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SUMMARY

GENETICS AND BREEDING

Biological characteristics and pathogenicity of avian *Escherichia coli* strains from albanian poultry flocks - SHTYLLA Tana, CIRCELLA Elena, MADIO Anna, BOCI Jonida, ÇABELI Pranvera, KUMBE Ilirian, CAMARDA Antonio 11
Genetic investigations using immuno-biochemical markers in a black spotted cattle population - Nicoleta IŞFAN, Tomița DRĂGOTOIU, Monica MARIN, Ştefania DOROBANŢU, Dana POPA, Carmen Georgeta NICOLAE ……..... 15
More about on local differentiation of Albanian local sheep populations - Lumturi PAPA, Kristaq KUME, Fehmi XHEMO 20
Animal biodiversity conservation, a key of sustainable agriculture. Case study: The romanian Pinzgau breed in Transilvania region - Răzvan POPA, Dana POPA, Marius MAFTEI, Dorel DRONCA, Vasile BĂCILĂ 25
Muscle content in pig carcasses of different genotypes - Ilie ROTARU, Serghei SECRIERU 30

NUTRITION

Effect of use of premix in dairy cows - Ylli BIÇOKU, Valbona YLLI 37
Achievement, testing and evaluation a mathematical model to optimize swine nutrition - Radu BURLACU, C. NIȚU, Ioana RĂDULESCU …….. 42
Growth and development of broiler chickens under the use of the adsorbents "Primix-Alfasorb" and the probiotics "Primix-Bionorm-K" in mixed fodders - Larisa CAISÎN, Alexei KOVALENKO, Ludmila BIVOL, Natalia GROSU 46
Assessment nutritional value and efficiency for use of a new source of vegetable fodder - Sergiu COŞMAN, Mihail BAHCIVANJI, Valeriu COCIU, Valentina COŞMAN, Sava BĂLĂNESCU 53
Determination of romanian alfalfa crude protein and crude fiber contents as well as *in vitro* organic matter digestibility by nir spectrometry - Laura M. DALE, André THEWIS, Ioan ROTAR, Christelle BOUDRY, Roxana VIDICAN, Anamaria MALINAS, Vasile FLORIAN, Bernard LECLER, Richard AGNEESSENS, Juan A. FERNÁNDEZ PIERNA, Vincent BAETEN 57
The effect of Kombucha tea on egg fat and meat fat in laying quails *(Cortunix cortunix japonica*) - Lovita ADRIANI, Tuti W., Elvia H., Endang S., Hendronoto A.W. Lengkey, S. Darana 63
Effect of feed on the basis of soybean in pig nutrition - Dragan MILIĆ, Vladislav STANAĆEV, Ana MARJANOVIĆ - JEROMELA, Vidica STANAĆEV, Nikola PUVAČA, Sava ZARIĆ ……. 67
Effect sof feeding different levels of guar meal on performance and blood metabolites in Holstein lactating cows - Morteza Salehpour, Khosro Qazvinian and Vasco A. P. Cadavez 73
Contribution of both soluble and insoluble fractions of untreated and treated *Acacia saligna* and *Leucaena leucocephala* with different levels of urea to rumen fermentation, *in vitro* - NASSER MOHAMED EMAD ABD EL-WAHAB 78
Ochratoxins - fooder contaminants an impact on animals and human health - Cătălina POSEA, Alexandru ŞONEA, Alin BÎRȚOIU, Monica ROMAN, Mihaela VASILE 87

Research on the activity of complex mixes of foliar fertilizers and herbicides on the nutrition value of fodder - R. TARAŞ, D. DRĂGOTOIU, Lucica NISTOR, Camelia HODOŞAN …….. 90
Study on the action efficiency of the mixes of herbicides and fertilisers in the corn crops - R. TARAŞ, D. DRĂGOTOIU, Lucica NISTOR, Camelia HODOŞAN ……. 94
The influence of supplementary feeding of lambs concerning their body weight at the end of the lactation - Nicoleta TEODORESCU (ROŞU), Ion CĂLIN, Rodian Paul TĂPĂLOAGĂ, Ion ROŞU 96
THE YIELDING CHARACTERISTIC OF SENTUL CHICKENS FED DIET CONTAINING PAPAYA LEAVES MEAL (*Carica papaya* L. Less) - T. Widjastuti, E. Sujana, S. Darana 99

REPRODUCTION, PHYSIOLOGY, ANATOMY

Some typical symptoms of mulberry silk worm poisoning with the neonicotinoid insecticides C*onfidor* and A*ctara* - Krasimira AVRAMOVA, Dimitar GREKOV, Rumyana IVANOVA, Hristo HRISTEV 107
A comparison of duck and chicken egg yolk for cryopreservation of egyptian buffalo bull spermatozoa - Ibrahim El-Shamaa, El-Shenawy El-Seify, Ahmed Hussien, Mohamed El-Sherbieny and Mohamed El-Sharawy 109
Effect of low density lipoproteins in extender on freezability and fertility of egyptian buffalo bull semen - El-Sharawy, M. E.; El-Shamaa, I. S.; Ibrahim, M.A.R.; Abd El-Razek, I.M.; El-Seify, E.M. 114
The interrelation between the reproductive performance and the dairy productivity level of the Moldavian Black Spotted cattle breed population of "south" subtype - Vera GRANACI, Alisa MORARI-PÎRLOG 121
The management of water state in glycerinated rat heart the role of 1H NMR spectroscopy - Bogdan Paltineanu, Cosmin Sonea, Cătălina Pena, Flory Revnic, Cristian Romeo Revnic 127
The management of calcium balance in rat skeletal, cardiac and vascular smooth muscle function the role of calcitonin - Bogdan Paltineanu, Alexandru Sonea, Catalina Pena, Flory Revnic, Cristian Romeo Revnic 131
Essay on estimation of undemonstrative spare outputs disclosed by reproduction biotechnologies in sheep breeding - Marcel Theodor PARASCHIVESCU, Alexandru ŞONEA, Alexandru BOGDAN, Marcel PARASCHIVESCU, George Florea TOBĂ 134
The management of ^{45}Ca uptake in skeletal, cardiac and smooth muscle tissue in rats of different ages. the impact of acute and long term treatment with D3 vitamin - Flory Revnic, Bogdan Paltineanu, Cosmin Sonea, Cristian Romeo Revnic, Cătălina Pena 140
Analyze of reproduction activity in dairy cows in Vrancea region - Paul TĂPĂLOAGĂ, Dana TĂPĂLOAGĂ, Alexandru ŞONEA, Iuliana NEAGU, Monica MARIN 142

TECHNOLOGIES OF ANIMAL HUSBANDRY

Bee colonies comfort in different types of hives - Valentina CEBOTARI, I. BUZU 149
State and priorities of livestock in private households - S. CHILIMAR …….. 154

