method described by Ionescu et all, 1992.

Cooking losses were calculated with the formula:

$$P= [Mi-Mf] \times 100/ Mi$$

where Mi= initial weight of the sample (raw meat);

Mf= final weight of the sample (after thermal treatment).

pH was determined according to A.O.A.C. method, (1984) with a Hanna digital pH-meter.

Water holding capacity was determined according to the method described by Fujimaki and Tsuda, cited by Thomson and col., 1997.

RESULTS AND DISCUSSIONS

pH evolution depending ontype of artificial tenderization and storage time at 4^0C

The pH of the meat has a special importance in its processing, directly influencing shelf life, color and quality of the meat (Simela, 2005). The tenderization of *Biceps femoris* muscle by injecting with $CaCl_2$, exogenous proteolytic enzyme (papain extracted from papaya) or

marinating in wine marinade has changed the pH values. pH values were dependent on the type of treatment applied and time of storage at refrigeration temperature of 4^0C (Figure 1).

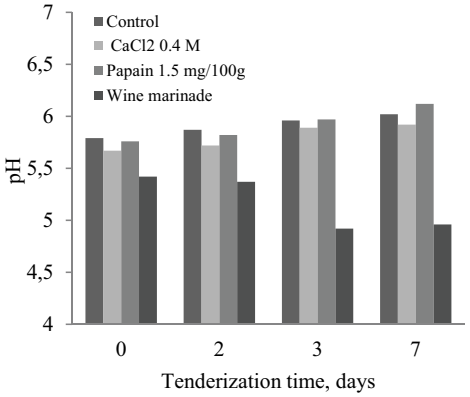

Figure 1. Influence of artificial tenderization of *Biceps femoris* muscle and storage time at 4^0C on pH values

The experimental data showed the increasing of pH values with the increasing of ageing time in control samples and experimental samples tenderized with papain and $CaCl_2$ 4M. At the samples marinated in wine marinade had recorded a decrease of pH values between 0 and 3 days of storage with a slight increase after 7 days of storage. The highest value of pH was achieved at the samples injected with papain for maximum ageing time of 7 days. The pH at the samples marinated in dry red wine was maintained at low values because of the marinade composition (dry red wine contributes to the low pH of the marinade).

The decrease of pH values in samples marinated in wine marinade may be explained by organic acids from wine absorption by meat and lactic acid production by lactic acid bacteria. The honey from marinade was the nutritive substrate for lactic acid bacteria. Lactic acid accumulation in time, led to a decrease in pH values in marinated samples.

According to Koohmaraie et al., 1990 and Morgan et al., 1991 increased values of pH may play an important role in the activation of calpain system calcium-dependent and in improvement of meat tenderness. pH value of meat products is highly important because it has a major influence on water holding capacity (WHC), tenderness and juiciness (Goli et al., 2007).

Influence of artificial tenderization of *Biceps femoris* muscle on water holding capacity (WHC)

One of the characteristics that define the quality of meat is its ability to retain its own water and water added. Other attributes of meat, such as juiciness, flavor and color are related to water holding capacity. Also, a close correlation is between water holding capacity and weight losses, which take place in meat during storage or thermal treatment. At the initial moment the best Water holding capacity had samples marinated in wine marinade which were followed by the control samples and the samples injected with $CaCl_2$ solution and papain (Figure 2).

Experimental data, showed in Figure 2, indicate the negative effect of exogenous proteolytic enzyme treatment and injection with $CaCl_2$ on water holding capacity of samples. Water holding capacity decreases with the increasing of aging time at refrigeration temperature of 4^0C. Thus, the best water holding capacity was recorded at initial time by the samples marinated in wine marinade, and the lowest value of water holding capacity was observed at the samples injected with papain for 7 days of aging. Reducing the amount of water bound of the enzymatic tenderized meats can be explained by the changes of myofibrillar protein structure as a result of exogenous proteolytic enzyme action. The degree of fragmentation of structural proteins was higher, the water holding capacity of meat was lower, and that is the case of samples treated with papain (Figure 2).

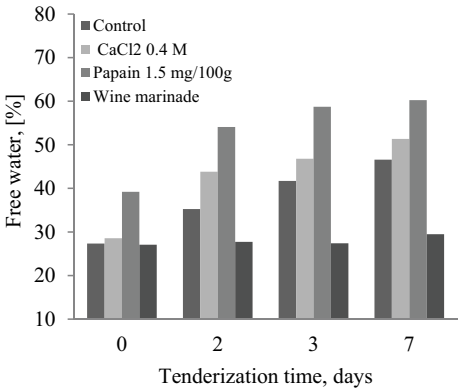

Figure 2. Influence of artificial tenderization of *Biceps femoris* muscle and storage time at 4^0C on water holding capacity (WHC)

Decreased water holding capacity for the samples treated with $CaCl_2$ may be due to reduction of water absorption in the presence of Ca^{2+} ions (Gerelt et al., 2002). Wheeler et al., (1993) indicated that the addition of exogenous calcium activates calpain enzymes meat, which reduces WHC by myofibrillar proteolysis. Koohmararie et al., 1990 reported that calcium ions modify the native conformation of proteins myofibrillar promoting their denaturation. Also, water-holding capacity of fresh beef increases rapidly as pH values decrease below the isoelectric point of muscle (5.1), which increases the relative number of positive-charged protein groups (Hamm & Deatherage, 1960). Furthermore, the number of these reactive groups available to bind water is dramatically increased when muscle pH values were greater than 6.0 or below 4.0 (Gault, 1985). Additionally, Rao and Gault (1989) pointed out that acidification of meat below its isoelectric point favored the swelling and moisture retention by stromal proteins, whereas greater meat acidification, to pH levels of 4.0 and below, resulted in the swelling and retention of added moisture by the myofibrillar proteins.

The influence of artificial tenderization of *Biceps femoris* muscle on cooking losses

During the thermal treatment applied to beef take place a series of more or less intense chemical and physical changes. The meat texture is altered during the thermal treatment as a result of protein denaturation, meat dehydration, collagen hydrolysis, fat expulsion from fat cells and their dispersion in to the meat mass.

Figure 3 shows the evolution of cooking losses depending on the type of treatment applied to beef samples and the storage time at 4°C (storage time = 0 – 7 days). Artificial tenderization of *Biceps femoris* muscle led to significant changes in the cooking losses as compared to the control samples.

Experimental data indicate the negative effect of injection with $CaCl_2$ on cooking losses. The highest value of cooking losses was achieved at the samples injected with $CaCl_2$ at 2 days of ageing at refrigerated temperature. Koohmaraie et al. (1990) reported that injection of beef loins with $CaCl_2$ within 1 h postmortem resulted in increased cooking losses.

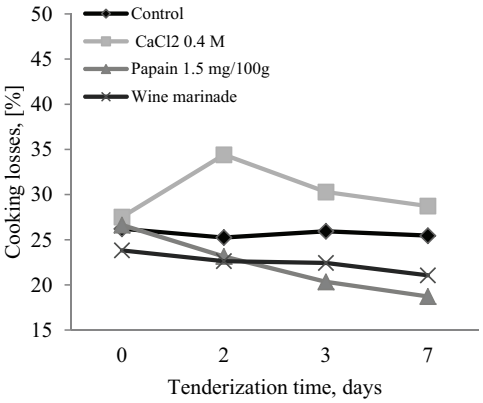

Figure 3. Influence of artificial tenderization of *Biceps femoris* muscle and storage time at 4^0C on cooking losses

The result obtained in this study agreed with the work of Omojola (2007) who reported an increase in cooking loss as time post mortem increased. In a similar way, Wheeler et al. (1992) reported higher cooking loss values in beef injected with $CaCl_2$ 24 h post mortem compared with 0 h post mortem.

Experimental data indicate a decrease of cooking losses both in the samples tenderized with papain and the samples marinated in wine marinade, the lowest values being recorded at the samples marinated in wine marinade for the maximum time of ageing 7 days. Evolution of cooking losses of the samples injected with papain and marinated in wine marinade was closely related to the evolution of pH. The results from several studies have shown that lowering the pH of beef also reduced moisture losses during cooking contributing to an overall improvement in the juiciness of the cooked product (Aktas et al., 2003; Gault, 1985; Onenc et al., 2004).

The influence of artificial tenderization and storage time at 4^0C on rigidity index of beef cuts thermal treated by boiling

Rigidity index represents the resistance opposed by meat to compression (in the present study rigidity index was determined at boiled beef cuts). Meat samples injected with solutions containing papain and $CaCl_2$ and marinated in wine marinade, stored at 4^0C, showed rigidity index values higher than control samples, so indicating an improvement of meat tenderness (Figure 4).

The increase of the ageing time at refrigerated temperature resulted in significant increase of the rigidity index in the samples injected with papain. The highest values of rigidity index were recorded at the samples injected with papain for the maximum time of tenderization 7 days. Also, the lowest values of rigidity index were recorded at the control samples at initial time. Calcium chloride improves meat tenderness by increasing intracellular calcium ion concentration leading to activation of calpain enzymes and increasing fragmentation of muscle fibers. The mechanism of the tenderizing action of acidic marinades is believed to involve several factors including weakening of structures due to swelling of the meat, increased proteolysis by cathepsins and increased conversion of collagen to gelatin at low pH during cooking (Berge et al., 2001).

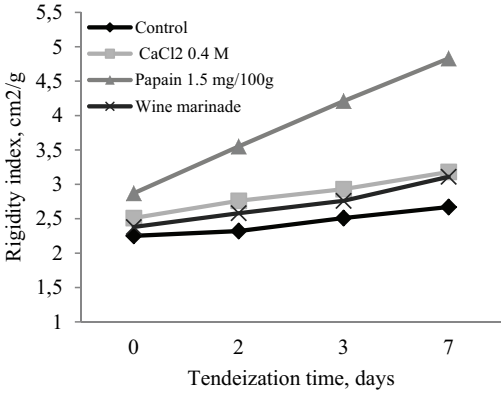

Figure 4. Influence of artificial tenderization of *Biceps femoris* muscle and storage time at 4^0C on rigidity index

Insignificant increases of rigidity index of the samples injected with $CaCl_2$ and marinated in wine marinade can be explained by the high content of collagen in adult beef meat. The meat with a high content of collagen is generally tough and requires a prolonged heat treatment (Perez-Chabela et al., 2005).

CONCLUSIONS

Injection of beef cuts with papain cause an important improvement of functional properties of *Biceps femoris* muscle as compared with the injection with $CaCl_2$ and marination in wine marinade. Papain is a powerful proteases preparation, with great under-layer specificity, catalyzing the breaking of the peptidic bonds in the protein molecules and their degradation products to amino acids.

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MILK - COMMODITY OR NECESSITY

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Abstract

The paper was carried out in order to present the production of milk mixture (cow and buffalo) obtained during 2008 - 2011 in the private and state sector, that it was delivered to market capitalization directly or indirectly through processing industrial units and family consumption.

Keywords*: total production, raw milk, market capitalization, statistics*

INTRODUCTION

Milk is one of the animal products with importance in human nutrition.
In animal products, the milk production ranks second after meat as food and economic importance, being one of the cheapest sources of animal protein with high biological value (Georgescu et al., 2000).
The aim of the paper was to present the milk production of cow and buffalo during 2008-2011 in the private and state sector.

MATERIALS AND METHODS

The research was conducted by collecting databases from the Agricultural County Departments (DAJ) on the production of mix cow and buffalo milk, cow milk and buffalo milk and also from the National Institute of Statistics (INS).

After the data was collected, they were processed as total values, representing the total milk production, the milk quantities sold on the market from the private and the state sectors, capitalized directly and indirectly through industrial processing units, the family consumption on county level, the economic development regions and then for the whole country. The milk consumption per capita was established based on operational data from DAJ and from INS (Banu et al., 1998).
The period under study is 4 years, respectively 2008-20011.

RESULTS AND DISCUSSIONS

From the data in Table 1, it can see a steady decrease in the milk production over the entire period the fluctuation in milk production occurs due to the decrease in the number of production animals.

Table 1. Dynamics of production of milk mixture (cow and buffalo)

Specifications				unit	Years of study			
					2008	2010	2011	Total
Cow and buffalo milk	State Sector	Total production		mii hl	51591.144	44106.432	43806.863	**139504.439**
		Market capitalization		mii hl	103.050	97.584	99.694	**300.328**
		Of witch:	Direct	mii hl	28.706	27.758	30.467	**86.931**
			Unit. ind	mii hl	74.344	69.826	69.227	**213.397**
	Private Sector	Market capitalization		mii hl	26055.528	22531.246	22221.159	**70807.933**
		Of witch:	Direct	mii hl	13752.255	12661.200	12804.610	**39218.065**
			Unit. ind	mii hl	12303.273	9870.046	9416.549	**31589.868**
		Family Consumption		mii hl	20376.627	17129.561	17378.572	**54884.760**

Studying milk production from the two sectors, the public sector and the private sector, it can be seen that the private sector contributes the most to milk market capitalization with a quantity of 70807.933 thousand hl, comparing to 300.328 thousand hl ensured by the state sector. Analyzing the quantities of milk sold on the market both directly and through industrial units it is apparent that in the private sector, the largest quantity of milk was capitalized by direct sales in the detriment of sales to industrial milk processing units, while in the public sector capitalization of raw milk was first made through industrial units and less milk through direct market sales. A strict analysis of the public sector would show that this sector has recorded in direct marketing, in 2011, a larger quantity of milk (30 467 thousand hl) than in 2008 (28 706 thousand hl), a sign that this sector oriented towards the free market where commercial transactions are made much faster.

Table 2. Dynamics of cow milk production

	Specifications			Unit	Years of study			
					2008	**2010**	**2011**	**Total**
Cow milk	State sector	Total production		mii hl	51160.482	4381.178	43548.639	138520.901
		Market capitalization		mii hl	102.390	96.034	98.344	296.768
		Of which	direct	mii hl	28.696	27.758	30.467	86.921
			unit. ind	mii hl	73.694	68.276	67.877	209.847
	Private Sector	Market capitalization		mii hl	25859.593	22408.939	22108.239	70376.771
		Of which	direct	mii hl	13599.917	12569.276	12720.834	38890.027
			unit. ind	mii hl	12259.676	9839.663	9387.405	31486.744
		Family consumption		mii hl	20198.752	16999.353	17272.158	54470.263

Regarding the production of cow's milk, it can observe a total production during 2008 - 2011 of 138,520.901, in the evolution of milk production it can be seen a decline, from 51160.482 thousands hectoliters in 2008 to 43548.639 thousand hectoliters in 2011. Market capitalization has a sinuous curve in both the private and state sector. The family consumption during 2008-2011 was 54470.263 thousand hectoliters, of which, in 2008 of 20198.752 thousand hectoliters, and in 2011 a quantity of 17272.158 thousand hl. An analysis of the evolution of milk production in the Table 2 shows a concave curve because in 2008 there was a production of 20198.752 thousand hectoliters, and in 2010 there was a decrease to 16999.353 thousand hl, and 2011 presents an upward curve, a shy increase in the amount of milk to17272.158 thousand hl milk.

Table 3. Dynamics of buffalo milk production

	Specifications			Unit	Years of study			
					2008	2010	2011	Total
Buffalo milk	State Sector	Total production		mii hl	430662	294652	258224	**983538**
		Market capitalization		mii hl	660	1550	1350	**3560**
		Of which:	direct	mii hl	10	0	0	**10**
			unit. ind	mii hl	650	1550	1350	**3550**
	Private Sector	Market capitalization		mii hl	195935	122307	112920	**431162**
		Of which:	direct	mii hl	152338	91924	83776	**328038**
			unit. ind	mii hl	43597	30383	29144	**103124**
		Family consumption		mii hl	177875	130208	106414	**414497**

Analyzing tabular data on buffalo milk production, the milk quantities for market capitalization and the amounts intended for consumption, it can observe a progressive decrease during 2008-2011. Family milk consumption presents the same diagram.

Studying milk production, milk quantities intended for family consumption and market

capitalization on economic development regions shows:

- Total milk production in the period under study was 139504.439 thousand hl, of which we can say that most milk production was recorded in the North - East (31370.112 thousand hl), followed by the North - West (25803.199 thousand hl), Center (22132.104 thousand hl), South - Muntenia (21372.924 thousand hl), and the lowest production was recorded in Ilfov - Bucharest (956 828 thousand hl).