The effect of algal suspension "Clorella Vulgaris" using in artificial raising of queens - Nicolae EREMIA, Mihail BAHCIVANJI, Andrei ZAGAREANU 158
Organoleptic, chemical and microbiological quality of table eggs obtained in different exploitation systems for laying hens in Romania - Anca-Maria GALIŞ, Ilie VAN, André THÉWIS 162
Policy of knowledge management in universities. From theory to practice - João GOUVEIA, Tudor STANCIU, Jan SJOLIN 167
THE EFFECT OF STORAGE TIME IN DIFFERENT TEMPERATURE ON NATIVE CHICKEN EGG HAUGH UNIT AND YOLK INDEX - Hendronoto Arnoldus W. Lengkey, Tuti Widjastuti and Sjafril Darana 173
Correlations between phenotypic associations Hb/K and quantitative production traits in the Botosani Karakul sheep - Gheorghe HRINCĂ, Petru Gabriel VICOVAN 176
Water buffalo for our next generation in Egypt and in the world - MOSTAFA ABDEL RAHMAN IBRAHIM 183
Study about the productive charachteristics of quails from the "Baloteşti" population - Lucian IONIŢĂ, Elena POPESCU MICLOŞANU, Ioan CUSTURĂ, Minodora TUDORACHE 193
Preliminary results about the effect of storage period on the hatching process of the hen eggs - Mihai PÎRVULEŢ, Elena POPESCU-MICLOŞANU, Cristina PÎRVULEŢ, Ioan CUSTURĂ, Ilie VAN, Minodora TUDORACHE 198
Quality evaluation of wells water from Teleorman county - Dana POPA, Cristiana DIACONESCU, Răzvan POPA, Marius MAFTEI, Andra ŞULER 205
Study on the hourly frequency of exploring and social behavioral manifestations in dogs housed in a specialized shelter in relationship with the sex proportion and animals age in the group - Elena POPESCU-MICLOŞANU, Carmena ŞERBĂNOIU 208
Comparative study about production and slaughtering performances in an industrial company with ROSS 308 standard chicken hybrid - Carmen Viorica RADU, Elena POPESCU- MICLOŞANU 214
Research on the sheep breeding in organic farming system in Romania - Ion RĂDUCUŢĂ 219
The evolution and current situation of goat breeding in Romania - Ion RĂDUCUŢĂ, Ion CĂLIN, Şerban PURDOIU 223
Hydrolysis of sago (Metroxylon Sago Rottb.) pith powder by sulfuric acid and enzyme and fermentation of its hydrolyzate by *Pichia stipitis* CBS 5773, *Saccharomyces cerevisiae* D1/P3Gi, and *Zymomonas mobilis* FNCC 0056 into bioethanol - Ratu Safitri, Bambang Marwoto, Fenny Firstianty, Jetty Nurhajati 227
Study of magnetic field and ultraviolet activation in geese eggs hatching - Elena SCRIPNIC, Suzana MODVALA 235
Researches concerning the organization of a craft manufactory for processing unreeling silk cocoons - Viorica SLĂDESCU, Georgeta DINIŢĂ, Claudia MUŞAT, Alexandra MATEI 239
Horse heart rate values at different times of training, recorded immediately after exercise and 10 minutes after exercise - Eugenia SOVAREL, Paula POSAN ... 244
The techno-economy dynamic system on broiler farming industries in West Java region - Taslim Dawan, Sjafril Darana 247
Study on unit cost of certificate-type broilers - Minodora TUDORACHE, Ilie VAN, Ioan CUSTURĂ, Elena POPESCU-MICLOŞANU, Antoaneta POPA 250

Rabbit general anesthesia for cataract surgery using cisatracurium as neuromuscular blocking drug. Case study - Alexandru Cosmin TUTUNARU, Alexandru ŞONEA, Charlotte SANDERSEN …… 255
Determination of Zerdava dog (Kapi Kopegi) raised in northeast of Turkey - Orhan YILMAZ, Mehmet ERTUGRUL 258

TECHNOLOGIES OF THE AGRO FOOD PRODUCTS PROCESSING

Effects of cooking methods on the heavy metal concentrations in the fish meat originating from different areas of Danube river - Cristiana DIACONESCU, Laura URDEŞ, Ştefan DIACONESCU, Dana POPA …… 265
Study regarding the sensorial and physico-chemical characteristics of certain wines produced in Romania - Gratziela-Victoria BAHACIU, Lucica NISTOR, Daniela BĂDESCU 268
The contaminated milk and influences on human health - Nela DRAGOMIR ... 272
The influence of using different proteolytic enzymatic produces over the characteristics of flour breading products - Monica MARIN, Dumitru DRĂGOTOIU, Carmen NICOLAE, Georgeta DINIŢĂ, Paul Rodian TĂPĂLOAGĂ, Dana TĂPĂLOAGĂ, Nicoleta ISFAN 276
Scientific evidences that pig meat (pork) is prohibited for human health - MUHAMMAD FIAZ QAMAR, IFRAH RAZA 281
The quality of meat in some hybrides pig - Consuela ROIBU, Ionuţ BEIA, Răzvan POPA, Dana POPA 287
Effect of thawing methodson physical characteristic and chemical composition of rib eye meat ongole crossed – Kusmajadi SURADI, Azeisha Diena RAHMANI, Maria Sri HARTATI, Nani DJUARNANI 290
Robust regression models for predicting the lean meat proportion of lambs carcasses - Cristina XAVIER, Vasco CADAVEZ 296

WILD LIFE MANAGEMENT, FISHERY AND AQUACULTURE

Preliminary results on the lower Danube sturgeon migration monitoring - Alin Marius BĂDILIŢĂ, Bogdan TĂNASE, György DEÁK, Marius RAISCHI, Radu SUCIU, Ştefan HONŢ, Ştefan DIACONESCU, Carmen NICOLAE 301
Research on sperm quality of north american sturgeon species *Polyodon spathula* - Mioara COSTACHE, Cecilia BUCUR, Daniela RADU, Daniel OPREA, Mihail COSTACHE, Carmen Georgeta NICOLAE 306
Study concerning the evaluation of the genetic determinism of some bioeconomic and ecoeconomic characters in Ineu carp breed - Carmen Georgeta NICOLAE, Horia GROSU, Monica MARIN, Mioara COSTACHE, Nicoleta IŞFAN, Victor NIŢĂ 312
Study concerning the heritability estimation for some bioeconomic and eco-economic characters in Ropsa carp breed - Carmen Georgeta NICOLAE, Horia GROSU, Mioara COSTACHE, Georgeta DINIŢĂ, Monica MARIN, Victor NIŢĂ 316

GENETICS AND BREEDING

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BIOLOGICAL CHARACTERISTICS AND PATHOGENICITY OF AVIAN *ESCHERICHIA COLI* STRAINS FROM ALBANIAN POULTRY FLOCKS

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Abstract

A total of 129 pathogenic Escherichia coli strains (APEC) isolated from hens and broilers suffering from colisepticaemia and ovaritis were studied regarding their biological and pathogenic characteristics such as serogroups and virulence associated genes. For comparison were studied also other 100 poultry fecal E.coli strains originated from apparently healthy birds. Serotyping demonstrated that most of E.coli strains were untypable in both colibacillosis clinic division groups (62% for and 34%, whereas in 129 of E. coli strains the searched eight virulence genes for their presence showed relationship with colibacillosis infection outbreaks in poultry. Serotyping identified a very wide variety of serotypes according to APEC and AFEC strains. Serotypes most often associated with the presence of clinical signs resulted O86 (8, 75%), O2 (4, 86%), O8 (6, 77%), O15 (3, 88%), O139 (2, 92%) ; O157 (2.92%) 78 O (1, 94%), while those with apparently healthy birds: O8 (11, 53%), O157 (7, 69%), O73 (3, 85 %), O86 (3, 85%), O115 (3, 85%) and O2 (3, 75%). The lack of virulence factors in APEC strains resulted 18, 05%, while in AFEC strains 81, 95%. This study identified significant differences of virulence factors among strains isolated from lesions, compared to those from apparently healthy subjects. Anyway the detection of virulence genes present in serotypes O15, O86, O73, O101, O147, O157, brings a wider variety of APEC in serogroups classification. These data obtained from genetic characterization of avian E.coli strains constitute the first report in Albania, for colibacillosis infection outbreak in poultry flocks. The presence, appearance and distribution of virulence genes in poultry flocks, provides basic information for the control and eradication of the colibacillosis infection. Application of molecular biology methods to further knowledge of the serotyping data now is a time requirement for the prevention and eradication of avian colibacillosis or other bacterial poultry infections.

Key words: *Avian Pathogenic Escherichia coli, Serotyping, Genotyping, colibacillosis, virulence genes*

INTRODUCTION

Colibacillosis caused by Avian Pathogenic *Escherichia coli* strains, is the main cause of economic losses in poultry industry, worldwide [1]. This acute infection is clinically localized and systemic, with variety of lesions in organs viscera.

Currently, pathogenic *Escherichia coli* infections are more frequently encountered in intensive poultry breeding flocks. Colibacillosis presence and its outbreaks are considered as an important indicator of the level of poultry productivity and growth.

Initial studies on avian *E. coli* strains have shown that O1, O2 and O78 serotypes, are mostly associated with colibacillosis outbreaks [3]. While half of the strains examined in many studies are not fully elucidated to settle in classical serogrouping classification, making these strains untypeable. In now days serotyping does not constitute a basis for *E. coli* diagnosis and identification, especially it does not specify the fact if a serotype expresses the virulence of the strain, but this test is important for epidemiological studies.

Clear identification and differentiation of Avian *Escherichia coli* with opportunistic *E. coli* remains a lack for the veterinarian research science of laboratory diagnosis. For this reason the serotyping of 129 avian *E.coli* isolates would help to situate a database of epidemiological evidences of the actual situation in the poultry industry about the circulation *E.coli* strains in Albania.

MATERIAL AND METHOD

On 129 avian *E.coli* isolates was conducted the serotipization using a standardized panel of 40 monoclonal specific antiserums against the somatic O antigen (O1, O2, O3, O4, O6, O8, O9, O10, O11, O15, O18, O20, O21, O22, O26, O45, O49, O64, O68, O73, O75, O78, O83, O85, O86, O88, O92, O101, O103, O109, O111, O115, O128, O132, O138, O139, O141, O147, O153 and O157){2}.
Serotyping was performed at Experimental Zooprophilactic Institute of Brescia, Italy, which uses the O anti serums cited above, in accordance with *E.coli* strains circulating in poultry in the area concerned.

RESULTS AND DISCUSSIONS

The serotipization of 129 *E.coli* strains, isolated from colibacillosis affected and aparently healthy birds allowed the characterization of only 31, 21% of them. Untypeable strains resulted 81 or 62, 79% of them (Tab. No. 1).

Table 1: Serogrups identified in APEC and AFEC strains (num = 129*)*

Untyped Serotyped	81 48[a]	62,79[b] 37,21
O2	6	4,65
O8	10	7,75
O15	4	3,10
O73	3	2,33
O78	2	1,55
O86	10	7,75
O88	2	1,55
O101	1	0,77
O115	1	0,77
O139	3	2,33
O147	1	0,77
O157	5	3,89

Serogrouping identified 12 different serotypes with the following percentages: O8 (7.75%) O86 (7.75%) O2 (4.65%) O157 (3.89%); O15 (3.10 %), O73 (2.33%), O139 (2.33%), O78 (1, 55%), O88 (1, 55%), O101 (0, 77%), O115 (0, 77%) and O147 (0, 77%).
More than half of *E.coli* strains (62, 79%) were not serogrouped because they failed to react with the standard antisera panel [2]. Thus, previous data on similar levels of untypeable levels of *E.coli* strains, comes up the discussion whether or not serogrouping is an efficient method of characterizing avian *E.coli.*
Shortcomings of this method relate to the existence of autoagglutinating strains or cross reacting ones with more than one O antiserum, all this depending on specific geographic regions [6].
Results of previous epidemiological research, in many countries indicate that only 15% of strains belonging serogroups O1, O2, O35, O36 and O78, or are associated with *E.coli* infections, the rest is part of unknown serogrups (untyped) or represent new serotypes. This should be taken as a signal for the presence of new pathogenic serotypes not yet studied {4}. If the untyped strains resulted 62, 79%, this does not mean that they should be considered as non-pathogenic, as many of them contained in bacterial genome responsible for virulence genes and originated colibacillosis affected birds.
Serotyping identified a very wide variety of serotypes according to APEC and AFEC strains. Serotypes most often associated with the presence of clinical signs resulted O86 (8, 75%), O2 (4, 86%), O8 (6, 77%), O15 (3, 88%), O139 (2, 92%) ; O157 (2.92%) 78 O (1, 94%), while those with apparently healthy birds: O8 (11, 53%), O157 (7, 69%), O73 (3, 85 %), O86 (3, 85%), O115 (3, 85%) and O2 (3, 75%).
Serotypes O2, O8, O73, O86 and O157 were present as in APEC, so in AFEC. While serotype's O15, O78, O88, O101, O139 and O147 presence resulted associated only with APEC. Given that serotypes: O2, O8, O15, O78, O115 and O139, were related to APEC strains, while AFEC had the presence of O2, O8, O157 serotypes, we can say that serotyping serves as a method of classification and not to define the pathogenicity of *E.coli* strains.
Our results support the fact of the existence of the wide serological diversity among avian *E.coli* isolates, may come as a result of the opportunistic nature of most infections.
Predisposing factors (Mycoplasmosis or viral infections and environmental factors) may be responsible for this diversity.

Table 2: The distribution of serotypes according to APEC and AFEC strains

Serotypes	APEC	%	AFEC	%
O2	**5**	**4,86**	**1**	**3,85**
O8	**7**	**6,77**	**3**	**11,53**
O15	**4**	**3,88**	**0**	**0,00**
O73	**2**	**1,94**	**1**	**3,85**
O78	**2**	**1,94**	**0**	**0,00**
O86	**9**	**8,75**	**1**	**3,85**
O88	**2**	**1,94**	**0**	**0,00**
O101	**1**	**0,97**	**0**	**0,00**
O115	**0**	**0,00**	**1**	**3,85**
O139	**3**	**2,92**	**0**	**0,00**
O147	**1**	**0,97**	**0**	**0,00**
O157	**3**	**2,92**	**2**	**7,69**
NT	**64**	**62,14**	**17**	**65,38**
TOTAL	103	100,00	26	100,00

Since in this study was revealed a large number of serotypes, previous studies recommend that the use of an effective vaccine which should summarize in a wide range avian *E.coli* serotypes [5]. Serotyping is not the only method of defining the pathogenic behaviour of *E.coli,* because it not always coincide with a wider genetic diversity among strains of a serotype [3; 7; 8]. For this reason in this research was studied the co-relation between the identified the serotypes with the presence of virulence associated genes as presented in table no.3.

Table 3: The co-relation of virulence associated genes with the identified serotypes on 129 E. coli strains

	8 virulence associated genes							
Serotypes	astA1%	iss%	Irp2%	iucD %	papC %	tsh %	Vat%	CvaA/B %
O2 (no = 6)	50,00	66,66	50,00	66,66	33,33	66,66	33,33	33,33
O8 (no = 10)	30,00	50,00	30,00	40,00	20,00	20,0	20,00	20,00
O15 (no = 4)	25,00	75,00	75,00	25,00	0,00	0,00	0,00	25,00
O78 (no = 2)	0,00	100,00	100,00	100,00	50,00	0,00	0,00	0,00
O86 (no = 10)	20,00	20,00	10,00	10,00	0,00	0,00	0,00	0,00
O73 (no = 3)	66,66	66,66	0,00	0,00	0,00	0,00	0,00	0,00
O101 (no = 1)	0,00	100,00	0,00	0,00	0,00	0,00	0,00	0,00
O115 (no = 1)	0,00	100,00	100,00	100,00	0,00	100,00	0,00	100,00
O139 (no = 3)	66,66	66,66	66,66	66,66	66,66	0,00	66,66	66,66
O147 (no = 1)	0,00	100,00	0,00	0,00	0,00	0,00	0,00	0,00
O157 (no = 5)	0,00	20,00	0,00	20,00	0,00	0,00	0,00	0,00
O88 (no = 2)	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
NT (no = 81)	23,40	53,09	35,8	40,74	20,99	24,69	8,64	8,65

The co-relation between virulence associated genes and the identified serotypes proides a clear picture of the genetic diversity within certain serotype or further more a serogroup. As shown on the table no. 3 serotypes O2, O8 and O78 which are frequently related with avian colibacillosis infection contain virulence factors in moderate leveles.

Serotypes O139 and O115, although bear in a significant degree of virulence factors, are not reported in previous studies [2] as implicated in avian colibacillosis. This result may be a reflection of regional differences related to the prevalence of different serogroups in different geographical areas. The presence of virulence genes present in serotypes O15, O86, O73, O101, O147, O157, except those known as pathogens,

shows a wider variation of APEC in serogrouping classification.

CONCLUSIONS

Serogrouping identified 12 different *E.coli* serotypes. The untyped strains resulted at a level of 62, 79%. Serotypes: O15, O73, O86, O101, O115, O139 and O147 represent new entrance in colibacillosis patogenesis. This should be taken as a signal for the presence of new pathogenic serotypes not yet elucidated.

Serotyping identified a very wide variety of serotypes according to APEC and AFEC strains. Serotypes O139 and O115 carried significant levels of virulence factors, although they have not been identified previously as avian colibacillosis implicated serotypes. This result indicated that the prevalence of clonal *E.coli* groups depends on the specificity of a geographical area.

This study results suggest that serotypes known as pathogens that have correlation with virulence factors and are closely associated with the colibacillosis outbreak in poultry. This conclusion applies those untypeable strains too, which in their genome contain important virulence genes.

The application of molecular biology methods to further knowledge of the serotyping data now is a time requirement for the prevention and possible eradication of avian colibacillosis or other bacterial poultry infections.

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GENETIC INVESTIGATIONS USING IMMUNO-BIOCHEMICAL MARKERS IN A BLACK SPOTTED CATTLE POPULATION

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Abstract

The study of the genetic markers and identifying new markers involves an increasing number of research projects in the fields of genetics of immunology, biochemical genetics, molecular genetics, quantity genetics and the genetic improvement of animals.Having considered the importance of genetic polymorphism of biochemical structures we considered a study of the genetic characterization of a sample in a cattle population, based on the information offered by the genetic polymorphism at hemoglobin and transferrine loci and the analysis of the serum. Two phenotypic category, hemoglobin A (79,4%) and AB (20,6%), has been identified for hemoglobin locus. Five categories of individuals, homozygous for Tf^A/Tf^A (2.9%), Tf^D/Tf^D (58,8%) and heterozygous for Tf^A/Tf^D (20.6%), Tf^A/Tf^E (5,9%), Tf^D/Tf^E (11,8%) have been identified on seric transferine loci; determined of existence of interest locus for three kinds of genes Tf^A, Tf^D and Tf^E with codominant effect. Blood factors were described in order to use them as genetic markers with the purpose of determine the homogenity or heterogenity degree for two cattle population.Serologic relations were observed between the factors of B system. The most relevant was the complex BGK. These tree factors were observed in five different combinations, namely BGK, BG, B, G, and their total absence.