Table 4. Production dynamics of milk mixture (cow and buffalo) by development regions

	Specification Unit			Geographical development region								
				West	North - West	Suth -West	North - East	South - East	Suth - Muntenia	Center	Ilfov - Bucuresti	**Total**
Cow and Buffalo milk	**Total production**		thousand hl.	11261.139	25803.199	12392.031	31370.112	14216.102	21372.924	22132.104	956.828	**139504.439**
	Market capitalization State sector		thousand hl.	50.489	35.659	24.970	78.133	7.531	6.975	96.571	0	**300.328**
	Of which	direct	thousand hl.	43.714	28.161	2.169	0.733	1.051	4.158	6.945	0	**86.931**
		Unit. ind	thousand hl.	6.775	7.498	22.801	77.400	6.480	2.817	89.626	0	**213.397**
	Market capitalization private sector		thousand hl.	4490.326	14907.840	4550.920	14858.260	6709.534	10065.848	14739.437	485.768	**70807.933**
	Of which :	direct	thousand hl.	2749.723	8446.612	4277.142	6758.166	4125.064	5903.099	6665.454	292.805	**39218.065**
		Unit. ind	thousand hl.	1740.603	6461.228	273.778	8100.094	2584.470	4162.749	8073.983	192.963	**31589.868**
	Family consumption		thousand hl.	5288.628	8043.038	6393.976	13475.958	6301.158	9572.411	5388.565	421.026	**54884.76**

- Regarding the market capitalization of raw milk, it can be seen that the largest quantity of milk is provided by the private sector (70807.933 thousand hl) than the state sector (300 328 thousand hl). The geographical areas of development with the greatest amount of milk for market capitalization are: North - West (14907.840 thousand hl), North - East (14858.260 thousand hl), Center (14739.437 thousand hl) and the lowest amount was recorded in Ilfov - Bucharest (485 768 thousand hl). The state sector presents as areas rich in milk, with large amounts intended for market recovery, the areas: Centre (96 571 thousand hl), North - East (78 133 thousand hl), West (50 489 thousand hl), and the lowest was registered in Ilfov - Bucharest (0 hl).
- Analyzing the quantities of milk intended for market by direct selling on the geographical development zones it can be seen that the largest quantities were delivered in the areas West, North - West, South - Muntenia in both the state and private sectors. Geographical areas of development Southwest, Southeast and Center have used milk in the field of state into a larger share through industrial units and less through direct sales, geographical development regions Northeast and Central regions unlike the other regions has capitalized milk in general through industrial units and less through direct sales, Bucharest-Ilfov through private sector has capitalized milk in general through direct sales.
- Regarding family consumption, the largest amount was recorded in the North-East (13475.958 thousand hl), followed by South-Muntenia (9572.411 thousand hl), North - West (8043.038 thousand hl), the smallest amount of milk intended for consumption was registered in region Ilfov - Bucharest.
- According to INS milk consumption per capita in 2011 was 243 liters, if we relate the quantity of milk intended for the market capitalization of both the free sale and through industrial units and family consumption we can say that on the entire period through own production of cow milk plus buffalo milk produced by the livestock existing at that time provided a quantity of 590 liter and 196 liter / capita / year.

CONCLUSIONS

- The high milk productions are recorded in the regions of economic development with tradition in animal breeding;
- The market capitalization varies by economic development regions, respectively in the South-West, Southeast and Central where prevails capitalization through industrial units, therefore in these regions activates multinational companies, who have a collection network well established.
- In the other regions of economic development, where prevails the big cities, the sale is done directly at market.
- The family consumption varies from one economic region to another, in areas where we have high productions, development of the region is mainly based on agriculture development, respectively animal breeding. The industry is underdeveloped, agricultural incomes are small, and therefore in the food predominates milk and milk product.
- In regions where free market capitalization predominates is recommended investment through rural development programs (RDP) to increase efficiency by diversifying production (cheese, cream) and corresponding packaging with consumer requirements.

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EFFECT OF RICE STARCH AND WASTE PRODUCT OF TOMATO PROCESSING ON SOME PHYSICO-CHEMICAL AND SENSORY PROPERTIES OF SAUSAGE

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Abstract

The producing low-calorie products is an important issue in this industrial world to reduce different diseases. This includes low-calorie meat products because they have a specific place in daily usage. The rice starch and waste product of tomato processing used in this formulation, as fat replacements, were (0.5, 1, 1.5, 2, and 2.5%) and (1, 2, 3, 4, and 5%) respectively. Fat reduced from 21.09% in control to 9.8%. The physical properties (texture), chemical properties (moisture, protein, fat, and ash content), sensory properties, and caloric value were evaluated in produced samples. Results obtained from chemical tests showed that moisture, ash, and protein content increased in treatments containing rice starch and tomato waste powder compared with the control. The results from caloric tests illuminated that the calorie of the samples significantly reduced coincide with fat reduction. The texture analysis tests indicated that all samples were firmer than the control. The sample containing 1.5% rice starch and 3% tomato waste powder had the highest shear stress compared with other samples. The colorimetric tests showed that fat replacing with tomato waste powder and rice starch led to an increase in yellowness (b) and redness (a*) and a decrease in lightness (L*) in samples. According to organoleptic properties, all samples got higher score than the control. However, the sample containing 2% rice starch and 4% tomato waste powder was recognized as the best one. The panellists reported that adding tomato waste powder and rice starch not only didn't have negative effects on sensory properties but also they increased total acceptability. Therefore, considering the results obtained from instrumental and sensory analysis, using tomato waste powder and rice starch respectively 4% and 2% resulted in desirable consequences in fat reduction.*

Keywords: *fat replacement, low-fat sausage, organoleptic analysis, rice starch, powder of tomato waste*

INTRODUCTION

Epidemiological studies have shown that there is a direct relationship between diet (high fat products) and occurrence of some diseases like colon cancer, obesity, and cardiovascular diseases. Like other countries, cardiovascular disease is widespread in Iran which is the cause of 40% of mortality. Since the most important factor is kind and amount of used fat, this has led to rapid changes in eating habits. Therefore, one of the controversial issues in today industrial word is producing low-fat (low-calorie) products containing low-fat meat products. Different researches have been done on low-fat sausages using fat replacers.

Many studies have suggested using tomato waste, tomato powder, tomato peels and lycopene in raw meat and meat products such as beef patties (Sanchez-Escalante et al., 2003), minced meat (Østerlie and Lerfall, 2005), hamburgers (García et al., 2009), fermented sausages (Calvo et al., 2008), fresh sausages (Mercadante et al., 2010) and frankfurters (Deda et al., 2011).

In this study, two fat replacers based on rice starch (has fat like properties) and waste product of tomato processing (fibre) were used

in order to improve the customer acceptability. Significant part (more than 60%) of tomato is used in manufacturing industries leading to a huge waste product. According to previous researches, the tomato waste product in Iran is more than 1.5×10^5 tone (Aghajanzadeh-Golshani et al., 2010). Despite having high bioactive compounds and nutritional value, most of this waste product is dumped and just a limited amount is used to feed livestock and poultries while this can be considered a by-product in tomato industries leading to high investment and new products.

The purpose of this paper is to use of rice starch and tomato waste powder as fat replacers in sausage formulation and study their effect on chemical, physical, sensory, and caloric value of the sausage.

MATERIALS AND METHODS

The tomato waste powder was dried using vaccume oven (Enretvts-70, Germany) in 55°C for 72 h to reach 12.5% of moisture content. It was then milled using a miller (Retsch-Rhcimische-36-D-4278-Haa, Germany).

The obtained powder was sieved with a 40-mesh sieve, packaged in polyethylen bags, and stored in -18°C.

The lean beef was obtained from boneless round and trimmed from all subcutaneous and inter muscular fat as well as thick, visible connective tissue and maintained frozen (0-18˚C). Fat content of the lean meat and fat portions were determined prior to the manufacture of sausages. Rice starch was obtained from National starch and Atame Pars Company. The tomato processing wastes were obtained from Ataco Co. for Food Processing. The control sausages were formulated to contain 60% lean beef with 15% kidney fat. Different levels of vegetable oil (12.5, 10, 7.5, 5, 2.5 and 0%) were replaced by rice starch/tomato waste combinations at different ratios (Table 1). Then, the beef was ground through a 3 mm plate electric grinder (Sky sunPSE-10). Then, the powder of waste product of tomato processing was added to the ground meat and mixed until a homogeneous distribution was obtained. The ingredients of each formulation were weighted (Table 1) and transferred into a cutter (Muller, Saarbrucken, W. Germany) using low speed (1400 rpm). When chopping resulted in a homogenized mixture, garlic, salt, phosphate, nitrate, rice starch and ice was added and chopping continued for 2-3 min with a speed of 2000 rpm. Other ingredients were mixed using a mixer for 3 min at 4-6°C. After mixing, the mixtures were stuffed in to synthetic cellulose casings (approximate diameter of 30mm) using a stuffer (H15, TALSA, Zaragoza, Spain). The farsh was chilled and stored for 1 hour at 0°C to achieve a better filling. The samples were then heated in 72±2°C for 30 min in a steam chamber (SAA10, Absury, Berlin, Germany). The cooked meat batters were cooled with cold water (15°C) and stored at 0-4°C. All analyses were carried out in triplicate for each formulation.

The proximate composition of the sausages samples was determined according to the AOAC standard methods (2011).

Total caloric values (kcal) were calculated using method of Watt and Mersil **(**1975**)**, where 4.27 kcal for g protein and 9.02 kcal for g lipid and 4.10 kcal for g carbohydrate.

Shear force values of raw samples were determined using a computer controlled Hounsfield H5KS Universal testing machine existed in Agricultural Engineering Research Institute. The samples of 5 cm thickness were prepared from central part of each sausage and texture resistance to shear stress was measured using stainless steel flat blade at zero temperature. The colour of the samples was measured using a colorimeter (Minolta spectrophotometer CM 3500d, Japan) and the colour reading included lightness (L*), redness (a*) and yellowness (b*).

The sensory analysis was performed by 10 trained panellists to evaluate the sausages for appearance, colour, texture, taste, smell, mouth feel by ranking, indicating score 0 as very bad and score 5 as excellent (Desmond et al., 1998).

Data were analyzed using SPSS 16.0 for one-way ANOVA. Duncan's new multiple range tests was used to resolve the difference among treatment means. A value of $p<0.05$ was used to indicate significant difference.

RESULTS AND DISCUSSIONS

Table 2 shows the chemical composition of sausages formulated with rice starch/tomato waste combinations.

Table 1. Sausages formulation containing of rice starch and powder of tomato waste

Ingredients	Control (%)	Treatments				
		T1	T2	T3	T4	T5
Lean meat	60%	60%	60%	60%	60%	60%
Oil	12.5	10	7.5	5	2.5	0
powder of tomato waste	0	1	2	3	4	5
Rice starch	0	0.5	1	1.5	2	2.5

Table 2. Proximate composition and calorie content of sausages formulated with different levels of rice starch and powder of tomato waste

Parameters	Control	Treatments				
		T1	T2	T3	T4	T5
Lipid (g/100g)	21.09 ± 3.12^{a}	18.75 ± 2.87^{b}	14.73 ± 2.14^{c}	11.1 ± 1.87^{d}	10.50 ± 1.20^{e}	9.8 ± 10.2^{f}
Moisture (g/100g)	57.8 ± 5.12^{e}	59.20 ± 6.35^{d}	62.24 ± 5.41^{c}	65.60 ± 6.23^{a}	65.50 ± 7.21^{b}	64.46 ± 6.1^{b}
Protein (g/100g)	15.45 ± 3.11^{e}	15.77 ± 2.26^{d}	16.06 ± 2.45^{c}	16.20 ± 2.31^{c}	16.80 ± 1.01^{b}	17.4 ± 1.01^{a}
Ash (g/100g)	2.16 ± 0.20^{d}	2.44 ± 0.31^{c}	2.47 ± 0.34^{bc}	2.50 ± 0.12^{bc}	2.57 ± 0.45^{ab}	2.62 ± 0.98^{a}
Energy (kcal/100g)	645.9 ± 12.31^{a}	570.6 ± 10.21^{b}	509.8 ± 11.41^{c}	2.50 ± 0.12^{bc}	2.57 ± 0.45^{ab}	2.62 ± 0.98^{a}

Different superscripts in the same column indicate significant differences ($p<0.05$).

Table 3. Shear force of sausages formulated with different levels of rice starch and powder of tomato waste

Parameters	Control	Treatments				
		T1	T2	T3	T4	T5
Shear Force (N)	17.08 ± 3.14^{f}	18.10 ± 3.65^{e}	19.20 ± 2.7^{d}	21.45 ± 2.14^{a}	20.93 ± 3.24^{b}	20.11 ± 2.56^{c}

Different superscripts in the same column indicate significant differences ($p<0.05$)

The fat content significantly decreased ($p< 0.05$) in all samples compared with control which is because of reduction of fat content in formulations. Fat content of the control was 21.09. It was decreased to 18.57, 14.73, 11.1, 10.50, and 9.8 in treatments 1, 2, 3, 4, and 5 respectively. Fat reduction was respectively 11.94%, 47.36%, 50.21%, and 53.53% of total fat in final product. The moisture content significantly increased compared to the control ($p<0.05$). The increase in moisture is due to reduction in fat and replacing it starch and fiber (tomato waste powder). Caceres et al. (2006) reported that low-fat frankfurters (40% fat content) had higher moisture content (68%) compared to the control (56%). There was a significant difference in protein and ash content ($p<0.05$) between samples and the control. All samples had higher protein and ash content which was due to higher amount of protein and ash in tomato waste powder.

Table 2 shows that amount of calorie significantly reduced in all treatments compared with the control ($p<0.05$). Amount of calorie evaluated in the control was 645.9 kcal/100 g which reduced to 570.6 kcal/100 g, 509.8 kcal/100 g, 478.4 kcal/100 g, 460.6 kcal/100 g, and 444.8 kcal/100 g in samples 1, 2, 3, 4, and 5 respectively.

The fat reduction and fibre addition resulted in a significant increase in shear force of all samples (Table 3). In other words, texture of the sausages was harder and more compact. Fat content has a key role in the quality of a meat product. Researchers have found that there is a strong relationship between fat content and firmness of the sausages in a way that fat reduction leads to harder texture (García et al., 2009). Moreover, being absorbed part of available moisture for meat proteins by non meat ingredients cause a harder product. The increase of hardness could be explained by the presence of fibre in tomato by-product. It has been reported by Knoblich et al. (2005) which has acid detergent fibre content close to 30 g/100 g of dry matter.

Table 4. Lightness (*L**), redness (*a**) and yellowness (+*b**) values for beef patties formulated with different levels of rice starch and powder of tomato waste

Parameters	Control	Treatments				
		T1	T2	T3	T4	T5
L*	51/14±4/21[a]	51/03±3/65[a]	50/90±2/45[a]	50/68±3/12[a]	49/92±4/11[b]	48/42±3/15[c]
a*	13/03±3/41[c]	13/35±2/42[e]	14/59±2/75[d]	16/74±1/56[c]	18/03±2/75[b]	18/98±2/15[a]
b*	12.65±1.38[d]	13.091±.89[d]	14.06±.3.14[c]	14.49±1.98[b]	15.60±2.54[a]	15.63±2.13[a]

Different superscripts in the same column indicate significant differences ($p<0.05$).

Table 5. Sensory properties of sausages formulated with different levels of rice starch and powder of tomato waste

Parameters	Control	Treatments				
		T1	T2	T3	T4	T5
Flavor	3.04±0.33[d]	3.09±0.54[d]	3.62±0.97[a]	3.65±0.29[c]	4.41± 0.67[b]	4.93±0.98[a]
Color	2. 81±0.53[e]	3.50±0.72[b]	3.49±0.35[b]	4.95±0.94[a]	4.96±0.73[a]	4.23±0.88[b]
Texture	4.69±0.96[a]	3.14±0.32[c]	3.09±0.85[c]	3.10±0.66[d]	3.11±0.31[c]	3.11±0.87[c]
Mouth feel	3.78±0.82[b]	3.75±0.81[a]	3.68±0.52[a]	4.01±0.58[b]	4.57±0.68[b]	4.54±0.69[a]
Acceptability	3.11±0.84[c]	3.08±0.43[d]	3.60±0.22[a]	4.01±0.97[b]	4.82±0.69[a]	4.06±0.83[b]

Different superscripts in the same column indicate significant differences ($p<0.05$).

Adding different amounts of tomato waste powder and rice starch parallel to fat reduction significantly increased b^* and a^* in samples 2, 3, 4, and 5 ($p<0.05$) (Table 4). Because of the addition of lycopene to meat products, through the addition of tomato peel and tomato waste, yellow color increased providing an orange tone (Calvo et al., 2008). The addition of tomato waste powder and rice starch did not affect lightness or darkness of samples 1, 2, and 3, but significantly reduced L^* (lightness) in sample 4 and 5 ($p<0.05$).

The sensory evaluation showed that the fat replacing with different amounts of tomato waste powder and rice starch did not have any unfavourable effects on color, flavour, mouth feel, and total acceptability (Table 5). In contrast, according to the panellists, control got the highest score in texture. Eyiler and Oztan (2011) reported that addition of tomato powder increased the acceptability of the frankfurters. Finally, the sample 4 containing 2% modified rice starch and 4% tomato waste powder was reported as the best sample by the panellists.

CONCLUSIONS

The results obtained from chemical tests showed that moisture, ash, and protein content increased in treatments containing rice starch and tomato waste powder compared with the control.

The results from caloric tests illuminated that the calorie of the samples significantly reduced coincide with fat reduction.

The texture analysis tests indicated that all samples were firmer than the control. Sample containing 1.5% rice starch and 3% tomato waste powder had the highest shear stress compared with other samples.

The colorimetric tests showed that fat replacing with tomato waste powder and rice starch led to an increase in yellowness (b*) and redness (a*) and a decrease in lightness (L*) in samples.

According to organoleptic properties, all samples got higher score than the control. However, the sample containing 2% rice starch and 4% tomato waste powder was recognized as the best one.