Key words: *reagents, bloods phenogroups, immuno-serological, haemoglobin, transferinne*

INTRODUCTION

Theoretical and practical achievements in husbandry are continuously conditioned in a great extent by the genetics evolution as a biology field.

Economical and food crisis as a result of the contemporary demographic explosion, leads to the fact that the material resources obtaining and revaluation will become the major objective of the „biological revolution", which will affect humanity much deeper than „mechanical revolution" of the XIX –th century or technological revolution of our century.

The complex knowledge and using of the high productive potential of living matter need fundamental and appliable researches, finally justified by a high efficiency obtained with minimum expenses.

In this context, genetics offer to the mankind new possibilities, both by reevaluating natural resources by animal genetical improvement , and by creating new organisms using modern biotechnologies [2,6].

One of these possibilities is offered by genetic markers, which are object of several researches within immunogenetics, biochemical genetics, molecular genetics and even quantitative genetics fields [1,3].

MATERIAL AND METHOD

The studied material included 34 individuals of Black spotted cattle population.

The blood samples collection was accomplished in heparinized, standard test tubes. Determination of blood phenogroups was realized according to the standard methodology, by using the set of 44 reagents existing in the immuno-serology laboratory. To realize the hemolytic test, Plexiglas plates were used, with buckets of 7 mm depth and 5 mm diameter, into which was dropped a drop from each monoserum used to test the eritrocitary suspensions. Then it is added, over these, a drop from the searched eritrocitary suspension; afterwards, the plates were shaken to realize the mixture between the antiserum and the erythrocytes in the suspension. After 10

minutes rest at room temperature, it was dropped over the mixture in each bucket a drop of complement. After the three components were introduced in the buckets, the plates were shaken again; afterwards they were incubated at 25^0C. The reaction reading was realized at ½ hours from incubation, at 2½ hours and at 5 hours. After each reading the plates were shaken [2].

The various degrees of hemolysis realizing were estimated by reading:

-negative reaction: all erythrocytes are deposited, the above liquid is clear;

-positive reaction: it was appreciated according to the lysed red cell utilized in the following four values scale: light hemolysis, accentuated hemolysis, net hemolysis, complete hemolysis.

For establishing the types of hemoglobin, we used the technique of vertical electro-phoresys, using polyacryl amidae as a migration support, the same technique as used by Meriaux J.C. (1992), adapted to the conditions in the bio-chemistry laboratory the Faculty of Biology of The University of Bucharest [8].

RESULTS AND DISCUSSIONS

The locus of haemoglobin

Statistical analysis of the studied sample revealed two types of haemoglobin, namely A and AB genetically determined by the following genotypes: homozygous Hb^A/Hb^A and heterozygous Hb^A/Hb^B. Identification of genotypic categories on haemoglobin locus allowed establishing genetic structure within this sample. Homozygous Hb^A/Hb^A individuals presents the highest occurrence within genetic structure, namely four times higher than heterozygous ones (Fig. 1).

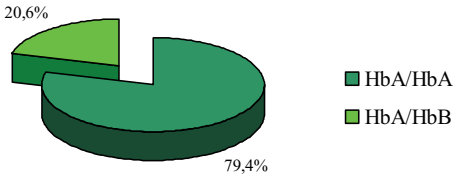

Fig. 1. The genotypic category distribution on haemoglobin locus (%)

The presence of two genotype categories in the group demonstrates the presence of two categories of genes, Hb^A, Hb^B, identified with different frequency (Fig. 2).

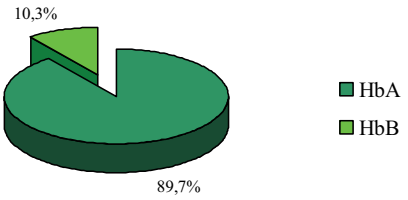

Fig. 2. Gene category distribution haemoglobin locus (%)

Hb^A gene was determined with a high frequency (89,7%), respectively compared to about 9 times higher than Hb^B.

The analysis of this sample equilibrium state, using χ^2 test (table 1), lead to the conclusion that this sample has a balanced genetic structure. We may notice that because after comparing table value χ^2 (5,99) with calculated value χ^2, the calculated value is smaller comparative with table value.

Table 1. *Equilibrium state estimation on haemoglobin locus*

Genotypes	No. observed genotypes	No. expected genotypes	d^2/A
Hb^A/ Hb^A	27	27,36	0,005
Hb^A/ Hb^B	7	6,28	0,083
Hb^B/ Hb^B	0	0,72	0,360
Total	34	34	χ^2 =0,448

The locus of serum transferines

The interpretation of electrophoresis graphs for the 34 individuals has detected five categories of individuals: homozygous for genes Tf^D, Tf^E, heterozygous Tf^A/Tf^D, Tf^A/Tf^E and Tf^D/Tf^E (figure 3).

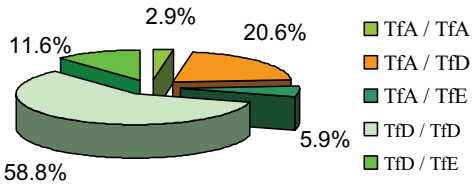

Fig. 3. The genotypic category distribution on transferrine locus (%)

The highest share was found in heterozygous individuals Tf^D/Tf^D (58,8%), with 55,9% higher than homozygous Tf^A/Tf^A (2,9%), which were emphasized with the smallest frequency.

The presence in the analyzed population of five genotypic categories emphasize that three gene categories, namely Tf^A, Tf^D and Tf^E, existed in the analyzed sample identified with different frequencies.

According to cathegory distribution, no homozigous Tf^E/Tf^E individual has been found in this sample. From this reason, after the gene category frequency determination, the gene Tf^E showed the lowest percent (figure 4)

Tf^D gene is characterized by a frequency about 77% higher than the frequency of gene Tf^E, which was described with the lowest procent.

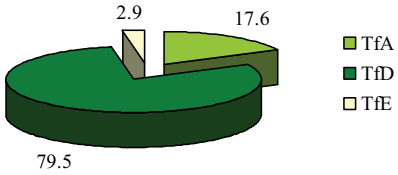

Fig. 4. Gene category distribution transferrine locus (%)

Determining the ratios of the genotype categories and, as a result, the genetic structure of the sample in this study allowed building an estimate for the status of genetic equilibrium, for locus analyzed.

The results of equilibrum state estimation for this sample show a balanced genetic structure, calculated χ^2 value (1,650) being smaller than the table value (5. 99). Results are presented in table 2.

Table 2. Equilibrum state estimation on transferrine locus

Genotypes	No. observed genotypes	No. expected genotypes	d^2/A
Tf^A/ Tf^A	1	0,887	0,014
Tf^A/ Tf^D	7	8,236	0,185
Tf^A/ Tf^E	2	0,972	1,087
Tf^D/ Tf^D	20	19,125	0,040
Tf^D/ Tf^E	4	4,514	0,058
Tf^E/ Tf^E	0	0,266	0,266
Total	34	34	χ^2 =1,650

In this effective there were not emphasized individuals with the I_2, E'_3 and G'' factors.

The M factor is associated with a low milk production. This factor was noticed at 2,9% of individuals [7,9].

For 13% from the emphasized erythrocytary, a net hemolytic was obtained. The most positive reactions (79%) were of complete hemolytic. For the rest of positive reactions (8%) it was found that the hemolytic was of 50%, the above liquid being pink-reddish colored.

The most reactions were observed within system B (table 3), known as the most complex blood group system at cattle [5]. The most relevant was the complex BGK. These tree factors were observed in five different combinations, namely BGK, BG, B, G, and their total absence. Another type of relations, namely the linear relations were observed between the factors of systems A (A_1, A_2), B (G_1, G_2, G_3; O_1, O_2, E'_1, E'_2), C (C_1, C_2; X_1, X_2), FV (F_1, F_2). The genetic explanation of these subtypes of blood group factors consists of the different antigenic structure of the respective factors. Between the subtypes of the same factor crossed reactions may appear, these being characteristic for each system.