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STARTUP STAGES OF A LOW-TECH AQUAPONIC SYSTEM

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Abstract

The goal of the project was to establish a low-tech cheap sustainable food production system that combines traditional fish farming with hydroponics (cultivating plants using mineral nutrient solutions, in water, without soil), in a symbiotic environment. We used local fish and plants species only. The project was conducted in two different stages: "construction" stage and "fish" stage. The goal of the start-up stage was to build the grow bed for the plants, to establish the water circuit, to set the lights and to prepare the fish tank for the fishes. For the "fish" stage we used local fish species (carp and caras). The goal of the stage was to build the nitrogen-fixing bacteria colonies in order to provide a constant NO_3^- rich water (around 50 mg/l), with as low as possible traces of NH_3 / NH_4^+ and NO_2^- (below 0,5 mg/l). This was established by testing the NH_3 / NH_4^+, NO_2^- and NO_3^- values under several environmental changes (pH, temperature, lighting, quantity of fish in the tank etc.) in order to identify the best combinations to achieve the goal. Upon completion of the second stage we'll test different local species of plants with the already established system. The goal of the project is to find the best combinations of fish, plants and environmental conditions in order to have a cheap, sustainable symbiotic food production system, easy to be replicated by anyone.

Keywords: *aquaculture, aquaponics, hydroponics, nitrogen-fixing bacteria, sustainable food systems.*

INTRODUCTION

Nowadays, some of the acute problems humanity must face are related to health and to food security. Unfortunately millions of people currently suffer of hunger. The causes that led to this situation are many and diverse, like world population continuously increasing, local conflicts, over-exploitation of land by intensive agriculture, pollution, climate change effects and so on. Furthermore, the lack of available healthy food combined with poverty leads to diseases, exclusion and social inequality. We seek a solution which will provide healthy food to these communities, making them food-independent, and which will also bring them a steady income on hands. The solution we seek will also need to be able to be implemented in areas with water shortage, degraded soils and under the unpredictable conditions of climate changes as well. As "the first step in a thousand miles journey" such a solution has been identified among the new innovative agriculture technologies: **the aquaponic agriculture**, solution which "ticks" all the requirements mentioned above (Figure 1).

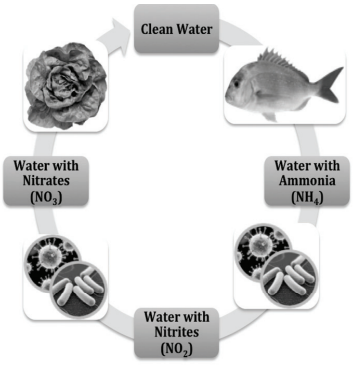

Figure 1. The Aquaponic Solution

The **aquaponic systems** were first described in the early 1980s through the article published by Watten and Busch in 1984 (Connolly and Trebic, 2010). After this first publication interest in aquaponics systems increased and a number of researchers have been working for the development and diversification. One of the most prolific researcher and author of many articles (more than 20 papers) in this field is James Rakocy from University of the Virgin Islands (Rakocy et al., 2006). The most implementations of aquaponic systems are in Australia and America. In Europe the use of

aquaponics is scarce. It seems that Italy is among the first European country where attention was given to this food production system.

Aquaponics is a combination of aquaculture and hydroponics for food production that uses nutrient – rich water from fish culture to irrigate and fertilize plants, while the plants clear the water before being re-circulated to the fish tank. Thus this system is a closed – loop system which re-circulates fresh water in which plants and fish grow together symbiotically. In this symbiosis the fish provide most of the plants' needed nutrients and the plans act as bio-filters cleaning the water for fish. Thus the wastes secreted by fish, such as urine and ammonia, are converted by denitrifying bacteria in the hydroponic grow bed into forms readily uptaken by plants for energy and growth (Diver, 2006; Considine, 2007; Nelson, 2008). The most commonly grown species in aquaponic systems are lettuce as plant and tilapia as fish. Systems used for commerce in majority of cases grow tilapia. However, in some systems, experiences have used channel catfish, largemouth bass, crappies, rainbow trout, pacu, common carp, koi carp, goldfish, Asian sea bass (barramundi), Murray cod and ornamental fish. Regarding the cultivated plants the most used are green leafy plants such as lettuce, basil, cilantro, chives, parsley, portulaca 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).

MATERIALS, TOOLS AND METHODS

In achieving its purpose the project is oriented to search the most viable combinations of local plants and fish combinations for real local conditions and to research and deliver blueprints and practical solutions to build customized aquaponics systems, adapted to the climate conditions in Romania, for micro-farms and backyards, in both rural and urban scenarios.

In this respect, the summary of the **main activities** of the project includes:

- to develop aquaponic systems solutions for temperate climate for domestic use (in both urban and rural deployments);
- to chose the appropriate local plant and fish species for the established aquaponic systems;
- to provide adequate seminars accompanied by demonstrations on field, workshops and guidelines on aquaponic agriculture to farmers in disadvantaged areas;
- to advice the future aquaponic systems users how to capitalize further on by emphasizing that the aquaponic systems can be an independent source of steady income and even a provider for new jobs in the community.

After its finalization, the project is expected to trigger a positive impact on the awareness of general public that it is really easy to use modern technologies that can offer healthy food and an extra income.

The system was build out of easy to be acquired, common (even recycled) materials. The main consideration was that the system would be **cheap**, **accessible** and **easy to be reproduced** by anyone (Table 1).

Table 1. List of materials

Materials	Retail Value per Unit (lei)	Nr. of Units
Fish Tank	100	1
Flower Stand	10	1
Water Pump	60	1
Water Heter	65	1
Water Filter	130	1
Fluorescent Lamp	30	1
Timed Power Supply	20	2
Floating Bio Balls	0,5	100
Plastic Tubing	1	1 m
Hydroton	35	10 l

The fish tank was provided for the project by the University. However, the two halfs of a 400 litre plastic barrel (around 100 lei retail value) could have be used instead.

The total cost of the build materials was around 400 lei.

The only **tool** used for the build was a pointed knife, used to cut the plastic tubing and to pierce the flower stand.

To assess the water parameters, the following were used (Table 2):

Table 2. List of water assesment tools

Water Assessment Tools	Retail Value per Unit (lei)	Nr. of Units
Dropper	4	1
Thermometer	17	1
Consumables		
pH Minus	30	1
pH Plus	30	1
Nitrite Removal	25	1
pH Test Kit	40	1
NH_3/NH_4^+ Test Kit	40	1
NO_2^- Test Kit	40	1
NO_3^- Test Kit	40	1

The total cost of the test kits was less than 200 lei. The kits used were titration-type kits, which require the user to take a sample of water from the tank and slowly drip a reagent into the vial to produce a colour end point to be compared against a specific colour-code scale.

The following **methodology** was used to acquire water parameters:

- all test kits were produced by the same company (Sera GmbH). Moreover, all of them belong to the same class of products;
- all tests were conducted in the same conditions of lighting. The pictures were taken with the same camera and under the same conditions (light, angle, flash, hour of the day). The maximum interval between measurements was 2 days;
- the fishes were always feeded only upon completion of the tests;
- the pictures were not edited and were labelled with the date when were aquired.

For the build, the following steps were taken:

- the fish tank was cleaned up and cleansed, then it was filled with regular tap water (around 400 litres);
- in order to reduce Chlorine the water was allowed to sit a few days. The test used was the *smell test*: if one can smell the water, then he water need to be allowed to sit longer;
- the fish tank was then populated with a few small fishes (fingerlings) of different species, (carp, caras and one xifo, on total weight of 50 g) in order for the ammonia/ammonium to build up and the bacteria cultures to start colonize. This process is called "***system cycling***". Toward this purpose, ***Bio Balls*** were added to the system;
- **after two weeks** the flower stand and the water pump were added to the system. The pump was set to continuously circulate the water between the fish tank and the flower stand ("*continuous flow*" timing scheme);
- the solution for the water to return from the flower stand to the fish tank was accomplished by setting up a small waterfall, instead of using the classical pump system. This solution was chosen in order to achieve both water aeration and a constant water stream in the fish tank. Besides that, another water pump was not needed to be added to the system.

Bio Balls is a special designed growth medium for bacteria colonies. The Bio Balls can be placed inside the water filter (for the anaerobic or photophobic bacteria, like *Nitrosomonas*), or can be let float and tumble along with the water current (for the aerobic bacteria, like *Nitrobacter*). This way the waterflow bring more nitrites to the bacteria and, by drifting on the water surface, the necessary Oxygen is also provided to them.

- Hydroton (an expanded clay aggregate with honeycomb core) was used to establish the grow bed;
- a few flower seeds and seedlings were planted. While the seeds were placed on a wool bedding to prevent to be washed out by the stream, the seedlings were placed into small plastic mesh potts (0,5 lei a piece) and secured with hydrotone.
- retail market price for hydroton (10 liters) is 35 lei.

The Tests

The water was sampled and probed during a period of 32 days, between 2013, December 18th and 2014, January 18th. The results are shown in the table below. Later on, detailed graphs will be also provided, in order to relate the measured values for water quality to the activities that took place during the project (Table 3).

Table 3. Water's chemical characteristics and temperature during the test period

Day Nr.	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*	*12*	*13*	*14*	*15*	*16*	*17*	*18*	*19*	*20*	*21*	*22*	*23*	*24*	*25*	*26*	*27*	*28*	*29*	*30*	*31*	*32*
NH_3/NH_4^+ (mg/l)	0,2	0,2	0,2	0,2	0,3	0,3	0,6	0,5	0,5	0,5	0,5	0,4	0,4	0,4	0,4	0,4	0,3	0,3	0,2	0,2	0,2	0,4	0,4	0,3	0,2	0,2	0,2	0,2	0,2	0,2	0,2	0,2
NO_2^- (mg/l)	0,7	0,7	0,7	0,7	0,3	0,4	3,5	4,0	3,0	2,7	1,8	0,9	0,9	0,9	1,0	0,8	0,6	0,5	0,3	0,2	0,2	0,7	0,7	0,6	0,6	0,6	0,5	0,5	0,4	0,3	0,2	0,2
NO_3^- (mg/l)	30	30	30	30	25	20	30	40	40	45	45	40	45	45	45	45	45	45	40	40	40	40	40	40	45	45	45	45	50	50	50	50
pH	7,8	7,8	7,8	7,8	7,6	7,5	7,4	7,3	7,4	7,4	7,4	7,4	7,4	7,3	7,3	7,4	7,4	7,4	7,3	7,3	7,3	7,3	7,3	7,3	7,3	7,3	7,3	7,3	7,2	7,2	7,2	7,2
Temperature (^{O}C)	22	22	22	22	22	22	22	22	21	21	20	20	19	19	24	25	25	24	23	22	22	22	22	22	22	22	22	22	22	22	22	22

System Cycling

Cycling started when fishes first added ammonia (NH_3) in the water as a product of their respiratory and digestive processes. The uneaten fish food, while decomposing, also provides ammonia in the water. In the water ammonia (NH_3) continuously shifts to ammonium (NH_4^+) and then the ammonium shifts back to ammonia, each one's concentration being related to water's temperature and to pH level (more NH_3 at higher temperatures and pH values). Because ammonia is very toxic to fish (while ammonium is relatively harmless to it), relatively low temperature and pH values are to be preferred. Another factor related to water temperature is to be taken into consideration also: the effect of water temperature on bacteria cultures. Studies show that the optimal temperature for their growth is between 25 and 30 ^{O}C, while at 18 ^{O}C the growth rate decreases by 50% and at 10 ^{O}C by 75%. Around 0 ^{O}C the bacteria population dies. After taking all above into consideration, a convenient temperature of 22 ^{O}C was chosen.

Water Temperature

Regarding the temperature evolutions, during the survey some temperature variations have occurred due to equipment failure. First, the water heater has stopped working, which leaded to water temperatures towards 19 ^{O}C. Afterwards, after a "successful" fix at the pet shop, the water heater refused to stop at the predetermined temperature and the temperature reached 25 ^{O}C. To conclude, the water heater was finally replaced, and the temperature finally has stabilized to its designated value (Figure 2).

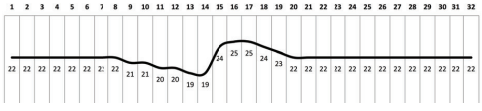

Figure 2. Temperature evolution during the test period

The water heater used was a 300 W Hot 2005 model, with a retail value of 63,99 lei. We weren't able to identify measurable effects of water cooling than heating, except for a adverse reaction (signs of stress) of the flowers when the water was above 23 ^{O}C.

pH

Generally known as "power of hydrogen", pH measure the ratio between hydrogen (H^+) and hydroxil (OH^-) ions in the water, on a scale between 0 and 14 (value of 7 being known as "neutral"). A higher level of hydrogen ions makes the water "acid", with a pH value between 0 and 7, while a higher level of hydroxil ions makes the water alkaline, with a pH value above 7. To establish a successful aquaponic system a proper pH value of water is critical to be identified and kept at all times for all system inhabitants: fish, plants and bacteria. Toward this purpose, the following are to be taken into consideration:

- Fish have an internal pH value of 7,4;
- Bacteria can operate between 6,5 to 8 pH values;
- Plants are on the "acid" part of the scale, around 6.5 value;
- Nitrification processes tend to lower the value of pH (due to the constant release of hydrogen ions in water)

On our system the pH started at a value of 7,8, dropped toward the 7,5 pH value after bacteria colonies were established, with a continuous lowering trend toward the value of 7,2 during the survey period (Figure 3).

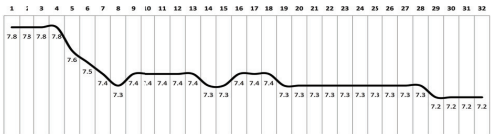

Figure 3. pH evolution during the test period

The "Good" Bacteria

Establishing an aquaponic systems have nothing to do with plants or with fish. Instead, it is all about "growing" bacteria. Not any bacteria, but the so-called "nitrifying" bacteria: ***Nitrosomonas*** and ***Nitrobacter***, the engine of any aquaponic system. They are special because they perform those chemical reactions in the water which make it usable for the plants, and harmless for the fish. To build their own cells they need elements present in the water (oxigen, nitrogen, phosphorus, carbon, potassium and calcium). To be able to use this elements and to run their own metabolism processes, they need energy. In order to get that energy, they drive chemical reactions that release energy. Some of these reactions are the conversion of ammonia to nitrites (NH_3 to NO_2^-) or the nitrites to nitrates (NO_2^- to NO_3^-). The reason for these chemical reactions is not that the bacteria "like" better the nitrogen in nitrites than the nitrogen in ammonia, or that bacteria cares about the welfare of our fishes or plants. The reason is the release of energy that follows the chemical reactions (for example, when the hydrogen ions are replaced by the oxygen atoms).

The first bacteria, ***Nitrosomonas***, is lured by the nitrogen found in ammonia or in other organic amines and, if finds plenty of it, will populate the system. As a result of its action, the ammonia is converted to nitrites (NO_2^-) which, at this stage, worsen the situation for the fish: the nitrites are even more toxic to fish than ammonia. Moreover, because the plants can't feed on the nitrogen found in ammonia or in nitrites, the water becomes even most poisonous for the fish. Fortunately, the nitrites attracts the real "good" bacteria, ***Nitrobacter***, which converts the nitrites to nitrates (NO_3^-). Nitrates are not only harmless for fish but also an excellent source of food for plants. The process of biological oxidation from ammonia to nitrates performed by autotrophic bacteria in the presence of O_2 is known as **nitrification**.

Nitrification Process:

Nitrosomonas:

$2\ NH_3 + 3\ O_2 \rightarrow 2\ NO_2^- + 2\ H_2O + 2\ H^+$

Nitrobacter:

$2\ NO_2^- + O_2 \rightarrow 2\ NO_3^-$

At this point, the water in a successful implementation of an aquaponic system will show:

- as little as possible (less than 0,5 mg/l) ammonia / ammonium: *it means that **Nitrosomonas** feeds on almost all ammonia / ammonium available*;
- traces of nitrates: *it shows that **Nitrobacter** colonies are established*;
- as little as possible (less than 0,5 mg/l) nitrites
- ***Nitrobacter*** *feeds on almost all nitrates available*;
- a slow yet constant drop of pH value, due to the release of hydrogen ions in water as a byproduct of nitrification process.

How the amount of nitrates (NO_3^-) in water help to *asses* an aquaponic system

The amount of nitrates in the water gives value information regarding the equilibrium of the system:

- if the nitrates are very close (but not equal) to 0 mg/l, it means that the system is in equilibrium (no fish or plants are needed to be added or removed) - **the best scenario**;
- if the nitrates are high, it means that more plants have to be added to the system;
- if the nitrates are equal to 0 mg/l, it means:
  - if the nitrites are at normal values (close to zero), the plants need more nitrates than the available amount provided by the bacteria (*more fish have to be added to the system, or some of the plants have to be harvested*);
  - if the ammonia and / or the nitrites are also high, the bacteria cultures are probably dead (*countermeasures are to be taken, or the fish will die. In this case further research is needed to find out what happened. Based of the conclusions of the research the appropriate measures should be taken to stabilize the system further more)*

The System

In the end, a final configuration was chosen and the start-up stages of the aquaponic system were successfully completed (Figure 4). No fish was lost during the setup process.

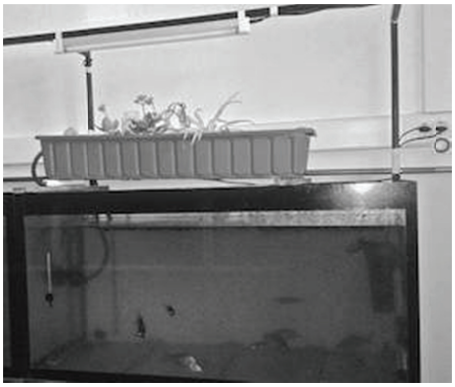

Figure 4. The assessed aquaponic system

RESULTS AND DISCUSSIONS

A correlation may arise between the events which had happened and the values for the measured water quality parameters, easy to be identified on the timeline of this stage of the project (Figure 5).