Table 3. Share of blood group systems category

Blood group system	Factor of blood group system	N 34	Factors distribution of blood group system
A	A_1	31	0,911
	A_2	27	0,794
B	B	10	0,294
	G_1	15	0,411
	G_2	25	0,735
	G_3	21	0,617
	I_1	26	0,764
	K	9	0,264
	O_1	27	0,794
	O_2	19	0,558
	T_1	13	0,382
	Y_2	27	0,794
	D'	23	0,676
	E'_1	23	0,676
	E'_2	25	0,735
	O'	20	0,588
	G'	32	0,941
	I'	19	0,558
	J'	14	0,411
	Q'	26	0,764
	Y'	17	0,500
C	C_1	18	0,529
	C_2	10	0,294
	E	23	0,676
	R_1	8	0,235
	W	29	0,852
	X_1	10	0,294
	X_2	28	0,823
	L'	27	0,794
FV	F_1	25	0,735
	F_2	1	0,029
	V	16	0,470
J	J	9	0,264
L	L	21	0,617
M	M	1	0,029
SU	S	28	0,823
	H'	32	0,941
	H''	16	0,470
	U'_1	4	0,117
	U''	3	0,088
Z	Z	30	0,882

CONCLUSIONS

1. Two categories of individuals have been described within the lot and they are as following: homozygous Hb^A/Hb^A and heterozygous Hb^A/Hb^B. The homozygous individuals Hb^A/Hb^A represent more than two thirth of the sample
2. The polymorphism study from the seric protein locus pointed out five genotypic categories: Tf^A/Tf^A, Tf^A/Tf^D, Tf^A/Tf^E, Tf^D/Tf^D and Tf^D/Tf^E
3. Following the analysis of the genetic balance conditioner seric transferine and haemoglobin locus, was concluded that a genetic balance condition already exist for the analyzed population.
4. The most reactions were observed within system B, known as the most complex blood group system at cattle.
5. The lowest gene frequency was present in F_2 and M factors (2,9%), and highest one in G' and H' (94,1%).

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MORE ABOUT ON LOCAL DIFFERENTIATION OF ALBANIAN LOCAL SHEEP POPULATIONS.

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Abstract

The estimation of archaism index was used to study the local differentiation of seven Albanian local sheep populations. The estimation of archaism index was carried out using the morph-metric data of several features: whither height, tail length, ears length, horns and wattles presence, coat color, frontal-nasal profile. The cluster analyses was carried out using the Euclidian's distances between populations in the plan of two first principal components. The cluster analyzes showed the existence of four distinguish sheep groups: first group - Ruda, Bardhoke, Baca, second group – Shkodrane, third group - Lara of Polisi, Syska of Mati, and fourth group - Recka. Based on the geographic distances between the regions where are bred the animals of these local sheep breeds and their geographic isolation can be concluded that the presence of local differentiation of Albanian local sheep populations is caused by the isolation in distance. These results show that in currant sheep population of Albania it is possible to find direct descendants of animals that have populated the Balkan regions in the form of three migratory successive waves. Currant results could be consider as preliminary one because of limited number of features included in the study and the complicity of this topic. They can serve as a bases for in-depth studies on local differentiation hypotheses of Albanian local sheep population.

Key words: *index of archaism, local breed, local differentiation, sheep*

INTRODUCTION

The biodiversity of farm animals constitutes an important resource for food security of human beings. Its sustainable conservation and use are among the main objectives of Global Action Plan for Animal Genetic Resources [3]. In Global Action Plan the identification, characterization, evaluation of risk of extinction trend, the conservation, the farming and sustainable use are determined among the most priority fields.

The sheep species produces about 7 % of total annual milk yield. About 30% of meat production (live weight) is secured by small ruminants in Albania. There is a very old tradition for sheep farming in Albania.

Currant Albanian sheep population is characterized by a high level of genetic variability [4]. About 62.3% of sheep population are imported breeds and their crossbreds with local one [8]. Recka local sheep breed apart and all together other autochthonous breeds like as Ruda, Bardhoke, Shkodrane, Baca, Lara of Polis and Lara of Mati constitute respectively about 21.6 % and 17.2% of hole sheep population [8]. At the beginning of its domestication process the sheep had a small body, with short ears and short tail changing annually its wool covering [1]. The sheep with great body, with long, thin and fat tail, with wool of high variability of type, length and quality are currently frequent. A successful approach to study the domestication process and geographic spread out scenarios of sheep species is based on the variability of these qualities and archaism level of different features. Lauvergne [5] [6], to study the population scenarios of Mediterranean regions was based on the evaluation of the archaism of morph-biometric features of different sheep population or breeds conceiving it as a process developed in the form of concentric waves, sequentially, with the differentiation and distribution centre of Middle East. Under

this hypothesis, the first wave, which was the most eccentric and greater geographic coverage wave, was composed by sheep breeds with small body and short tail. These breeds are considered as most archaic in the region. Populations of the first wave were subject of successive overlapping process from more genetically evolved sheep breeds (breeds with long wool and thin tail), which were spread out in the region in the form of a second wave. With the third wave, sheep breeds with long and fat tails were scattered in Mediterranean regions. The archaism indices could be successfully used to judge about the belonging to the corresponding wave of currant sheep breeds. These indices are used by Lauvergne [1] to judge about the wave to which belonged several autochthonous sheep breeds of Franc. They were also used by Bonacini et al., [2] to explain the genetic effect of improving breeds on local breeds of Arc Alpin in Italy. Pares et al., [7] gives results of a comparative study of 14 European sheep breeds based on the estimation of archaism index.

In this study the archaism indices estimated for seven Albanian local sheep breeds are used to judge about local differentiation level between these breeds. The relation and belonging of autochthonous sheep breeds with ancient sheep populations spread out in these regions during their concentric waves diffusion is also judged.

MATERIAL AND METHOD

Seven Albanian autochthonous sheep breeds are included in the study. Measurements and observations were done in adult animals separately for each breed as follow: 56 animals (50 females and 6 males) of Ruda breed, 98 animals (91 females and 7 males) of Bardhoke breed, 62 animals (54 females and 8 males) of Baca breed, 58 animals (52 females and 6 males) of Shkodrane breed, 91 animals (84 females and 7 males) of Lara Matit breed, 67 animals (60 females and 7 males) Lara Polisit breed and 83 animals (75 females and 8 males) of Rrecka breed. Geografic regions where these breeds are farmed are presented in Fig. 1.

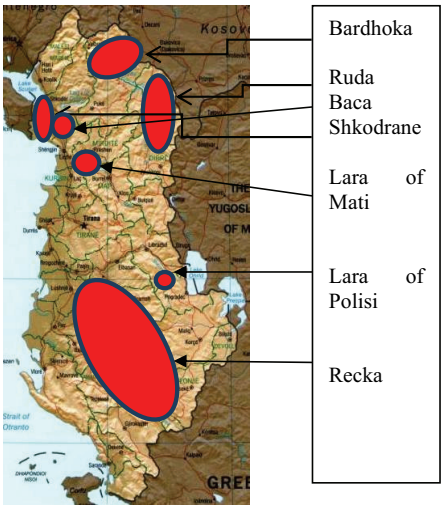

Fig. 1. Geographic distribution of seven local sheep breeds.

The features taken in consideration to estimate the archaism were treated as discrete variables with values (marks) 0-1, 0-1-2 or 0-1-2-3-4. The more archaic was the feature higher would the corresponding variable value be(mark). The marks determination was done based on the data of direct measurements for example: the interval where the average value of feature falls; indirect data for example the interval where average value of index auricular falls (the ratio between ear length and tail length) or from the observation of the presence or not of the feature (for example the horn presence in male and female animals). The features taken into consideration to estimate archaism were: length of ear, horn presence, wattles presence, whither height, tail length, frontal-nasal profile and coat color. Three indices were calculated: (i) index caudal = tail length /whither height, (ii) index auricular =ear length/whither height and (iii) Index of convexity = arc length /length of chord. To determine the archaism point of analyzed features the protocol described by [1] was used. (Table 1).

The archaism index was calculated as the very simplest of values (marks) of found archaism of all traits and indices taken into consideration [1]. The values of archaism index were used to make the first

classification(ranking) of breeds according to archaism level.
The analyses of principal components was used to judge about distances between breeds and their local differentiation

Table 1. The protocol to estimate the archaism marks for each feature.

Character	Mark	Degree
Ear length	0	Index auricular >0.19
	1	Index auricular from 0.16 to 0.19
	2	Index auricular <0.16
Horns	0	Horned males and females
	1	Horned males, polled females
	2	Horned females and polled females,
Wattles	0	Random presence
	1	Absence
Body format	0	Whither height >70 cm
	1	Whither height from 60cm up to 70 cm
	2	Whither height <60 cm
Tail length	0	Index caudal > 0.6
	1	Index caudal from 0.5 to 0.6
	2	Index caudal < 0.5
Frontal - nasal profile	0	Index of convexity <0.95
	1	Index of convexity from 0.95 to 1
	2	Index of convexity = 1
Colour	0	Completely white
	1	White fleece, colored legs.
	2	Colored fleece and colored legs.
	3	Two tones coloration
	4	More than two tones coloration.

RESULTS AND DISCUSSIONS

The evaluations of the averages of three indices calculated for each one of breeds are presented in Table 2. The archaism marks for each one analyzed features and the archaism index calculated as very simplest sum of these marks for each breed are presented in Table 3.The archaism index varies from 2 (Bardhoke breed) up to 12 (Rrecka breed). Shkodrane and Rrecka sheep breeds have higher archaism index. Currant populations of these two breeds are direct descendents of that part of corresponding populations that have been bred as pure for centuries until nowadays. These two breeds could be grouped in short tail breeds referring to the values of Index caudal. Bardhoke, Ruda and Baca breeds have lower values of archaism index.

Table 2. Number of animals and means of indices

Breed	N	Index auricular	Index caudal	Index of convexity
Bardhoke	8	0.21±0.02	0.57±0.27	0.92±0.08
Ruda	8	0.20±0.02	0.61±0.32	0.94±0.12
Baca	2	0.18±0.02	0.65±0.21	0.92±0.07
Lara of Matit	1	0.15±0.02	0.49±0.22	0.95±0.09
Lara of Polisit	7	0.14±0.02	0.49±0.31	0.95±0.11
Shkodrane	8	0.19±0.01	0.46±0.28	0.96±0.13
Rrecka	3	0.13±0.02	0.42±0.12	0.96±0.09

The graphic of the archaism indices frequencies (Fig. 2) gives an approximate vision regarding to groupings of local sheep breeds taken in analyses. There are evidenced three groups: Rude, Rude, Bardhoke and Baca breeds with archaism index of 2-3; Lara of Polisit and Lara of Mati breeds with archaism index of 8, Shkodrane and Rrecka breeds with archaism index 11-12. Starting from these groupings the hypothesis which may be raised is that Shkodrane and Recka sheep breeds belong to the first wave of population. Other sheep breeds could be considered as descendents of animals that have populated Albanian regions during the second waves. Besides that based on archaism marks of coat color and wattles presence features of 0 could be confirmed that these breeds could be grouped in standardized traditional breeds.
The analyzes of principal components (Fig. 2) identifies three groups according to which could be classified seven local sheep breeds taken into consideration.

Table 3. Marks of archaism and indices of archaism for seven Albanian sheep breeds.

Breed	Code	Note of archaism							Index of archaism
		Index auricular	Horns	Wattles	Format	Index caudal	Index of convexity	Colour	
Ruda	1	0	1	0	1	0	0	0	2
Bardhoke	2	0	1	0	0	1	1	0	3
Baca	3	1	1	0	1	0	0	0	3
Lara of Matit	4	2	2	0	1	1	1	1	8
Lara of Polisit	5	2	1	1	1	1	1	1	8
Shkodrane	6	1	2	1	2	2	1	2	11
Rrecka	7	2	2	1	2	2	1	2	12

.

The differences between different breeds, their distances in the plan of two first principal components and the formed groups evidence the local differentiation phenomena that characterizes the local sheep population in Albania. Referring to their geographical breeding location (Fig. 1) and given groupings could be confirmed that this differentiation is result of isolation in distance and completeness of the factors that have formed over the centuries farmers behaviours and their preferences for these breeds.

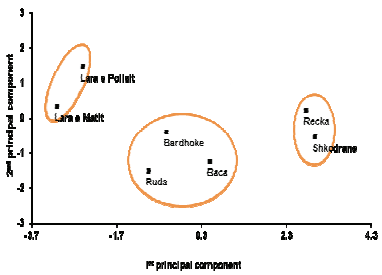

Fig. 2. The presentation of local breeds groupings on the plan of two first principal components.

The populations of Lara of Polisit and Lara of Mati sheep breeds can be considered geographically isolated in distance. Meanwhile these breeds are a group referring to archaism index. The observed differentiation level could be explained or could be result of conservative attitude of farmers towards these breeds. In both cases these breeds are conserved as pure breeds although in small number. Referring to archaism level the differentiation between them is small although they can be considered as isolated population in distance. For Ruda, Bardhoke and Baca sheep breeds the differentiation between their archaism index are similar to those associated with the effects of isolated in distance. Furthermore it should be noted that in the configuration of this group a considerable effect must have brought the genetic closeness of Baca and Bardhoke sheep breeds. Baca breed is relatively new one created by crossbreeding of Shkodrane breed with Bardhoke breed.

The above judges regarding to the archaism level of Albanian local sheep breeds and their local differentiation need to be fulfilled with more detailed studies. The study and evaluation of polymorphism in DNA level and their combination with the visible genetic profile study data and archaism index can results in more complete and accurate characterization of local sheep breeds in Albania.

CONCLUSIONS

Using the mark system of archaism for different features and archaism index for characterisation of local sheep breeds help to judge about the affiliation of currant sheep populations with incomes breeds during the process of sheep population of these regions.

Shkodrane and Rrecka sheep breed are among the most archaic sheep breeds in Albania. They can be considered to have arrived during the first wave of sheep population of Mediterranean region.

The differentiations of archaism indices can serve to judge about the differentiations level of local sheep breeds. The differentiation between sheep breeds could be explained by the action of factors related with isolation in distance and the farmers attitudes and preferences for pure breeds farming.

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ANIMAL BIODIVERSITY CONSERVATION, A KEY OF SUSTAINABLE AGRICULTURE. CASE STUDY: THE ROMANIAN PINZGAU BREED IN TRANSILVANIA REGION

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Abstract

Pinzgau breed or Pinzgauer is called after its region of origin (place Pinzgauer, near Salzburg, Austria) and is a mountain breed of cattle. The breed appeared in the 19th century from local mountain breeds and was developed in three directions: traction, milk and meat. Recent research has shown that specimens of the Pinzgau breed feed recovered well from such areas, farmers, to get average yields, having to buy a small amount of concentrated feed for animal ration to complete. In Romania, Red Pinzgau breed formed after absorption crosses made between local breeds of cattle (Grey Steppe and Mocăniţa) and Pizgau of Austria, since the second half of the nineteenth century, and black Pinzgau named "Cow of Dorna" by crossing local cattle with various mountain improved breeds (Pinzgauer, Mölltal, Zillertal, Dux-Zillertal, Dutch, Brown, etc.). The breed is exploited in three areas: NW of Moldavia, SW of Transylvania and W of Transylvania - Apuseni Mountains. Transylvanian Pinzgau breed has a sound constitution, lively temperament, docile character, precocity mediocre, high longevity, good capacity of adaptation, resistance to disease and weather. Has a multilateral skill (milk, meat, traction). These things are the main reasons why race should be kept in a form of active conservation. Moreover, in order to preserve the tradition and traditional products in Romania, is required to maintain this breed and even the formation of its national park.

Key words**:** *Transylvanian Pinzgau, active conservation, sustainable agriculture.*

INTRODUCTION

Around 1820, cows of Pinzgau were exported to countries like Romania, Yugoslavia, Czech Republic and Slovakia. Moreover, currently breed is present in over 25 countries worldwide. In South Africa, Canada, USA and Australia, Pinzgau prospered even in the harshest environmental conditions. Resistant hooves, able to cover great distances, even coat color, which allows UV protection, are what made this population of cattle to be appreciated by farmer's cows on five continents [2].
The ancestors of the breed were brought by the Celts around 800 DC. In time, have evolved several types of race, according to the development area (Salzburg, Tyrol, Carinthia, Bavaria and Styria), showing a predominantly mottled coat colour on brown background, but black. In 1857, Baron Freiherr von Mesnil described Pinzgau specimens with a full coat with a brown or white line on the abdomen and the upper line [2].
Unique colour mottled red-brown spots on the body side and white line became race character. Individuals presenting black and white robe, called the "lucky line" survived like animals that every farmer was proud to have. But breeders associations preferred animals with red-brown with white robe which meant that in time black variety become very rare [2].
During the Austrian Empire, the breed was quickly spread to other parts of it and today, can still be found in Austria, Slovakia and Romania. When used as traction animals have lost importance, the breed was developed in two directions: milk and meat, in alpine areas, while exploiting the ability of animals to long marches on rough terrain. This last feature was

the main reason for the export of pure-bred specimens. In countries like South Africa, Australia and the United States of America, Pinzgau was raised for one purpose, that meat breed.