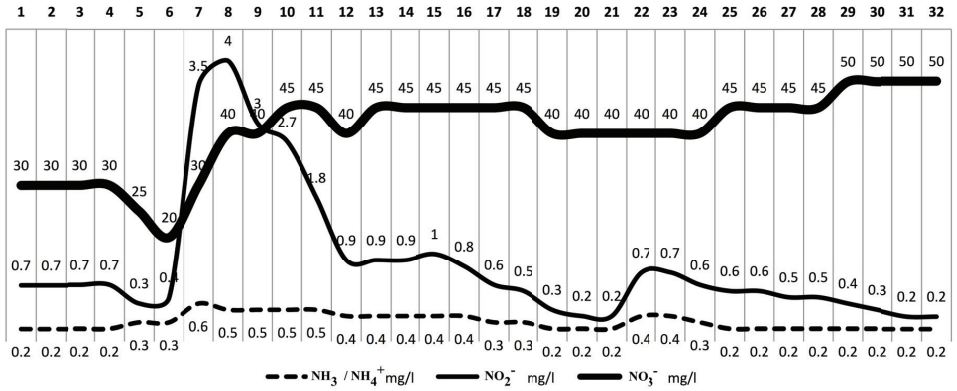

Figure 5. The dynamics of NH_3 / NH_4^+, NO_2^- and NO_3^- concentrations during the test period

- on December 20th - the flower stand and the water pump were added to the system. The pump was set to "*continuous flow*" timing scheme.
- on December 21st - the pump was set to "*flood & drain*" timing scheme (15 min "on" / 30 min "off"). The grow bed was established, and three flower seedlings were placed in plastic mesh pots and secured with hydrotone. The juice from a lime (pH 6,4) was added to lower the pH value.

Discussed results: NO_3^- decreased from 30 mg/l to 20 mg/l between December 21st and 23rd;

Explanation: the seedlings started to feed on NO_3^-;

Discussed results: NH_3 value increased from 0,2 mg/l to 0,3 mg/l, while NO_2^- value decreased from 0,7 mg/l to 0,3 mg/l. The variations on both parameters were around the same margin (50%);

*Explanation: the Citric Acid ($C_6H_8O_7$) in lime juice killed some of the **Nitrosomonas** colonies (responsable to reduce the quantity of NH_3 while providing NO_2^- instead);*

Discussed results: pH value decreased from 7,8 to 7,5

Explanation: Lime juice effect.

- on December 23rd - a new 500 g batch of fishlings was added to the system;

Discussed results: NH_3 value increased from 0,3 mg/l to 0,6 mg/l (on Dec 24th).

Explanation: The new fishes in the system added to the NH_3 level.

Then, on December 25th, **NO_2^- value suddenly jumped from 0,3 mg/l to 3,5 mg/l and soon reached the 4,0 mg/l mark**;

*Explanation: NO_2^- value skyrocketed because a very small amount of **Nitrobacter** colonies were left in the system to break NO_2^-, probably as a late effect of the lime juice.* This situation called for rapid countermeasures, otherwise the fish population could have been lost.

– on December 25th- JBL Denitrol was added to the system, and the NO_2^- value rapidly started to decrease from 4,0 mg/l to 1,0 mg/l (on Dec 29th).

Discussed results: Between December 29th and January 7th, the amounts of NH_3 and NO_2^- decreased steadily, under almost the same gradients;

Explanation: Both bacterial cultures have adapted to the new fish population.

– January 7th: a new 300 g batch of fishlings was added to the system;

Discussed results: increased values of NH_3 and NO_2^- were measured on January 8th and 9th. Afterwards, a steady decrease of NH_3 and NO_2^- values occurred, again, both under a very similar gradient;

Explanation: The bacteria cultures compensated the new conditions;

Other events have had occurred during the project, but apparently they did not caused any measurable changes to water quality parameters:

– December 25th - decrease of pump waterflow;
– December 27th - increase of pump waterflow;
– December 29th - a timed lighting system was added to the system (lights "on" between 6 a.m. and 9 p.m.);
– January 7th - the "flood & drain" timing scheme was altered to 30 min "on" / 30 min "off".

CONCLUSIONS

An aquaponic system is an easy to build system for anyone with some basic practical skills. No special tools or skills are required in the process.

The cost of such a system may vary with the type of the materials used and the scale of the project, but one can setup a basic system for as low as 100 euro.

Labor wise, the system needs more attention during its cycling, when you have to run all the tests on daily bases. However, sampling and testing the water will not require more than one hour per day. Afterwards, on a balanced running system, the tests should be run no more than once a week.

Spare parts are not mandatory, but it is recommended to have some spares always on hand (the water pump is the most obvious one). A spare power source may also be a recommended acquisition, along with a set of "first aid" tools (pH Plus, pH Minus, Denitrol etc.) to be used in case of emergency.

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QUANTITATIVE AND QUALITATIVE EVALUATION OF PHYTOPLANKTON IN THE SITE ROSCI0066 DANUBE DELTA - THE MARIN AREA - A CASE STUDY IN SEPTEMBER 2012

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Abstract

The phytoplankton is a fundamental link in the trophic chain and is one of the indicators to assess the status aquatic ecosystem. To assess the qualitative and quantitative structure of Phytoplankton in September 2012 has been used samples collected from marine area of Danube Delta Biosphere Reserve with plastic water sampler (Niskin type). The qualitative structure of phytoplankton was characterized by the presence of 53 species belonging to six algal taxonomic groups (Bacillariophyta, Dinoflagellata, Chlorophyta, Cyanobacteria, Chrysophyta and Euglenophyta). The diatoms have dominated the number of qualitative structure of phytoplankton species in most areas, their share ranging between 39% and 54%. The dinoflagellates were the second group that diversity, the proportion ranging from 27% and 44%. September 2012 was characterized by values of densities between 67 - 453·10^3 cel·l^{-1} and for biomasses between 0.22 – 1.08 g·m^{-3}. The diatoms have dominated for more than 70% in density and 50% in biomass. The conclusion of this case study, confirmed by previous researches, is that the phytoplankton is not presented as an integral whole viable, but is rather the appearance of a heterogeneous floating tanatocenosis. The site ROSCI0066 Danube Delta - the Marin Area does not have a specific phytoplankton, because the mixed waters from here are always pushed by winds and currents in all directions, especially towards South.

Keywords*: ecosystem, marine water, Natura 2000 sites, phytoplankton.*

INTRODUCTION

ROSCI0066 Danube Delta - the Marin Area besides SCI status, it has the status of the natural protected area in Natura 2000 network and Ramsar and Unesco sit. The ROSCI0066 site is located in the Romanian Black Sea area and covers an area of 121697 ha. It corresponds with the geographical unit of Biosphere Reservation - the Black Sea coast, at the mouth of the Danube - Chilia channel to Cap Midia, to the South, and up to 20 m isobaths, to the East (Figure 1).

The characterization habitats by conducting an inventory of species provides a true picture of the state of preservation and is the basis to achieve an appropriate management of the protected area and halting the loss biodiversity and natural resources. By making an inventory of species and habitats shall ensure the development of a framework for efficient and sustainable management of marine protected area Natura 2000. Through adequate measures can be perform both protection of the natural environment, as well as the conventional livestock by organic farming (Răducuță, 2011).

MATERIALS AND METHODS

The phytoplankton is the link of the aquatic trophic chain, representing the food for microscopic organisms (zooplankton) and higher vertebrate animals (fish). Apart from the positive effect on aquatic life, phytoplankton may also cause inconvenience, as precursor disease or death of aquatic organisms and beyond.

In order to characterize the quantitative and qualitative structure of phytoplankton, the samples have been taken from the Mila 9, Sahalin, Periteașca, Portița and Vadu areas in

September 2012 (Figure 2), using the research ship "Starfish", property of the National Institute for Marine Research and Development “Grigore Antipa” of Constanta.

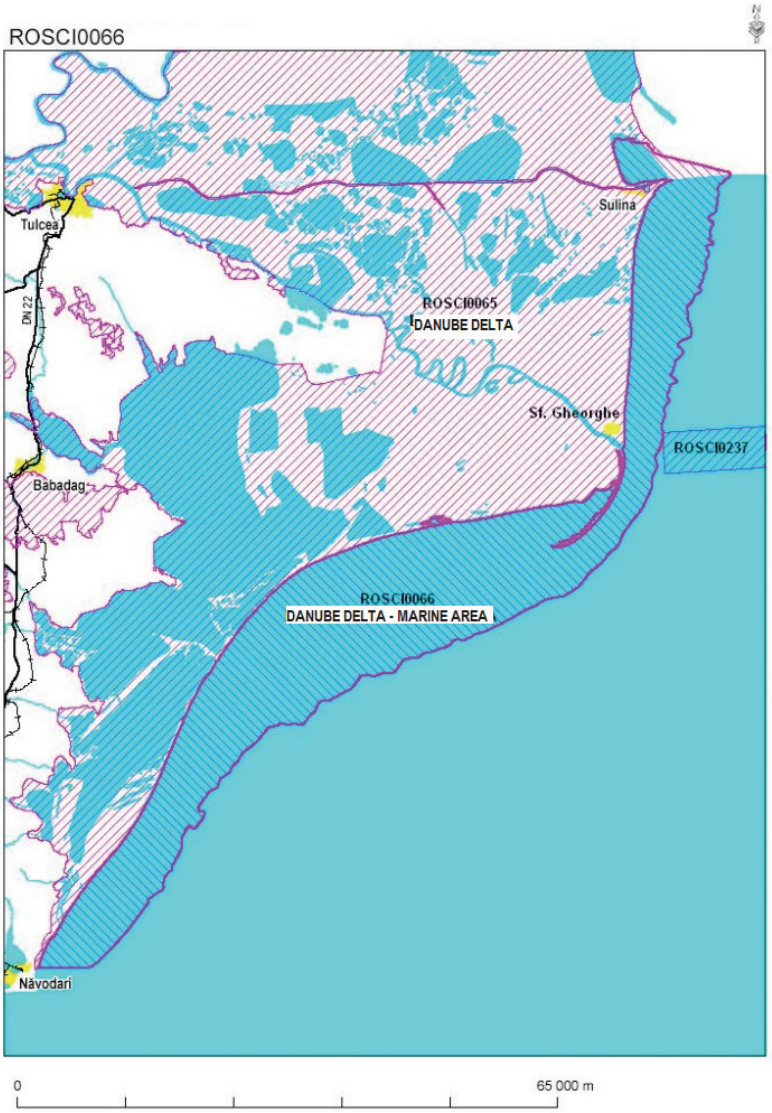
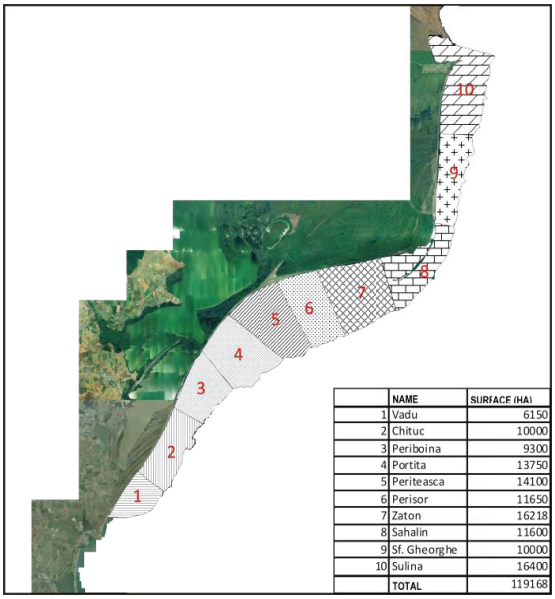


Figure 1. The location of ROSCI0066 Danube Delta - the Marine Area on the hydrographic map of Romania (Source: www.mmsm.usamv.ro, processed picture)

The samples have been collected using plastic water sampler batometers (Niskin type) on each standard horizon as 0, 10, 20 m. Once collecting, phytoplankton samples were stored in plastic containers of 500 ml, preserved with 4% buffered formaldehyde solution, transported to the laboratory and were left to sediment for 2 weeks (Morozova-Vodianitzkaia, 1954; Bodeanu, 1987-88).

In order to microscopic analyze, samples were concentrated by siphoning to a volume of 100 ml. Qualitative and quantitative analysis was performed by extracting from the sample of some subsamples of 5, 10, 20 ml, according to the frequency of species. The determination

and cell counting by species in the analyzed sample fraction was achieved by inverted plankton microscope with 20x and 40x objectives. Depending on primary data obtained, were calculated number density (cells·l^{-1}) and wet biomass (g·m^{-3}) for each specific component for each of algae taxonomic groups and phytoplankton total.



	NAME	SURFACE (HA)
1	Vadu	6150
2	Chituc	10000
3	Periboina	9300
4	Portita	13750
5	Periteasca	14100
6	Perisor	11650
7	Zaton	16218
8	Sahalin	11600
9	Sf. Gheorghe	10000
10	Sulina	16400
	TOTAL	119168

Figure 2. Sampling perimeters of ROSCI0066 (Progress Report no. 8, SOP-ENV Project, CNRS SMIS Code 17162)

RESULTS AND DISCUSSIONS

The phytoplankton is one of the best indicators to assess the status of aquatic ecosystem. Black Sea waters have significant quantities of phytoplankton influenced by variations of environmental factors. Enrichment of specific composition in freshwater forms is due to the influence of fertilizing waters of the Danube, which also going to the low salinity (Boicenco, 2010).

Analyzing the phytoplankton samples has been identified the presence of 53 species of 6 algae taxonomic groups (*Bacillariophyta, Dinoflagellata, Chlorophyta, Cyanobacteria, Chrysophyta* and *Euglenophyta*). The rich represented were diatoms (39%) and dinoflagellates (38%), in relatively equal percentage, followed by cyanobacteria with 11% of total phytoplankton species identified. The most poorly represented in the total number of species identified in specific waters of ROSCI0066 were *Chrysophyta* (6%), *Chlorophyta* (4%) and *Euglenophyta* (2%) (Figure 3).

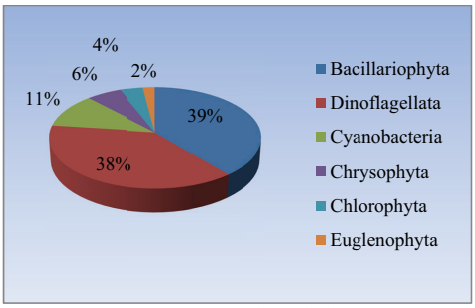


Figure 3. Composition per phytoplankton taxonomic groups in marine waters of ROSCI0066 site

Regarding the classification of species according to their resistance to salinity regime, marine and marine-brackish forms were 83% of the total number of species compared with freshwater and freshwater-brackish forms which do not exceed 17%.

As expected, freshwater populations have the greatest developments in areas in the immediate vicinity of the mouths of the Danube. The quantities there of are reduced in the vertical plane, with increasing depth, and in the horizontal plane as moves away from the source of sweetness, like for Chituc and Vadu (Boicenco, 2013).

In September 2012, the water of ROSCI0066 was characterized by values of densities between 67 - 453·10^3 cells·l^{-1} and for biomasses from 0.22 - 1.08 g·m^{-3}. The biggest share has had diatoms, over 70% in density and 50% biomass. The phytoplankton population was dominated in September, 2012, to the species *Cyclotella caspia* (231·10^3 cells·l^{-1} maximum density), *Cerataulina pelagica* (89·10^3 cells·l^{-1} maximum density) and *Emiliania huxleyi* (258·10^3 cells·l^{-1} maximum density).

The greatest diversity of phytoplankton organisms it was found in Sakhalin (28 species), Mila 9 (26 species) and Periteaşca (25 species) compared to Vadu (20 species) and Portiţa (18 species) (Figure 4).

The waters of Periteaşca and Sakhalin perimeters were characterized by the large quantities of phytoplankton, respectively 453·10^3 cells·l^{-1} şi 1.08 g·m^{-3}. At Periteaşca,

the dominant coccolitoforidee was *Emiliania huxleyi*, which had a maximum density of $258 \cdot 10^3$ cells$\cdot l^{-1}$, representing 62% of the total density. Regarding the biomass, the diatoms had the largest share (74%) and the majority was *Cerataulina pelagica* species.

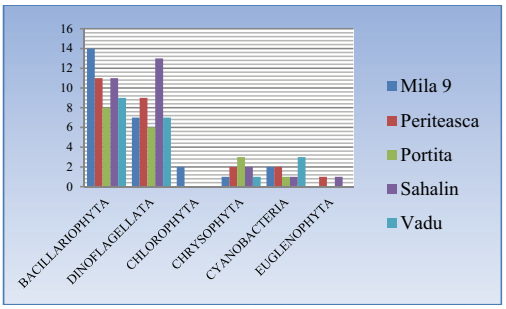


Figure 4. Number of phytoplankton species identified in the study areas, September 2012

In the Sakhalin area, the phytoplankton community was characterized by the dominance in the biomass of dinoflagellates, *Prorocentrum minimum* and *Prorocentrum micans* species reaching highs development to $18.4 \cdot 10^3$ cells$\cdot l^{-1}$, respectively $11.6 \cdot 10^3$ cells$\cdot l^{-1}$. The diatoms have dominated in density having 80% of the total density, the most abundant species as small *Cyclotella caspia* ($230 \cdot 10^3$ cells$\cdot l^{-1}$ maximum abundance). The phytoplankton community in September in Mila 9 and Portița perimeters was characterized by abundance between $124 \cdot 10^3$ cells$\cdot l^{-1}$ şi $170 \cdot 10^3$ cells$\cdot l^{-1}$. The diatoms were noticed by the largest number of species at Mile 9 (14 species), dominating the density (88%) and biomass (61%). Among dinoflagellates were found *Prorocentrum micans, Goniaulax polyedra, Ceratium furca* and *Ceratium fusus* species.