In last decades, even in the birthplace, the number of specimens of the breed declined drastically due to changes in "fashion" and intensive agriculture, which caused the race to be in danger. Pinzgau, only Austrian indigenous breed, worldwide famous, should receive special attention through the establishment of national park and through the use of race to achieve its organic productions.

In Romania, Red Pinzgau breed formed after absorption crosses made between local breeds of cattle (Grey Steppe and Mocăniţa) and Pizgau of Austria, since the second half of the nineteenth century, and black Pinzgau named "Cow of Dorna" by crossing local cattle with various mountain improved breeds (Pinzgauer, Mölltal, Zillertal, Dux-Zillertal, Dutch, Brown, etc.). The breed is exploited in three areas: NW of Moldavia, SW of Transylvania and W of Transylvania - Apuseni Mountains.

Transylvanian Pinzgau is characterized by mixed morphology type, variable body development, meeting three types: hypermetric (size 131cm, body weight 520 kg in the south-western Transylvania), eumetric (127 cm and 460 kg in Suceava County) and hypometric (122 cm and 400 kg in the Apuseni Mountains), mezobrevimorfe profiles and less harmonious conformation. It has a large head, full; strong neck with developed dewlap; trunk not too long, but deep, with left top line, croup narrow at the ischium, big abdomen, globular udder and solid states. The colour is typical for Pinzgau, that dark red with white features drawings. Dorna cow has size less than 1-2 cm than Red Pinzgauer, made more pronounced rectangular body, bones and muscles better developed and the background colour black (in Moldova there is a colour polymorphism of red and black, or black variety of Pinzgau). Black Pinzgau was seen as a distinct type (population), with different characteristics (Fisteag, 1958), although it is or not reproductive isolated [2].

The purpose of this paper is to estimate the number of Transylvanian Pinzgau individuals from the area of Suceava County, to characterize individuals in terms of production, to describe breeding systems and to question breeders about the purpose of exploiting breed in over others more productive.

MATERIAL AND METHOD

Research was conducted during 2001-2012, being a component of a complex project that aims to achieve a sustainable program for management of Pinzgau genetic resources. Based on official records provided by A.N.A.R.Z. (National Agency of Animal Recording), breed mapping was done, an estimate of the number of animals in pure breed and hybrid, and a characterization in terms of production ability. Also, by moving (February-March 2012) in the Transilvania Region tried to characterize the breeding systems and breeders asking about the exploitation of the breed. Research methods were observation, analysis and questionnaire.

RESULTS AND DISCUSSIONS

The situation of Transylvanian Pinzgau breed showing a higher proportion of crossbred versus pure breed (23120 heads in total, of which 46.24% pure breed and 53.76% crossbred), it have seen a redistribution of areas for growth and exploitation, by emergence of new ones. Thus present in Table 1 mapping of Transylvanian Pinzgau breed in early 2012.

Table 1. Mapping of Transylvanian Pinzgau breed in 2012 [5]

Specification	Total	Pure breed	Hybrid
Alba	1101	651	450
Bacău	28	-	28
Bihor	490	-	490
Bistriţa	893	447	446
Cluj	35	-	35
Hunedoara	391	140	251
Mehedinţi	6	-	6
Neamţ	1243	463	780
Sălaj	110	93	17
Sibiu	1330	818	512
Suceava	16460	8040	8420
Tulcea	983	14	969
Vâlcea	50	25	25

The dramatic situation of Pinzgau breed in Romania is reflected in the number of females registered in the herd book - Table 2.

Table 2. Number of cows registered in the herd book during 2005-2008 [5]

Herd book section breed	A	B	C	D	TOTAL
Brown	2032	3835	-	17050	22917
Romanian Simmental	2371	5263	375	38563	46572
Romanian Holstein Friza	7976	6522	39	45064	59601
Transylvanian Pinzgau	14	38	-	466	518
Buffolos	-	29	-	205	234

Situation of females entered in the Herd Book from different counties in Transylvania is presented in Table 3. Note that Bihor County has the highest number of females entered in Section D. We can underline that the situation of Pinzgau in Transylvania counties is dramatical, consequence of proportion of individuals entered in section D (crossbred).

Table 3. Number of cows registered in the herd book, from different counties during 2005-2008 [5]

County cod	County	Pinzgau breed				TOTAL
		A	B	C	D	
1	BIHOR	3	5		15	23
2	BISTRIȚA				9	9
5	MARAMUREȘ				2	2
6	MUREȘ				1	1
9	SIBIU				1	1
TOTAL		3	5		28	36

Note: - minimum 87.5% purity is in A, 75% B, 62.5% in C
- difference of up to 100% in A, B, C will be Red Holstein
- in D will enter crossbred at least 50% Pinzgauer, and Red Holstein and at least 25% Pinzgauer.

Distribution of Transylvanian Pinzgau animals of types of farms is presented in Table 4. Note that the largest weight of holding is owned by those of 1 or 2-3 heads, in subsistence farms. This confirms that the maintained of breed in exploitation was due to tradition and intuition of breeders.

Table 5 presents the number of lactation and average milk production (L), fat (G) and protein (P) on standard lactation and reproduction indicators (calving interval - CI and age at first birth - VP), depending on control lactation rank since 2008-2009.

Table 4. Number of recording farms from different counties according to the size of Pinzgau livestok, in 2009 [5]

County	The size of livestok								TOTAL
	1	2-3	4-7	8-15	16-31	32-63	64-127	>127	
BH	11	4	0	0	0	0	0	0	15
BN	12	3	0	0	0	0	0	0	15
CJ	1	0	0	0	0	0	0	0	1
MS	1	0	0	0	0	0	0	0	1
SM	1	0	0	0	0	0	0	0	1
SJ	5	0	0	0	0	0	0	0	5
SB	1	0	0	0	0	0	0	0	0

Milk production situation of Transylvanian Pinzgau in different counties of Transylvania region and according to the size of the herd (end of 2009) is presented in Table 6.

The data presented in Table 6, the conclusion that emerges is that the rule in terms of lactation number and size of holdings is owned by the county of Bistrita Nasaud.

These has a significant number of farms over the four heads that come out from under the stigma of subsistence farms. Also, noted higher average values for the quantity of milk in Bistrita Nasaud, probably due to a higher percentage of crossbred (Simmental x Pinzgau). The highest percentage of immigrant blood in Bistrita Nasaud (16.7%), explains the higher average amount of milk recorded here.

To consider the production main reason of Pinzgau breed drastic reduction number and risk status change is, in our opinion, an error. Not productive limitation is the reason.

We discus by the average values of milk production by 4100-4500 kg of lactations no III-IV, which provides a minimum value of economic efficiency of any holdings. If we add high quality of meat, the degree of adaptation to environmental mountain conditions, disease resistance (practically no leucosis), excellent recovery of alpine pastures, low concentrated demand, lower intake (a consequence of the waist), small investing shelters (tough/resistent breed), etc. we have a complete picture of a false problem.

Table 5. Romanian Pinzgau caracterisation in terms of productive and reproductive skills [5]

Lactation	No of lactations	L	G		P		VP		CI
		kg	kg	%	kg	%	mounth	days	days
1	17	4176	161	3,85	135	3,23	30	0	
2	28	4051	159	3,90	134	3,29			366
3	20	4046	155	3,83	130	3,21			345
4	28	4200	164	3,89	136	3,24			341
5	29	4279	169	3,93	141	3,29			367
6	22	4264	167	3,90	140	3,26			374
7	10	4139	160	3,87	135	3,27			378
8	9	4185	162	3,86	135	3,23			335
9	9	4480	171	3,81	143	3,19			390
10	5	4469	172	3,84	146	3,28			344
11	3	3760	143	3,78	121	3,30			345
Total	180	4188	163	3,88	137	3,26			359

Table 6. Number of lacations (N) and average milk production in Maturity Equivalent (L, kg) in different counties and according to the size of the farm (E) and percent of pure blood (%S) (source: ANARZ)

County	n.lact	E								Total	%S
	kg_L	1	2-3	4-7	8-15	16-31	32-63	64-127	>127		
BN	N	0	3	6	6	2	1	0	0	18	
	L	0	5069	5275	4571	4608	3403	0	0		83.3
MS	N	0	1	0	0	0	0	0	0	1	
	L	0	6136	0	0	0	0	0	0		100
SJ	N	0	1	0	1	0	0	0	0	2	
	L	0	3652	0	4298	0	0	0	0		50

In our opinion, the reason of Pinzagu vulnerability is fashion of indiscriminate “hollsteinisation and simmentallisation" of Romania, coupled with aggressive actions of economic agents involved in the importation of semen.