In Vadu perimeter was found weakest phytoplankton development. The diatoms have dominated in density (69%) along with a series of small flagellates, cyanobacteria and dinoflagellates, such as *Phormidium hormoides, Chroococcus minutus* and *Scrippsiella trochoidea*.

CONCLUSIONS

Following the study the conclusions are:

- In the marine site ROSCI0066 has been identified a total of 53 species of planktonic organisms belonging to six algal taxonomic groups (*Bacillariophyta, Dinoflagellata, Chlorophyta, Cyanobacteria, Chrysophyta, Euglenophyta*);
- The diatoms have dominated like number of species qualitative structure of phytoplankton in most analysis perimeters, their share ranging between 39 and 54%;
- The dinoflagellates were the second group of species as diversity, the proportion ranging between 27 and 44%;
- In September 2012, marine waters of ROSCI0066 were characterized by values of densities between 67 - $453 \cdot 10^3$ cells$\cdot l^{-1}$ and biomasses from 0.22 – 1.08 g$\cdot m^{-3}$;
- Changing the structure of phytoplankton in an aquatic ecosystem will cause major changes to the whole trophic chain. It will change the composition of the zooplankton that develops on his behalf and implicitly of fish that feed on phyto-and zooplankton

ACKNOWLEDGEMENTS

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DYNAMICS OF THE PRIMARY PRODUCTION IN ACCUMULATION "STREZEVO" IN THE REPUBLIC OF MACEDONIA

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Abstract

The level and dynamics of the primary production in accumulation "Strezevo" were investigated during the period from March until October 2009. Based on the obtained results from our investigation, the level of the primary production in the accumulation varies and indicates clear seasonal fluctuations. The highest level of the primary production was 9.29 mg/l water and was observed during the summer on the first investigated locality, where the river Shemnica flows into the accumulation, and the lower level was near the floodgate and was from 3.62 to 6.45 mg/l. During the autumn months the primary production level was higher in all profiles, mainly at the beginning of the accumulation and was 12.49 mg/l.

Keywords*: accumulation "Strezevo", primary production, water quality.*

INTRODUCTION

The research of the accumulation Strezhevo employed physico-chemical analysis of the water quality, as well as hydrobilogical, microbiological and ichthyological investigations. The goal of the research was finding a solution to the existing problem with the accelerated eutrophization of the accumulation lake Strezhevo through bilogical way, which means: improving water quality; inclusion of the excessive primary production into regulated processes of matter's circulation; reduction of the risk of unclean water penetration into the water supply system thus enabling permanent supply of quality drinking water for the population of Bitola City and the surrounding villages.

The water from the accumulation primarily used for watering the arable land in Bitola part of Pelagonia, in a great deal enables solving the problem of water supply of Bitola and the surrounding villages with drinking water. The use of the accumulation as a drinking water reservoir in great deal restricts the opportunities for direct action and demands solving the problem in a natural, biological way (without using chemicals and algaecides), with the use of biomanipulation methods. Biomanipulation is a practical biological method applied exactly in solving the eutrophization problem. In the last two decades this is a process that is intensively studied and applied with the goal of suppressing the development of macrophitae vegetation and plankton production in stagnant waters.

Biomanipulation can be defined as restructuring the biological communities with the goal of achieving favorable reaction, most often reduction of algae biomass and getting clean water. The term originally connects more techniques (Shapiro, 1990), however, today it is typically used when it comes to "top-down" manipulation with fish communities. For example, reduction of zooplantophaga and bentivora species or stimulation of piscivora species (Lammenes et al., 1990). Biomanipulation ought to be used in the theoretical context of two extremes of a stable equilibrium, as an extreme perturbation that requires elimination of the phitoplanktonic dominant state. Understanding of the nature and the mechanisms responsible for "turbid" water is crucial (important) if we want the biomanipulation to be successful. In the world literature it is suggested that only by sufficit of information it is possible certain fish community components to be part of biomanipulation. In principle, the goal of the biomanipulation in the accumulations is

creation of a period of clean water in sufficient duration of time. For this purpose, it is the best the biomanipulation to be conducted in winter and in early spring, in order to create clean water as early as possible in the season.

The concept of biomanipulation is ingrained in the ecological theory and is based on the concepts of two alternative stable trophic states (Irvine et al., 1989; Scheffer, 1990; Scheffer et al., 1993). At high concentrations of nutrients the phitoplankton is abundant, the water is turbid, and in the fish community populations of zooplanktivora and/or bentivora species prevail. In Europe these populations are most often ciprinidae like *Rutilus rutilus*, *Alburnus alburnus* and *Abramis brama*. At low concentration of nutrients with high illumination in the clean water the submergeous macrophitae dominate, and in the fish community there is a high percentage of piscivora and planktivora-bentivora fish species, most often *Perca fluviatilis* and *Esox lucius* (Persson et al., 1991; Jeppesen et al., 1997).

In the state of turbidity, zooplanktivora fish directly influence enhancing the phitoplankton by reducing the pass pressure on the phitoplankton (Brooks & Dodson, 1965), whereas bentivora fish can resuspend the sediment and pump phosphorus, which influences development of algae, directly into the water column (Tatrai and Ištvanović, 1986; Breukelaar et al., 1994).

Biomanipulation can have dramatic effects on the ecosystem and not all of them can be predictable (Bendorf, 1992; Meijer et al., 1994a; Moss et al., 1996). Quite often discussions arise as to whether the biomanipulation is efficient or not (Reynolds, 1994). It is considered that there shouldn't be any doubt whether the biomanipulation works or not, but it is quite another question whether it is performable or not in a given situation and how it is performed. Many setbacks in biomanipulation presented in the literature are generally a result of bad planning, inadequate or wrong goal-setting, technical and nonecological limitations, as well as little knowledge of the processes (Martin et al., 1997).

By plannned stocking with fish and catching the fish the nutritive resources can be used rationally, and in the same time it is possible to significantly improve the water quality. In comparison with the other solutions offered this one requires means which are a negligible investment.

The field activities were organized in seasonal intervals, and special attention was paid in the critical months (June, July, August) with the goal of following the dynamics of changes in the accumulation. Also, more attention was paid to the ichthyological investigations oriented to determining: all the fish species that live in the accumulation; the rate of fish growth in length and in weight; the provision with food; the condition and the reproductive characteristics of fish.

MATERIALS AND METHODS

Determination of biomass, i.e. of the primary algae production in Strezhevo accumulation, was performed following standard methods for determining chlorophyll in algae (Standard Methods, APHA, 19th Edition, 1995). The water was being taken from nine points (three points with three profiles – accumulation's middle, and both left and right banks). The samples of 0.5 l of water were concentrated in as short as possible time and the phytoplankton was isolated via membrane filtration using glass filter (Wathman GF/C), then the chlorophyll was extracted into acetone. The filter with the filtrate was macerated and centrifuged at 500 rpm in duration of 20 minutes. Then the extract was decanted and transferred into quartz kivettes for spectrophotometring. The corresponding absorbents with precisely defined wavelengths (662 nm and 644 nm) were read out on the spectrophotometer.

Chlorophyll *a* is a photosynthetic pigment specific to all green plants, both low-life (algae) and high-life (macrophyta). The role of the pigments, in the first place of the chlorophyll, is absorption of light and its transformation into chemical energy, and that's the base of the photosynthesis. The indirect method for determining the biomass i. e. the primary production of the low-life plants (phytoplankton), is determination of chlorophyll *a* whose content serves as a main parameter for algae's biomass.

RESULTS AND DISCUSSIONS

Data of the content of the chlorophyll *a* in the water of the Sterezevo accumulation during summer and autumn season are presented in Table 1.

Table 1. Chlorophyll *a* contents in Strezhevo accumulation (mg/l)

Sites		SI 1	SI 2	SI 3	SII 1	SII 2	SII 3	SII I_1	SII I_2	SII I_3
Season	summer	8.02	9.29	8.12	5.48	4.30	9.29	4.79	6.45	3.62
	autumn	8.97	9.31	9.12	10.21	10.00	10.26	12.29	12.49	12.20

The examined Strezhevo accumulation is an aquatic ecosystem of microphitic type, so that algae i.e. the phytoplankton are the main and only primary producers since macrophytic vegetation is not present at all.

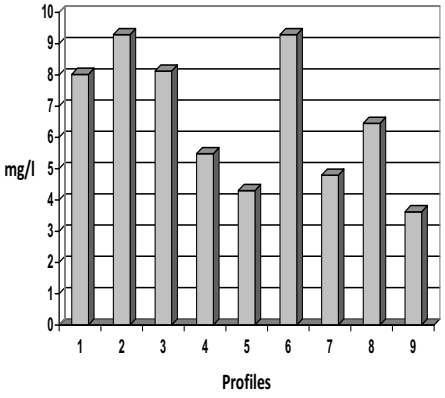

Figure 1. Chlorophyll *a* contents in Strezhevo accumulation in summer

From the Table (or the chart) it is evident that SI profile is of greatest productivity. At this point the river Shemnica flows in carrying various suspended matters of organic origin, this way burdening the accumulation. Here chlorophyll *a* content ranges up to 9.29 mg/l of water. At SII profile the primary production is something lower (5.48 mg/l and 4.3 mg/l) compared to the aforementioned profile, with significantly increased chlorophyll *a* concentration at the point SII3 (9.29 mg/l). The profile SIII, located at the dam, has something lower chlorophyll *a* concentration (from 3.62 to 6.45 mg/l).

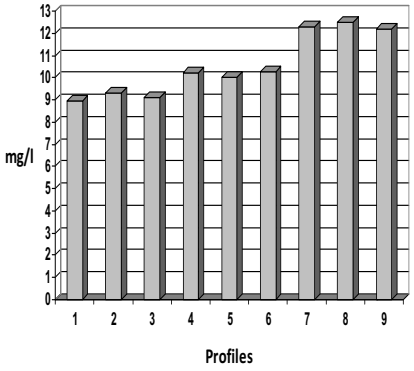

Figure 2. Chlorophyll *a* contents in Strezhevo accumulation in autumn

In the autumn period relatively higher chlorophyll *a* contents were registered on all three profiles, especially at the beginning of the accumulation by the water intake tower where the content of chlorophyll *a* is as high as 12.49 mg/l of water.

CONCLUSIONS

The present study presents results of the dynamics of the primary production in the accumulation Strezevo. In conclusions, the level of the primary production in the accumulation varies and indicates clear seasonal fluctuations. The highest level of the primary production was during the summer on the first investigated locality, where the river Shemnica flows into the accumulation, and the lower level was near the floodgate. During the autumn months the primary production level was higher in all profiles, mainly at the beginning of the accumulation.

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HELMINTH COMMUNITIES AND ECOLOGICAL APPRAISAL FOR THE CONDITION OF THE VELEKA RIVER, BLACK SEA REGION, BULGARIA

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Abstract

Biodiversity and ecological particularities of the parasite communities of the Black Sea Roach (Rutilus frisii (Nordmann, 1840)) and (Alburnus chalcoides (Güldenstädt, 1772)) from the Veleka River (Black Sea Region) were studied during 2013. 59 specimens of R. frisii and 97 specimens of A. chalcoides were examined with standard techniques for parasites and heavy metal contamination. The purpose of this research is to represent new data for the biodiversity, prevalence, intensity and mean intensity, mean abundance of parasite communities of both fish host species. Basic physicochemical indicators in fish, some endohelminth species as bioindicators and bottom sediments were analyzed. The obtained results for the parasite communities of R. frisii and A. chalcoides correspond and are in close connection with dependence of the biology and ecology of the determined species of helminthes and the place of the intermediate hosts as bioindicators for the status of the studied natural freshwater ecosystems. The results may be applied in the various monitoring systems for assessment and forecast of the Veleka River condition.

Key words: *parasite communities, bioindication, Rutilus frisii, Alburnus chalcoides, Veleka River.*

INTRODUCTION

The Veleka River is one of the biggest rivers in the Black Sea Water Basin. Veleka River is part of Sredna Gora Tectonic Zone in Nature Park Strandzha (1995). The geological phenomena that can be seen in the protected area have to do with the effects of the so-called Ahtopol Volcanic Structure from the late Cretaceous (70 million years ago). It is responsible for the formation of some of the most picturesque biotopes found on the Bulgarian Black Sea Coast: the volcanic fiord-shaped shores. The mouth of Veleka River is the most scenic spot on the Bulgarian Black Sea Coast. The waters of the Veleka are rich in flora and fauna, with more than 30 species of freshwater fish (*Anguilla anguilla*, *Cyprinus carpio*, *Rutilus rutilus*, *Atherina hepsetus*, *Alburnus chalcoides*, etc) being present. Five endangered animal species (*Phalacrocorax pygmeus*, *Phalacrocorax aristotelis*, *Cygnus olor*, *Falco vespertinus*, *Branta ruficollis*, *Tadorna tadorna*, *Tursiops truncatus*, *Monachus monachus*, etc.) inhabit the river, as well as important regional plants (*Otanthus maritimus*, *Stachys maritima*, *Nuphar lutea* etc.) (Patronov, 2010). Veleka River is included in the National monitoring program (Water Body Type BG2VEL09R001) (Regulation 1/2011).

Parasite species are interesting as indicators of the ecological status of the freshwater ecosystems. One part of parasitological studies discusses the problem for the health of fish species, but more of examinations are about the relationship between pollution and parasitism in the aquatic environment (Baruš et al., 2007, 2012; Cone et al., 1993; Daei et al., 2009; MacKenzie et al., 1995; Mohammad et al., 2011; Sures et al., 1999; Taraschewski, Sures, 1996; Turčekova, Hanzelova, 1996, 1999; Brázová et al., 2012).

This research presents the results from examinations of helminth communities of freshwater fishes and ecological conditions in the studied biocenoses under the influence of the anthropogenic effects (tourism, constructions, motor boats, logging, poaching, etc.) from the Bulgarian border part of the Veleka River (before town of Sinemorets and Black Sea).

MATERIALS AND METHODS

During June - September, 2013 sediments, fish and fish parasites were collect and examined from the Veleka River (before town of Sinemorets). The Veleka River is 147 km long, of which 123 km are in Bulgaria and 25 km are in Turkey. It rises from Demirkapu Peak in Turkey and crosses the Bulgarian border through beautiful gorges. River Veleka takes it sources from a number of Kartst springs in the Turkish part of the Strandzha (İstranca) mountain and flow into the Black Sea at the Bulgarian village of Sinemorets (42°04'10"N, 27°58'06"E). The river's width near the mouth is from 8 to 10 m and its depth ranges from 2 to 4 m. (Patronov, 2010).

The studied biotopes are situated on the riverside, about 2 km far away south-eastern from the town of Sinemorets (Southern Black Sea, Bulgaria). It is distinguished with a depth and slowly running water, with sands and slimes at some places. The waterside vegetation is represented mainly by *Quercus hartwissiana* Stev., *Salix* sp., *Populus* sp. and *Alnus glutinosa* Linnaeus, 1758, *Smilax excelsa* L., *Periploca graeca* L. etc. The region of river basin and the riverside are distinguished with significant diversity of highly protected species and territories declared as protected with national and international nature protective status (Patronov, 2010; Kirin et al., 2011).

A total of 9 sediment samples and 130 freshwater fish specimens belonging to the species *Rutilus frisii* (Nordmann, 1840) (59 specimens) and *Alburnus chalcoides* (Güldenstädt, 1772) (71 specimens) were collected and examined. The fish were caught by angling. The scientific and common names of fish host was used according to the FishBase database (Fröse and Pauly, 2012). Fish were weighed and measured.

Samples of sediments were collected according to the Guidance on sampling of rivers and watercourses - ISO 5667-6:1990, introduced as a Bulgarian standard in 2002. Heavy metal concentration of the water and sediment samples, fish tissues, organs and parasites were carried out according to standard techniques. The samples were analyzed for content Pb and Zn by atomic absorption spectrometry (Bíreš et al., 1995). Two host-parasite system: The Kutum (*Rutilus frisii* (Nordmann, 1840)), Danube bleak (*Alburnus chalcoides* (Güldenstädt, 1772)) and their nematoda species *Eustrongylides excisus* (Jägerskiöld, 1909) from the Veleka River were chosen as a model fish species and as a model helminth species for parasitological examination and for heavy metal content in this study.

Helminthological examinations were carried out following recommendations and procedures described by Byhovskaya-Pavlovskaya (1985), Moravec (1994, 2001), etc. The parasites, dissected organs and tissues of fish samples were weighed, packed in polyethylene bags and kept at -30 ^{0}C until analysis. The sample of muscle, liver, kidneys and bones were collected from all individuals.

The ecological terms prevalence (P%), mean intensity (MI) are presented for each species. Analyses of helminth community structure were carried out in both levels: infracommunity and component community. The infracommunity data were used to calculate the total number of species, mean number of helminths, etc. (Kennedy, 1993, 1997; Magurran, 1988). The infracommunity data were used to calculate the total number of species, mean number of helminth worms, the Brillouin's diversity index (HB) and evenness index of Brillouin (Maguran, 1988). The analysis of the dominant structure of the found fish parasite taxa were presented to the level of the component communities. The criterion of Bush at al. (1997) was used for determining the dominant structure of the component helminth communities. In order to determine the relative accumulation capability of the fish tissues in comparison to the sediments, bioconcentration factor (BCF=[Chost tissues]/[Csediments]]) were calculated (Sures et al., 1999). The bioconcentration factors were computed to establish the accumulation order and to examine fish for use as biomonitors of trace metal pollutants in freshwater environments. The differences in concentration factors were particularly discussed in respect to the bioavailability of trace metals from sediments.

RESULTS AND DISCUSSION

Fish communities

A total of 130 fish specimens from 2 species were collected and examined from the Veleka River: Kutum (*Rutilus frisii* (Nordmann, 1840) and Danube bleak (*Alburnus chalcoides* (Güldenstädt, 1772). In Bulgaria, Kutum is critically endangered (CR) and in International conservation for this fish species are not enough data (DD=Data Deficient; IUCN Red List Status). *Rutilus frisii* is included in Red Data Book of the Republic of Bulgaria (Golemanski (Ed.), 2011) and Bern Convention, Appendix III. Negatively influencing factors for the decline and the spread of *R. frisii* are water pollution, as well as the rapid expansion of tourism infrastructure along the Black Sea coast, leading to loss of the last habitats of the species in the country, introduction of alien species, which directly affect the species as competitors poaching. Kutum occurs mainly in permanent rivers such as dwell and their estuaries. *R. frisii* feeds on algae and small invertebrates (Golemanski, 2011).

Danube bleak is endangered fish species in Bulgaria EN but with no enough data in the world (DD; IUCN). *Alburnus chalcoides* is included in Red Data Book of the Republic of Bulgaria (Golemanski, 2011), in Bern Convention, Appendix III and in Habitats Directive, Appendix II. The species occurs mainly in permanent rivers, but also in estuarine, coastal brackish and freshwater lakes and permanent freshwater marshes. It feeds on zooplankton, insects and small fish. Negatively influencing factors for the decline and the spread of *A. chalcoides* are: pollution and increasing eutrophication, intensive development along the Black Sea coast, introduction of alien species affecting directly the species as competitors and poaching.

Helminth community structure

From the both fish hosts (Kutum (*Rutilus frisii* (Nordmann, 1840) and Danube bleak (*Alburnus chalcoides* (Güldenstädt, 1772)) a total 63 specimens of helminths *Eustrongylides excisus* (Jägerskiöld, 1909) were fixed. They are belonging to class Nematoda and family Eustrongylidae. *Eustrongylides excisus* (Jägerskiöld, 1909), larvae is developed with participation of the first intermediate host oligochets (blackworm *Lumbricus variegatus* Linnaeus, 1758, sludge worm *Tubifex tubifex* (Müller, 1774), *Limnodrilus* sp.) and the second fish species, amphibians (Marsh frog, *Pelophylax ridibundus* (Pallas, 1771) (=*Rana ridibunda* Pallas, 1771) and reptiles (Dice snake, *Natrix tesselata* (Laurenti, 1768)). The adult nematodes parasitic in the glandular stomach of cormorants (Great Black Cormorant *Phalacrocorax carbo* (Linnaeus, 1758) and Pygmy Cormorant *Microcarbo pygmeus* (Pallas, 1773) (=*Ph. pygmaeus* Pallas, 1773)) (Moravec, 1994). In Bulgaria, the species is presented of *Sander lucioperca* (Linnaeus, 1758) (=*Lucioperca lucioperca* Linnaeus, 1758) (as paratenic host) and of *Gobius* sp. (as intermediate host) of *Aspius aspius* (Linnaeus, 1758) from the Danube River (Kakacheva, Margaritov, Grupcheva, 1978; Margaritov, 1959); of *P. fluviatilis* from the Zhrebchevo Reservoir (Nedeva, Grupcheva, 1996) and from the Srebarna Lake (Hristov, 2010; Shukerova, Kirin, 2007; Shukerova et al., 2010); of *Silurus glanis* Linnaeus, 1758; *Lota lota* (Linnaeus, 1758), *Neogobius melanostomus* (Pallas, 1814) (=*Neogobius cephalarges* Pallas, 1814), *N. kessleri* (Günter, 1861), *P. fluviatilis* from the Danube River (Atanasov, 2012), etc.

E. excisus, which use fish as intermediate hosts represented the allogenic species for the helminth communities of the examined freshwater fish species of the Veleka River ecosystem. *E. excisus* of the parasite communities of *A. chalcoides* and of *R. frisii* of the Veleka River were distinguished with high values of prevalence (P=40.84% and P=28.81%, respectively) but with lower value of mean intensity for *E. excisus* (MI=1.47±0.14, 1-3, SE Mean 0.14, C.V. 49.79; MI=1.17±0.39, 1-2, SE Mean 0.09, C.V. 33.40, respectively). The two helminth species are component species of the helminth communities of the Kutum and Danube bleak, respectively.

Content of heavy metals in sediments, fishes and parasites

Table 1. Content of heavy metals (Cmg/kg±SD) of *R. frisii* and *E. excisus*

R. frisii	Veleka River	
	Pb	Zn
C $C_{E.excisus}$	24.12±0.27	163.33±0.31
BCF	1.92	0.31
C_{Skin}	2.98±0.23	41.22±0.10
$C_{Skin}/C_{Sediments}$	0.24	0.08
$C_{E.excisus}/C_{Skin}$	8.09	3.96
C_{Bones}	2.88±0.12	39.75±0.47
$C_{Bones}/C_{Sediments}$	0.23	0.07
$C_{E.excisus}/C_{Bones}$	8.37	4.11
$C_{Muscles}$	0.85±0.03	13.62±0.40
$C_{Muscles}/C_{Sediments}$	0.07	0.03
$C_{E.excisus}/C_{Muscles}$	28.37	11.99
Sediments mg/kg	12,55±0.35	534,22±1.23

Bioconcentration factor (BCF=[Chost/parasite tissues]/[Csediments])

Table 2. Content of heavy metals (Cmg/kg±SD) of *A. chalcoides* and *E. excisus*

A. chalcoides	Veleka River	
	Pb	Zn
$C_{E.excisus}$	32.5±0.29	321.22±0.11
BCF	2.59	0.60
C_{Skin}	4.31±0.16	57.42±0.25
$C_{Skin}/C_{Sediments}$	0.97	0.11
$C_{E.excisus}/C_{Skin}$	7.54	5.59
C_{Bones}	4.12±0.02	52.03±0.08
$C_{Bones}/C_{Sediments}$	0.33	0.09
$C_{E.excisus}/C_{Bones}$	7.89	6.17
$C_{Muscles}$	1.07±0.04	17.89±0.16
$C_{Muscles}/C_{Sediments}$	0.09	0.03
$C_{E.excisus}/C_{Muscles}$	30.37	17.96
Sediments mg/kg	12,55±0.35	534,22±1.23

Bioconcentration factor (BCF=[Chost/parasite tissues]/[Csediments])

The result of the chemical analyzes (Pb and Zn) of samples of muscle, skin, bones of *Alburnus chalcoides* and *Rutilus frisii* of the Veleka River were presented (Tables 1 and 2). The content of Pb and Zn in the parasite species *Eustrongilides excisus* was determined. The content of heavy metals in sediments was fixed. Based on the results of chemical analyzes, mean concentrations (mg/kg) in tissues, organs of the fish, parasites and sediments, as well as the bioconcentration factor (BCF = [Chost/parasite tissues]/[Csediments]) were defined (Table 1 and 2).

The highest mean content of Pb are defined in *E. excisus* from *A. chalcoides* (32.5 mg/kg), followed by those in *E. excisus* from *Rutilus frisii* (24.12) and in the sediments (12.55 mg/kg). Of tissues and organs, higher concentrations were obtained for the content of Pb in skin and bones (4.31 and 4.12 mg/kg, respectively). The mean content of Zn showed higher values in the sediments (534.22 mg/kg) than in *E. excisus* from *A. chalcoides* (321,22 mg/kg) and from *Rutilus frisii* (163.33). Of tissues and organs, the highest content was detected for skin (C_{skin}=57.42 mg/kg and 41.22 mg/kg for the both fish species, respectively), followed by those for bones (C_{bones}=52.03; C_{bones}=39.75 mg/kg, respectively). The lowest values of Zn are detected in the muscles of examined fish samples (C_{musles}=17.89 mg/kg and C_{musles}=13.62 mg/kg, respectively) (Table 1).

BCF of *E. excisus*, parasite species of *A. chalcoides* and *Rutilus frisii* of the Veleka River was the highest for Pb ($BCFC_{E.excisus}/C_{SedimentsPb}$=2.59 and $BCFC_{E.excisus}/C_{SedimentsPb}$=1.92). With regard to the examined fish tissues and organs, BCF was the highest for Pb in skin ($BCF_{skin/sedimentsPb}$=0.97 and $BCF_{skin/sedimentsPb}$=0.24) and for Zn in skin ($BCF_{skin/sedimentsZn}$=0.33 and $BCF_{skin/sedimentsZn}$=0.24). BCF was with the lowest values for the two trace heavy metals for fish muscles.

Accumulation of heavy metals in *E. excises* to their content in the fish organs and tissues was the highest of Pb from the muscles ($BCF_{E.excisus/musclesPb}$=30.37 and $BCF_{E.excisus/musclesPb}$=30.37, respectively), followed by those of Pb for bones ($BCF_{E.excisus/lbonesPb}$=7.89 and $BCF_{E.excisus/lbonesPb}$=8.37), of Pb for skin ($BCF_{E.excisus/skinPb}$=7.54; $BCF_{E.excisus/skinPb}$=3.96). Generally, the accumulation of the two trace heavy metals were the highest of fish parasite species *E. excisus*, compared to their contents in muscles.

As a result of this study (Tables 1, 2), the content of Zn was the highest in the sediments of the Veleka River (C_{Zn}=534.22 mg/кg, respectively) and the content of Pb was the highest in *E. excisus* (321.22 mg/кg; 163.33

mg/кg). With regard to organs and tissues, the content was the highest for lead and zink in the samples of skin (C_{skinPb}=4.12/2.98 and C_{skinZn}=57.42/41.22 mg/кg, respectively).

CONCLUSIONS

As a result of this examination a total of 130 fish specimens from 2 species were collected and examined from the Veleka River. *E. excisus*, parasitic in *A. chalcoides* and *R. frisii* is allogenic species. The received data for heavy metal contents in sediments, fish tissues and organs and fish parasites from the Veleka River were presented for the first time for both examined fish species and their parasites *E. excisus*. The highest mean content of Pb are defined in *E. excisus*, followed by those in the sediments. Of tissues and organs, higher concentrations were obtained for the content of lead in skin. Generally, the accumulation of the two trace heavy metals were the highest of fish parasite species *E. excisus*, compared to their contents in muscles of the two fish species, respectively. The high values of the bioconcentration factors and of the significant correlations determined *E. excisus* as sensitive bioindicator for lead.

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HELMINTH COMMUNITIES AND HEAVY METAL CONTAMINATION IN MACEDONIAN VIMBA AND FISH PARASITES OF THE MARITSA RIVER, BULGARIA

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Abstract

Biodiversity and ecological particularities of the parasite communities of the Macedonian Vimba (Vimba melanops (Heckel, 1837)) from the Maritsa River were studied during 2013. Thirty two specimens of V. melanops were examined with standard techniques for parasites and heavy metal contamination. The purpose of this research is to represent new data for the biodiversity, prevalence, intensity and mean intensity, mean abundance of parasite communities of V. melanops from the Maritsa River. Concentration of heavy metals in fish (muscle, liver, intestines and bones), some endohelminth species as bioindicators and bottom sediments were analyzed. The obtained results for the parasite communities of V. melanops correspond and are in close connection with dependence of the biology and ecology of the determined species of helminthes and the place of the intermediate hosts as bioindicators for the status of the studied natural freshwater ecosystems. The results may be applied in the various monitoring systems for assessment and forecast of the Maritsa River condition.

Keywords: *parasite communities, heavy metals, bioindication, Vimba melanops, river Maritsa.*

INTRODUCTION

The Maritsa River is related to the Aegean water collecting region. The Tunja and Arda rivers are its chief tributaries. The lower course of the Maritsa River forms part of the Bulgarian-Greek border and most of the Greek-Turkish border. The upper Maritsa valley is a principal east-west route in Bulgaria (Dakova et al., 2004).

The unnavigable river is used for power production and irrigation. The major negative anthropogenic impact on the Maritsa River ecosystem associated with the changes of the studied freshwater communities are farm activities, constructions, industry, etc. Maritsa River is included in the National monitoring program (Water Body Type BG3MA350R039 – Major rivers) (Regulation 1/2011).

Parasite species are particularly interesting as indicators of the ecological status of the freshwater ecosystems because the completion of their life cycle requires interactions with several host vertebrates and invertebrates, and the effects on each of the hosts differ according to the pollution level of the habitat in question (Baruš et al., 2007; Cone et al., 1993; Gelnar et al., 1997; Kennedy, 1997; MacKenzie et al., 1995; Marcogliese and Cone, 1997; Overstreet, 1997; Sures, Siddall, 1999; Thielen et al., 2004; Tieri et al., 2006, etc.).

Different studies show higher concentrations of heavy metals in some intestinal fish helmithes than in tissues and organs of their final host Macedonian Vimba (*Vimba melanops* (Heckel, 1837)). In Gabrashanska and Nedeva (1996) examined the contents of Cu, Cr and Zn in the host parasite system *Vimba melanops* – *Caryophyllaeus brachicollis* and report for higher concentrations of determined heavy metals in cestods than in the fish host. Kirin et al., 2013 reports the higest values of Cu, Pb and Zn in helminths *Caryophyllaeus laticeps* than in muscles in its host Macedonian Vimba (Arda River, Bulgaria), etc.

This paper presents the results from an examination of heavy metal content in sediments, fish tissues and organs, fish parasites and dominant structure of fish parasite communities from the Bulgarian part of the Maritsa River (after town of Plovdiv).

MATERIALS AND METHODS

During April - September, 2013 sediments, fish and fish parasites were collect and examined from the Maritsa River (after town of Plovdiv). The Maritsa River is with a length of 521 km and is the longest river that runs solely in the interior of the Balkans. It has its origin in the Rila Mountains (2°09'40"N, 23°36'00"E, 2378 m altitude, from Maritsa lakes and below Peak Mancho) in Western Bulgaria, flowing southeast between the Balkan and Rhodope Mountains, past Plovdiv to Edirne, Turkey and to Aegean Basin (41 m above sea level) (Dakova et al., 2004).

The studied biotope (village of Manole, 42.183N, 24.933E) is situated on the riverside, about 18 km far away north-eastern from the town of Plovdiv (42.15N, 24.75E). It is distinguished with a depth and speady running water, with sands and slimes at some places. The waterside vegetation is represented mainly by *Salix* sp., *Populus* sp. and *Alnus glutinosa* Linnaeus, 1758. The region of the town and the riverside are distinguished with significant diversity of highly protected species and territories declared as protected with national and international nature protective status (Assyov, 2012; Kirin et al., 2006).

A total of 15 sediment samples and 69 freshwater fish specimens belonging to the species *Vimba melanops* (Heckel, 1837) were collected and examined. The fish were caught by angling. The scientific and common names of fish host was used according to the FishBase database (Fröse and Pauly, 2012). Fish were weighed and measured.

Samples of sediments were collected according to the Guidance on sampling of rivers and watercourses - ISO 5667-6:1990, introduced as a Bulgarian standard in 2002. Heavy metal concentration of the water and sediment samples, fish tissues, organs and parasites were carried out according to standard techniques. The samples were analyzed for content of Cu, Pb and Zn by atomic absorpsion spectrometry (Bíreš et al., 1995). The Macedonian Vimba *Vimba melanops* (Heckel, 1837) from the Maritsa River and its cestoda species *Caryophyllaeus brachycollis* Janiszewska, 1951 were chosen as a model fish species and as a model helminth species for parasitological examination and for heavy metal content in this study.

Helminthological examinations were carried out following recommendations and procedures described by Byhovskaya-Pavlovskaya (1985), Dubinina (1987), Gusev (1983, 1985), Moravec (1994, 2001), etc. The parasites, dissected organs and tissues of fish samples were weighed, packed in polyethylene bags and kept at -30 ^{0}C until analysis. Samples of muscle, liver, kidneys and bones were collected from all individuals.

The ecological terms prevalence (P%), mean intensity (MI) are presented for each species. Analyses of helminth community structure were carried out in both levels: infracommunity and component community. The infracommunity data were used to calculate the total number of species, mean number of helminths, etc. (Kennedy, 1993, 1997; Magurran, 1988). The infracommunity data were used to calculate the total number of species, mean number of helminth worms, the Brillouin's diversity index (HB) and evenness index of Brillouin (Maguran, 1988). The analysis of the dominant structure of the found fish parasite taxa were presented to the level of the component communities. The criterion of Bush at al. (1997) was used for determining the dominant structure of the component helminth communities. In order to determine the relative accumulation capability of the fish tissues in comparison to the sediments, bioconcentration factor (BCF=[Chost tissues]/[Csediments]]) were calculated (Sures et al., 1999). The bioconcentration factors were computed to establish the accumulation order and to examine fish for use as biomonitors of trace metal pollutants in freshwater environments. The differences in concentration factors were particularly discussed in respect to the bioavailability of trace metals from sediments.

RESULTS AND DISCUSSION

Fish communities

A total of 69 fish specimens from the species *Vimba melanops* (Heckel, 1837) were collected and examined from the Maritsa River. *V. melanops* is included in Red Data Book of the Republic of Bulgaria (Golemanski (Ed.), 2011) as Vulnerable species (VU=Vulnerable). It is demersal fish species.

Macedonian Vimba distributed Northern Aegean basin from Maritza to Pinios drainages (Turkey, Greece, Bulgaria, Macedonia). Inhabits rivers and streams, usually with relatively swift current. Also occurs in lakes and lowland water courses with little current. Feeds on invertebrates and plants. Spawns among stones in large rivers (Kottelat, Freyhof, 2007).

Helminth community structure

A total 79 specimens of helminths belonging to species *Caryophyllaeus brachycollis* Janiszewska, 1951 (Platyhelminthes, Cestoda, Caryophyllidea) were fixed from 36 infected fish speciment.

Caryophyllidean (Platyhelminthes:Eucestoda) parasites represent a widely distributed group of intestinal helminthes of Cyprinidae and Siluridae fishes occurring in all zoogeographical regions except the Neotropics. Some cariophyllideans may be pathogenic for their fish hosts (Mackiewicz, 1994; Oros et al., 2010; Scholz and Hanzelová, 1998).

Caryophyllaeus brachycollis Janiszewska, 1951 is developed with participarion of the first intermediate host *Limnodrilus hoffmeisteri* Claparède, 1862 and *T. tubifex* and the second different fish secies. In Bulgaria caryophyllidean tapeworms were presented from different fish species and freshwater ecosystems: as *C. laticeps* (Pallas, 1781) - of *Barbus barbus* (Linnaeus, 1758) (Margaritov, 1959; 1966) from the Danube River; of *B. cyclolepis* Heckel, 1837 (Margaritov, 1959) from the Iskar River; of *A. alburnus* (Margaritov, 1959) of the Tunja River; of *B. barbus*, *Vimba carinata* (Pallas, 1814), *Abramis brama* (Linnaeus, 1758) and *Ballerus sapa* (Pallas, 1811) (=*A. sapa* Pallas, 1811) (Kakacheva, Margaritov, Grupcheva, 1978) from the Danube River; of *Leuciscus cephalus* (Linnaeus, 1758) (Cakic et al., 2004) from the Danube River; as *C. fennica* (Schneider, 1902) – of *B. barbus* (Margaritov, 1959; 1966) from the rivers Iskar and Tunja; of *B. cyclolepis* and *L. cephalus* (Margaritov, 1959) from the Iskar River; of *L. cephalus*, *V. melanops* and *B. cyclolepis* (Margaritov, 1963/64) from the rivers Maritsa and Topolnitsa; of *B. cyclolepis*, *L. cephalus*, *V. melanops* (Kakacheva, 1965) from the rivers Asenitsa, Harmanlijska, Topolnitsa, Syuyutlijka, Sushenitsa and Bedechka; of *B. barbus* and *S. lucioperca* (Margaritov, 1966) from the Danube River; of *B. petenyi* (Kakakcheva, 1969) from the rivers Nishava, Ogosta, Vodomerka, Buchinska, Vrabnishka, Barsiya, Chuprenska, Iskrecka, Botunya, Bebresh; of *L. cephalus* and *R. rutilus* (Margaritov, 1977) from the Shiposhnitsa River and Reservoir Iskar; of *V. carinata*, *A. brama*, *B. sapa*, *Ballerus ballerus* (Linnaeus, 1758) (=*Abramis ballerus*), *Blicca bjoerkna* (Linnaeus, 1758), *A. alburnus*, *B. barbus*, *S. lucioperca*, *Sc. erythrophthalmus* and *Pelecus cultratus* (Linnaeus, 1758) (Kakacheva, Margaritov, Grupcheva, 1978) from the Danube River; of *L. cephalus* and *R. rutilus* (Kakacheva, Menkova, 1978) from the Palakariya River; of *B. barbus* (Kakacheva, Menkova, 1981) from the Struma River; of *B. cyclolepis*, *A. alburnus*, *Sq. orpheus*(=*L. cephalus*) (Kirin, 2002b, 2003) from the Arda River; of *L. cephalus* (Cakic et al., 2004) from the Danube River; as *C. brachycollis* Janiszweska, 1953 - of *B. cyclolepis* and *L. cephalus* (Kakacheva, 1965) from the rivers Asenitsa, Sjujutlijka, Chepinska, Bedechka and Topolnitsa; of *L. cephalus*, *V. melanops*, *A. alburnus*, *B. cyclolepis*, *R. rutilus* (Margaritov, 63/64) from the rivers Maritsa, Vacha, Chepinska, Topolnitsa, Bistritsa; of *L. cephalus*, *Barbus petenyi* Heckel, 1852 and *B. barbus* (Kakacheva, 1969) from the rivers Vrabnishka, Nishava, Mirkowska, Botunya, Ogosta, Malak iskar; of *L. cephalus* (Kakacheva, Menkova, 1978) from the Palakariya River; of *B. petenyi*, *L. cephalus* (Kakacheva, Menkova, 1978) from the rivers Devinska, Sarneshka and Vacha; of *B. petenyi*, *B. barbus*, *L. cephalus* (Kakacheva, Menkova, 1981) from the rivers Blagoevgradska Bistritsa, Struma, Zheleznitsa and Gradevska; of *P. fluviatilis* (Nedeva, Grupcheva, 1996) from the Zhrebchevo reservoir; of *B. cyclolepis*, *A. alburnus*, *Sq. orpheus*(=*L. cephalus*) (Kirin, 2002b, 2003) from the Arda River; and of *L. cephalus* (Cakic et al., 2004) from the Danube River; as *Caryophyllaeus* sp. – of *L. cephalus* and *A. alburnus* (Kakacheva, 1965) from the rivers Maritsa, Syuyutlijka and Harmanlijska; of *Cyprinus carpio* Linnaeus, 1758 (Margaritov, 1975, 1976) from the Fish Farming–Yambol; of *C. carpio* (Kakacheva,

Menkova, 1981) from the Fish Farming–Blagoevgrad; of *V. melanops* (Kakacheva, 1965) from the Harmanlijska River; of *Cobitis bulgarica* (Drensky, 1928) (Margaritov, 1966) from the Danube River; as as *Caryophyllaeus* sp. juv. - of *Gobio gobio* (Linnaeus, 1758), *B. cyclolepis*, *V. melanops* (Kakacheva, 1965) from the river Maritsa, Chepinska and Harmanlijska; of *B. petenyi* (Kakacheva, Menkova, 1978) from the Palakariya River; of *C. carpio* (Margaritov, 1992) from the Fish farms-Yambol, Blagoevgrad, etc.

C. brachycollis of *V. melanops* of the Arda River were distinguished with prevalence P=55.35% and mean intensity MI=2.19±1.95, 1-9. *C. brachycollis* is a core species of the helminth communities of the Macedonian Vimba.

Content of heavy metals in sediments, fishes and parasites

The result of the chemical analyzes (Pb, Cu and Zn) of 60 samples of muscle, liver, kidneys and bones of *Vimba melanops* of the Maritsa River were presented (Table 1 and 2). The content of Pb, Cu and Zn in *Caryophyllaeus brachycollis* was determined. The content of heavy metals in sediments was fixed. Based on the results of chemical analyzes, mean concentrations (mg/kg) in tissues, organs of the fish, parasites and sediments, as well as the bioconcentration factor (BCF=[Chost/parasite tissues]/[Csediments]) were defined (Table 1, 2).

Table 1. Content of heavy metals (Cmg/kg±SD) of *Vimba melanops* and *Caryophyllaeus brachycollis*

Vimba melanops	River		
	Cu	Pb	Zn
$C_{C.brachycollis}$	52.04± 1.02	72.15± 1.26	22.31± 1.16
C_{Liver}	36.64± 1.18	3.76± 0.07	7.77± 1.38
C_{Kidney}	3.26± 1.15	4.81± 0.02	28.31± 0.20
C_{Bones}	8.61± 0.29	5.26± 0.22	6.25± 0.03
$C_{Muscles}$	1.26± 0.12	0.12± 0.02	2.21± 0.09
Sediments mg/kg	109.2± 2.95	35.88± 1.23	69,22± 1.33

The highest mean content of Cu showed the sediment samples of river (109.2 mg/кg), followed by those of the parasite species *R. acus* (52.04 mg/кg). From fish tissues and organs, with the highest content of Cu were distinguished the liver (36.64 mg/кg). The highest mean content of Pb are defined in *C. brachycollis* (72.15 mg/kg), followed by those in the sediments (35.88 mg/kg). Of tissues and organs, higher concentrations were obtained for the content of Pb in bones and kidneys (5.26 and 4.81 mg/kg, respectively). The mean content of Zn showed higher values in the sediments (69.22 mg/kg) than of *R. acus* (32.31 mg/kg). Of tissues and organs, the highest concentrations were differed of Zn. The highest content of this trace heavy metal was detected for kidneys (C_{kidney}=28.31 mg/kg), followed by those for liver and bones (C_{liver}=7.77; C_{bones}=6.25 mg/kg, respectively). The lowest values of Zn are detected in the muscles of examined vimba (C_{musles}=2.21 mg/kg) (Table 1).

Table 2. Bioconcentration factor of *Vimba melanops* and *Caryophyllaeus brachycollis*

Vimba melanops BCF	Arda River		
	Cu	Pb	Zn
$C_{C.br.}/C_{Sediments}$	0.476	2.020	0.322
$C_{Liver}/C_{Sediments}$ $C_{C.br.}/C_{Liver}$	0.335 1.420	2.011 19.188	0.112 2.871
$C_{Kidney}/C_{Sediments}$ $C_{C.br.}/C_{Kidney}$	0.029 15.963	0.134 15.00	0.409 0.788
$C_{Bones}/C_{Sediments}$ $C_{C.br.}/C_{Bones}$	0.079 6.044	0.146 13.716	0.090 3.569
$C_{Muscles}/C_{Sediments}$ $C_{C.br}/C_{Muscles}$	0.012 41.302	0.003 601.25	0.032 10.095
Sediments mg/kg	109.2± 2.95	35.88± 1.23	69,22± 1.33

BCF of *C. brachycollis*, parasite species of *V. melanops* of the Maritsa River was the highest for Pb (BCF $C_{C.br}/C_{SedimentsPb}$=2.020), followed by those for Cu (BCF $C_{C.br.}/C_{SedimentsCu}$=0.476) and Zn (BCF $C_{C.br.}/C_{SedimentsZn}$=0.322) (Table 2). With regard to the examined fish tissues and organs, BCF was the highest for Cu in liver ($BCF_{liver/sedimentsCu}$=0.335), followed by those for Zn in kidneys ($BCF_{kidneys/sedimentsZn}$=0.409) and for Pb in bones ($BCF_{bones/sedimentsPb}$=0.146). BCF was with the lowest values for the tree trace heavy metals for vimba's muscles. Accumulation of heavy metals in *R. acus* to their content in the fish organs and tissues was the highest of Pb from the muscles ($BCF_{C.br./musclesPb}$=601.25), followed by those of Pb for liver ($BCF_{C.br./liverPb}$=19.188), of Pb for

kidneys and of Pb for bones ($BCF_{R.acus/kidneysPb}$ = 14.923; $BCF_{R.acus/bonesPb}$ = 15.0). Generally, the accumulation of the tree trace heavy metals were the highest of fish parasite species *C. brachycollis*, compared to their contents in muscles.

CONCLUSIONS

As a result of this examination a total of 69 fish specimens *Vimba melanops*, collected from the Maritsa River. The determined helminth species *Caryophyllaeus brachycollis* is a core species for the helminth communities of *Vimba melanops* from the studied ecosystems.

The received data for heavy metal contents in sediments, fish tissues and organs and fish parasites from the Maritsa River were presented for the first time for *V. melanops* and parasite species *C. brachycollis*. The highest mean content of Pb was defined in *C. brachycollis* (72.15 mg/kg) than these in sediments, tissues and organs. From tissues and organs, the highest concentrations were obtained for the content of Cu in liver of the vimba. Generally, the accumulation of lead was higher of fish parasite species *C. brachycollis*, compared to their contents in sediments. The high values of the bioconcentration factors and of the significant correlations determined *C. brachycollis* as sensitive helminth species for heavy metal (lead, copper and zinc) content of freshwater ecosystems.

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ACCUMULATION OF LEAD (Pb) IN *SCARDINIUS ERYTHROPHTHALMUS* AND *CERATOPHYLLUM DEMERSUM* FROM FRESHWATER ECOSYSTEM BIOSPHERE RESERVE SREBARNA, BULGARIA

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Abstract

Concentration of heavy metals in Scardinius erythrophthalmus (L., 1758) found in Biosphere Reserve "Srebarna" (Northeastern Bulgaria) was examined in 25 fish individuals from every age group and season. The concentration of heavy metals was investigated by mineralization and subsequent atomic absorption spectrophotometry (according to the requirements of the methodology of the pollutants). To ensure the comparability of results the following standards were complied: 1. Sample collection and storage - ISO 5667-3/4:1995; etc. Fish sampling and analysis were carried out in compliance with National Program for Biomonitoring of Bulgaria (MEW, 1999). Particular the concentrations of Pb in Scardinius erythrophthalmus, Ceratophyllum demersum (L.), sediments and water were studied. Bioconcentration factor (BCF=C fish tissues;macrophytes/C water;sediments) for Pb in the tissues of fish and macrophytes were estimated and discussed. Biomagnification factors (BMF=C fish tissues/C macrophites) for Pb in the tissues of fish were estimated and discussed. The chemical analysis showed accumulation of Pb mainly in the fish bones as compared to the accumulation of the respective metal in the fish muscles. Biomagnification factors of Pb in Scardinius erythrophthalmus compared to Ceratophyllum demersum were evaluated. The mean concentration of Pb in the muscles of Scardinius erythrophthalmus was established to greatly exceed the reference values for lead in fish meat from Regulation № 31 of 29 July 2004 on the maximum levels of pollutants in food.

Keywords**:** biomagnification, fish, macrophyte, rudd, trace metal

INTRODUCTION

The circulation of heavy metals in the lake ecosystem has not been a subject of detailed and systemic studies. There is evidence of heavy metal content in sediments from 5 monitoring points of BR “Srebarna” for the period 2004 – 2005. These analyzes were carried out in close connection with the monitoring program on the territory of the reserve. The only published data are those on the content of heavy metals in sediments of Srebarna Reserve and the Bulgarian section of the Danube River (Ricking and Terytze, 1999). The content of heavy metals in the major species macrophytes (*Tipha phragmites*) of Lake Srebarna was monitored by Yurukova and Kochev (1996). These studies indicated exceeding by minimum the admissible levels of macroelements that provokes and motivates once again the interest in studying their circulation in freshwater ecosystems of Srebarna reserve.

Aquatic macrophytes are good indicators of water quality because of their exceptional abilities to accumulate chemical elements as the increased accumulation of individual elements in plant tissues may be associated with increased concentrations in the aquatic environment (Yurukova and Kochev, 1996).

The studies of Pajević et al. (2003a, b) and Borišev et al. (2006) showed *Ceratophyllum demersum* as a macrophyte with a great potential for accumulation of Pb.

Arnaudova et al. (2008) studied the accumulation of Pb, Zn and Cd in three fish species perch, rudd and bleak from Studen Kladenets and Kyrdzhali dams. In the organs and tissues of the fish species *Alburnus alburnus, Scardinius erythrophtalmus* and *Perca fluviatilis* were observed high amounts of Pb, Zn and Cd, exceeding the limit concentrations for these metals. The accumulation of heavy metals varied among the different fish species. The highest content of the three metals was detected in *S. erythrophtalmus.*

Information on the fish fauna of Srebarna Lake can be found in publications since the beginning of the XX century (Antipa, 1909;

Ivanov, 1910; Kovatchev, 1922; Morov, 1931; Bacescu, 1942; Drensky, 1951). The first detailed study of the ichthyofauna of Srebarna Lake was made by Bulgurkov (1958), which reported 31 species of fish. Assay of the content of heavy metals (Cu, Pb, Zn, Cd, Mn, Co, Ni и Fe) in the silt layer in one meter depth (Hristova, 2000) showed no reasonable contamination of the lake with trace metals.
Comprehensive analysis of burden on the aquatic ecosystem hasn't been carried out. The aim of the current study was to determine the concentration of Pb in the dominant species of fish fauna and the presence of Pb in the elements of the nutrient spectrum of the studied species.

MATERIALS AND METHODS

Srebarna Biosphere Reserve is situated in Northeastern Bulgaria (44°7'N, 27°5'E) and it includes the Srebarna Lake (Figure 1). The Srebarna Lake is a freshwater eutrophic lake connected through an artificial canal with Danube River and is characterized by a significant diversity of highly protected species (Michev et al., 1998; Uzunov et al., 2001; Pehlivanov et al., 2006).
For the period of the present study seasonal changes of the water level have been established. High water levels have been constituted during the spring due to the influx of water from Danube River and lower water level – during the following months.
The model fish species chosen for this study was the herbivorus fish, rudd *S. erytrophtalamus* L., 1758. The rudd is one of the most abundant fish species in the Biosphere Reserve Srebarna (Pehlivanov, 2000; Pehlivanov et al., 2005). The fishes were caught by nets in the lake.

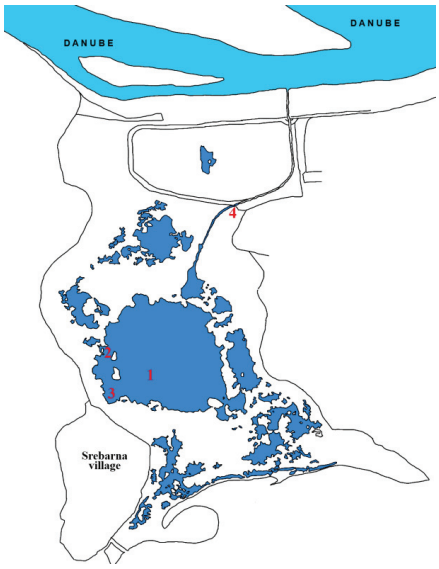

Figure 1. Srebarna Lake (source Map Google, 2009, with reductions)

Fish material, macrophytes, water and sediments were collected within 2010 year at three times during each of the spring (22 March-22 June), summer (22 June-22 September) and fall (September 22 to December 22). For the content of Pb 9 samples of *Ceratophyllum demersum* L., 8 samples of water and 8 samples of sediments were tested. A total 225 individuals of *S. erythrophthalmus* were examined (Table 1). The fish were weighed and measured. Samples of muscles, bones and skin were collected from all individuals.

Table 1. Characteristics of the fish studied - *Scardinius erythrophthalmus*

age	number of fish	Body weight (g)				Total length (cm)				Width (cm)			
		Range	Mean	SD	SE	Range	Mean	SD	SE	Range	Mean	SD	SE
1+	75	8.00-12.50	10.60	1.33	0.34	8.80-10.80	9.93	0.66	0.17	2.00-2.30	2.15	0.10	0.03
2+	75	16.50-38.00	24.93	6.36	1.64	9.70-12.90	11.97	0.94	0.24	2.90-4.10	3.41	0.28	0.07
3+	75	48.00-164.00	66.04	31.96	8.25	13.00-16.50	13.86	1.09	0.28	4.00-6.30	4.40	0.65	0.17

Determination of the age of the studied freshwater fishes was based on a standard method (Moyseev et al., 1975). The studied fish specimens were divided into 3 groups according to their age (Tables 1 and 2).
Each fish was determined to the species using the definers by Drensky (1951) and Karapetkova and Zhivkov (1995, 2000). For the systematic determination of fish species were used additional databases: GISD, ITIS, Fauna Europaea (http://www.faunaeur.org/), FishBase (http://www.fishbase.org/).
The fish weight was measured in grams and its dimensions in centimeters. For each studied

fish specimen were examined the following parameters (Zashev and Margaritov, 1966): Total length (L, cm) from the tip of the snout to the end of the beams of the caudal fin; Standard length (l, cm) from the tip of the snout to the end of caudal peduncle/ beginning of the caudal fin; Maximum height (H, cm), Weight (W, g), Sex and Age.

The material for the study was collected from four locations of the lake ecosystem (Figure 1): 1-Central water surface of the lake, 2-Puddle "Ribarnika", 3-"Below the village" and 4-South gateway.

The water samples were collected and when necessary were preserved following the hereafter mentioned standards and regulations: BSS EN 25667-2:2000; ISO 5667-3:1994; ISO 5667-4:2002. Two-liter sealed containers were used and were delivered to the laboratory for chemical analysis. Each sample was recorded and numbered in advance. Samples that could not be tested the same day were stabilized or preserved by the addition of 5 ml nitrogen acid or 1 ml sulfur acid to every 1 l of water sample. For short period preservation samples were cooled down to 4°C.

The sediment samples were isolated with the use of Bottom Sediment Grab Sampler of Ekman. They were collected in accordance with the regulations of BSS EN ISO 5667-15:2009. The sediments were kept in a temperature of 4°C until they were analyzed.

Samples of tissues from the examined fish were deeply-frozen (-25^{o}C) and posterior processed for trace element analysis. Samples of muscles, bones and skin (1 g) were dried under temperature of 110^{o}C and mineralized in the combustion mixture consisted of perchloric and nitric acid in ratio 3:2 and then burned. The concentration of heavy metal Pb was established by atomic absorption spectrophotometry (Bíreš, at al., 1995) (Table 2). Samples were analyzed in the Laboratory of atomic absorption spectrophotometry at IBER on AAS "Perkin-Elmer" - 3030 B.

The differences in concentration factors were particularly discussed in respect to the bioavailability of trace metals from macrophytes. A linear correlation coefficient, r_s was used to test the associations between sediments, water, fish tissues and macrophytes.

For the calculations and analysis were used MS Excel and BioDiversity Pro Softwares.

Table 2. Mean concentration of Pb (mg/kg) in muscles, bones and skin of *Scardinius erythrophthalmus* in age groups

age	number of fish	Muscles			Bones			Skin		
		Range	Mean	SD	Range	Mean	SD	Range	Mean	SD
1+	75	1.09-10.42	5.31	3.34	1.21-13.12	7.15	3.47	1.51-5.94	3.64	1.62
2+	75	0.86-7.40	3.70	1.72	0.36-11.53	4.87	2.70	0.18-3.65	2.20	0.87
3+	75	0.76-7.43	3.94	2.50	1.43-9.21	4.53	2.42	1.01-10.10	3.98	3.04

RESULTS AND DISCUSSIONS

The content of Pb in water and sediments of the freshwater ecosystem of the Lake Sreberna was established (Table 3). The results obtained in the present study for the content of Pb in water (35.00 µg/l) were higher than the values obtained by Yurukova and Kochev (1996), Hiebaum et al., (2012) for Lake Srebarna.

Table 3. Mean concentration of Pb in water (µg/l) and sediment (mg/kg) from Lake Srebarna

Elements	Water (µg/l)	Sediments (mg/kg)
Pb	35.00	28.29

The high values of the studied elements in the water of Lake Srebarna were thought to be due to the passage of contaminated water from Danube River. The problem with the burden of the ecosystem could be related to the improper use of different fertilizers and pesticides as well as the improper agricultural activities in the surrounding land used for agricultural needs. The high concentrations of Pb in *C. demersum* showed that there was a chance that they were present there from the surrounding agricultural areas indicated by Pajević et al. (2003a).

The content of Pb in sediments exceeded the element content in the water of Lake Srebarna. The values obtained from previous studies (Yurukova and Kochev, 1996) were similar and slightly lower than those in the present study. When comparing the content of heavy metals in sediment samples from Lake Srebarna by Hiebaum et al. (2000) with the results of this

study it can be seen minor increase in the content of Pb over the years (Table 4).

Table 4. Mean concentration (mg/kg) of Pb in sediments from Lake Srebarna

	Year	Pb
1	1999	35.855
	2002	41.707
	2006	15.25
2	1999	20.716
	2002	26.213
	2006	17.817
3	1999	26.056
	2002	29.438
	2006	16.527
X	**2010**	**28.29**

Legend: 1-3 points of comparison from Hiebaum and etc. (2012); X – data from the present study.

In all researches regarding the content of elements in Lake Srebarna (Hiebaum et al., 2012; Ricking and Terytze, 1999, others) the presence of technogenic pollution of the upper sediment layer of the lake was highlighted. Similar results were presented by a number of authors who studied Danube's basin (Milenkovic et al., 2005; Vignati et al., 2013; Vítek et al., 2012, etc.).

The accumulation of Pb in *Ceratophyllum demersum* was highest in spring followed by autumn and summer, with typical similar mean values respectively 23.46 mg/kg, 21.93 mg/kg and 21.52 mg/kg (Figure 2).

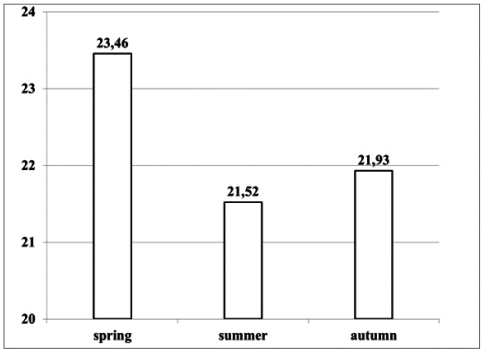

Figure 2. Seasonal variations in the concentration of Pb (mg/kg) in *Ceratophyllum demersum*

The mean values of concentration of Pb in *C. demersum* in this study (22.31 mg/kg) corresponds to the highest levels of accumulation of macrophyte survey of Predrag et al. (2006) (23.0 mg/kg) for chemical analysis of dominant aquatic macrophytes from DTD canal (Vlajkovac locality).

Studies on heavy metal content in macrophytes (Pajević et al., 2006) showed approximately 4-times lower levels of accumulation compared to the present study. The studies of Pajević et al. (2003a, b, 2005) and Borišev et al. (2006) showed the macrophyte *C. demersum* as a great potential for accumulation of Pb. The current study showed 4.5-times higher accumulation of Pb when compared to Borišev et al. (2006). The study of Jamnická et al. (2006) confirmed the high levels of accumulation of Pb in *Ceratophyllum demersum* determined in this study.

The content of Pb in *C. demersum* from Lake Srebarna were up to 8 times higher than the values obtained by Yurukova and Kochev (2000) at the same location.

Mean concentrations of Pb in *C. demersum* were very close to the studies of Branković et al. (2009) for the accumulation of heavy metals in different macrophytes of lake ecosystems.

Differences in the accumulation of Pb in the tissue of the *S. erythrophthalmus* were established. In general the accumulation of Pb in rudd followed the trend bones>muscles>skin (Figure 3).

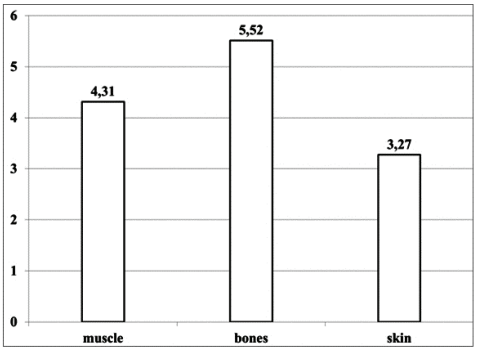

Figure 3. Mean concentration of Pb (mg/kg) in the tissues of *Scardinius erythrophthalmus*

In contrast to the results of the present study Arnaudova et al. (2008), Çiçek et al. (2009) and Sevcikova et al. (2013) established lowest concentrations of Pb in the muscles of *S. erythrophthalmus*. However, in the current study were found much higher mean values of Pb compared to study of Çiçek et al. (2009) (0.32 mg/kg).

We found that the accumulation of Pb differed in distinct age groups. Most Pb was accumulated in the youngest species (Figure 4), they were followed by 3+ and 2+ age groups where it was detected the lowest accumulation of Pb.

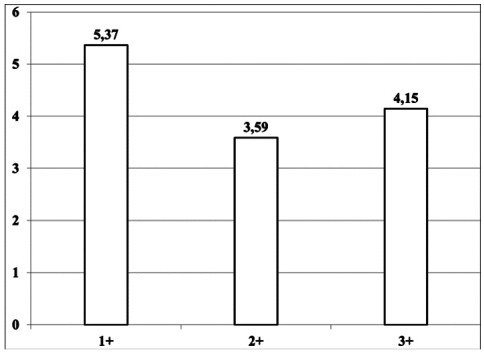

Figure 4. Mean concentration of Pb (mg/kg) at different age of *Scardinius erythrophthalmus*

The concentration of Pb in muscles varied between age groups. Age group 1+ had the highest measured values of accumulation. There was at least accumulation of Pb in age group 2+. The concentration of Pb in bones followed the same trend and it was highest in age group 1+, but the lowest concentration was in age group 3+ (the largest one). Overall, accumulation of Pb in bones was 2 times greater than in muscles (Table 2, Figure 5).

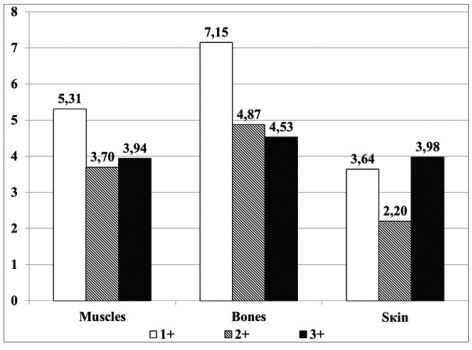

Figure 5. Mean concentrations of Pb (mg/kg) in tissues and organs in different age groups *S. erythrophthalmus*

Evaluation of the concentrations of Pb in different tissues and organs with respect to the age of the fishes showed that highest accumulation of Pb was in 1+ age group in the bones (7.15 mg/kg), followed by accumulation of Pb in 1+ age group in muscles (5.31 mg/kg). However, in the skin was detected higher level of Pb in 3+ age group than in 1+ age group.

Evaluation of the concentrations of Pb in the different tissues and organs with respect to the season showed that most Pb was accumulated in bones in spring (Figure 6). The second high value was again in bones but in autumn. In skin was detected the lowest content of Pb in autumn. The muscles accumulate Pb mostly in autumn.

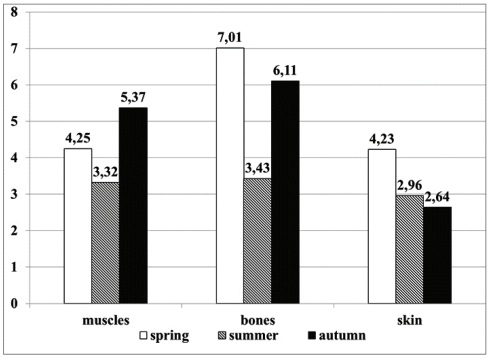

Figure 6. Seasonal variations in the concentration of Pb (mg/kg) in *Scardinius erythrophthalmus*

The concentration of Pb in *S. erythrophthalmus* from Biosphere Reserve Srebarna was higher than several other surveys on the accumulation of heavy metals in rudd (Kalyoncu et al., 2012; Özparlak et al., 2012).

Al Sayegh Petkovšek et al. (2012) who studied the accumulation of heavy metals in fish from the Šalek lakes, found repeatedly less accumulation of Pb in the muscle (0.02 mg/kg) compared to our study (4.31 mg/kg). The study of Sevcikova et al. (2013) also determined that the muscles accumulate in the least Pb to other organs, the average concentrations of Pb in muscles *S. erythrophthalmus* from Skalka reservoir are several times lower (0.05 mg/kg) than those of rudd from Srebarna .

The correlation (correlation coefficient of Spearman, r_s) between the content of Pb in the environment - water and sediments and dominant taxa of the biota of the lake ecosystem *S. erythrophthalmus* and *C. demersum*, as well as the factors of bioconcentration and biomagnification were all studied.

Positive correlations (r_s) to the content of Pb in macrophytes, water and sediments were found (Table 5).

Table 5. Correlation coefficient Spearman (r_s) and the levels of importance of the content of Pb in *S. erythrophthalmus, C. demersum,* water and sediments

x-y	r_s	p
S. erythrophthalmus - *C. demersum*	0.65***	<0.001
S. erythrophthalmus – water	0.21*	<0.05
S. erythrophthalmus – sediments	0.12*	<0.05
C. demersum – water	0.47**	<0.01
C. demersum - sediments	0.22*	<0.05

Note: *significant correlation p<0.5;
**highly significant correlation p<0.01;
***very significant correlation p<0.001;
ns non-significant correlation p>0.05.

The calculated values of bioconcentration factor (BCF) in *S. erythrophthalmus* and *C. demersum* were high compared with those for water (Table 6).

Table 6. Values of bioconcentration factor (BCF=C tissue /C water, sediments) for Pb to *S. erythrophthalmus* and *C. demersum* compared to their content in the water and sediment

BCF	Water	Sediments
S. erythrophthalmus	124.00	0.15
C. demersum	637.14	0.79

The mean values of BMF were the highest for bones and range as follows BMFPbbones>> BMFPbmuscle >>BMFPbskin (Table 7).

Table 7. Mean values of Biomagnification factor (BMF=C host tissues/C macrophytes) of Pb in *S. erythrophthalmus* compared to *C. demersum.*

Srebarna Lake	Fish tissues	BMF Pb
Scardinius erythrophthalmus	muscle	0.194
	bones	0.248
	skin	0.147

CONCLUSIONS

Increase of the content of Pb in sediments compared to previous studies of Lake Srebarna was found. The content of Pb in waters was up to 10 times higher than previous studies of the lake ecosystem. Seasonal fluctuations in the content of Pb in *Ceratophyllum demersum*, related to vegetation and development of plants were found. High values of BCF in *C. demersum* compared to the water and sediments were due to the high sensitivity of the species to Pb in that area. *Ceratophyllum demersum* is a reliable biomarker for monitoring the degree of contamination of aquatic ecosystems with Pb. The data obtained testify to the application of the studied macrophytes as a good indicator for biomonitoring of pollution with Pb.

Lake ecosystem is loaded with high content of Pb, caused by technogenic pollution of the upper layer of the lake sediment mainly from waters entering from the Danube River.

The mean concentration of Pb in the muscles of *Scardinius erythrophthalmus* was established to greatly exceed the reference values for lead in fish meat from Regulation № 31 of 29 July 2004 on the maximum levels of pollutants in food.

The ways in which Pb enters in *S. erythrophthalmus* are from the food sources, water and sediment.

Considering our results from the study of accumulation of Pb in *Scardinius erythrophthalmus* and *Ceratophyllum demersum* in Srebarna Biosphere Reserve we can conclude that the lake is burdened with heavy metals compared to other freshwater ecosystem.

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