To these can be added: the lack of specialized education, the existence of "blinders" of specialists and total indifference to promote organic and sustainable agriculture, and conservation of biodiversity in all its aspects (matter that is an education issue also) [4].

In Romania, as the result of a series of works [1, 2, 3] cattle production comes in over 80% from subsistence farms, family farms, small commercial farms.

In Transylvania region the breed is about 1% of all cows exploited, consequence of "simentallisation". A very small number o farmers preserved traditions and structure of agricultural land area. In this area, as shown in the show until now, holding over 4 heads have the smallest weight. The shelters are simple construction, made of wood. Very rarely, cattle feed concentrates.

By moving (February-March 2012) in the Transylvania region (in different counties) we ask the breeders about the exploitation of the breed.

When the farmers were asked why exploited this breed, most (96%) responded that the situation was inherited from parents; 2% said that cows are resistant to disease; 2% said they products obtained from milk have special qualities.

CONCLUSION

Due to its rusticity qualities, resistance to the specific environment hilly and mountainous areas, with a remarkable productive longevity and survival, expression of her genetic distinct from other breeds, Transylvanian Pinzgau must be considered a component of national genetic resources. It should go immediately into active conservation program [3, 4]. Otherwise we will lose soon a valuable genetic reserve for livestock of Romania.

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MUSCLE CONTENT IN PIG CARCASSES OF DIFFERENT GENOTYPES

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Abstract

The objective of this experiment was to determine the effect of diverse production systems on pig performance, muscle characteristics, and their relation to pork quality measures. Carcasses were evaluated whit two methods : the Zwei Punkte method and UE reference method (hams, chests, shoulders and chops were dissected into bone, skin, fat and lean). The received results certify a higher quality of meat in experimental groups, with significant differences.

Key word: breed, carcasses, genotype, hybrid, meat.

INTRODUCTION

Nowadays, the preferences of pig breeders are aimed for achieving fast growing animals and low specific consumption, while the consumers and processors request housing with higher percentage of muscle tissue and qualitative meat. [5] state that hybrids are animals obtained after crossing two, three or even four races or groups of animals. In comparison with forms from which it were obtained, hybrids result from a larger production use more efficient food, are more resistant to diseases and have more important advantages. [2], say that crossing different races of pigs with different qualities create the heterosis phenomenon. Heterosis is a phenomenon which appears at the hybridization between two or more animal races with different heredity, having as a result individuals with special qualities and exceptional productivity especially the growth of vitality or adjustment power. Heterosis is caused by heterozygous and represent a high value towards character average between descendants, causing an excess of positive characters towards parents and which appears as a result of hybridization [1] [3] sustain that the value of carcasses is represented by the content of muscle tissue in carcass, protein composition and the ratio of essential aminoacids, like digestability and assimilation of protein in the body. Thus, it was made a study, in what concerns the determination of muscle tissue percentage in pig carcasses, of different genotypes.

MATERIAL AND METHOD

The experiment was accomplished in the unit of swine growth and weight gain, Vergecom, Hincesti, r. Moldova. To obtain hybrids as maternal form there were used sows of Yorkshire race and the paternal form represented by Hampshire, Landrace and Pietrain breed boars and biracial boars of Landrace and Pietrain. To obtain experimental biologic material, there were formed five lots of piglets which will be presented in the scheme below

Table 1. The scheme of the experiment

Lot	Parental forms		Result	
	maternal	paternal	sows	Piglets at fattening
I	Yorkshire	Yorkshire	6	15
II	Yorkshire	Landrace	6	15
III	Yorkshire	Hampshire	6	15
IV	Yorkshire	Landrace x Pietrain	6	15
V	Yorkshire	Pietrain	6	15

During gestation, sows were fed in analog conditions and forms, using combined feeds of complete value which provided the needs in nutrients. The feeding recipe represented : 35% of corn, 35% of barley, 10% of soy, 10 of marc, 4% of meat-bone meal, 3% of bran, 1.5% of forage chalk, 1% of premix, 0.5 % salt.

After slaugher weight was determined using the total mass

In order to establish the muscular mass percentage value two methods were used:

1. Caracas section using the EU reference method which derivates from the german DLG approach and dissection of the main parts (leg, shoulder, chest, chop). These parts were sectioned by separation the muscles, bones, skin and fat (subcutaneous & inter muscular). The calculation of the meat percentage in carcass is made using the following formula:

Y = meat percentage in carcas;
C = 1,3 (constant factor);
J = the section mass before dissection;
SSF = the mass of subcutaneous fat + skin;
IF = the weight of intermusculat fat;
B = the bones weight;
T = sirloin mass;
Σ = suma greutăţilor porţiunilor: pulpa, spata, cotletul şi pieptul;
12
Σ = total weight of 12 components

2. Method Z.P. (Zwei Punkte) consist in performing of 2 linear measurements on the carcas: the thickness of the fat layer covering the muscle „Gluteus medius" which includes the skin and meat thickness on a straight line between the medullary canal and the anterior tip of the „Gluteus medius" muscle.

Based on the 2 methods described above, the muscular percentage is calculated by following the algorithm:

RESULTS AND DISCUSSIONS

In similar nutritional and keeping conditions, the growth and developing capacity of the experimental piglet's carcases showed a wide range of results:

The previous tables results demonstrate a higher developing rate of muscular mass in the whole range of carcases for the Yorkshire X Pietrain breed as following: 64.92% for legs, 55.31% for chop, 60.54% for shoulder, 55.17% in the chest area. Opposite results were obtained from the pure Yorkshire experimental pigs – they showed the lowest muscular mass and the highest fat value

Significant muscular mass differences were recorded in the lots # 5 & 1 : 973g (B>0.999) & 929g (B>0.99) accordingly. This difference is explained by the fact that the Pertrain breed swine have a better developed ham, with a higher perimeter and weight compared to other breeds.

Both methods showed that the muscular mass percentage had fluctuated in each lot and had registered a higher value for hybrids obtained by using the X Landrace & Pietrain pure breed bores

Table 2. Morphological pulp structure related to pig's genotype

Lot		I	II	III	IV	V
Weight	g	8584±255	8992±104	9108±156	9365±201	9557±187**
Muscles	%	61,46	62.92	63.02	64.11	64.92
	g	5276±153	5658±76	5740±105	6004±123	6205±107***
Skin and fat under skin	%	22.39	21.58	21.73	21.04	20.55
	g	1922±64	1941±34	1980±44	1971±23	1964±31
Bones	%	10.27	10.08	9.83	9.71	9.62
	g	882±26	907±24	896±16	910±22	920±17
Inter-muscular weight	%	5.46	5.04	5.07	4.81	4.63
	g	469±15	454±21	462±18	451±15	443±17
Loss	%	0.40	0.35	0.32	0.30	0.26
	g	35	32	30	29	25

(B>0,99), *(B>0,999)

Table 3. Morphological structure of the chop

Lot		I	II	III	IV	V
Weight	g	5830±187	6145±191	5850±165	6376±144	6425±137
Muscles	%	47,92	52,72	51,82	54,48	55,31
	g	2794±98	3240±86	3032±56	3474±61	3554±56
Skin and fat under-skin	%	30,15	26,44	27,07	25,20	24,87
	g	1758±38	1625±26	1584±19	1607±24	1598±21
Bones	%	12,31	12,33	12,63	12,24	12,07
	g	718±37	758±25	739±17	781±15	776±20
Inter-muscular fat	%	8,97	7,92	7,89	7,49	7,14
	g	523±17	487±15	462±14	478±15	459±13
Loss	%	0,63	0,56	0,56	0,56	0,59
	g	37	35	33	36	38

Table 4. Morphological structure of the shoulder depending on the pig's genotype

Lot		I	II	III	IV	V
Weight	g	4612±151	4678±134	4639±178	4712±166	4745±211
Muscles	%	56,48	57,31	58,59	59,04	60,54
	g	2605±102	2681±112	2718±67	2782±89	2873±65
Skin and fat under skin	%	17,71	17,22	17,00	16,59	16,12
	g	817±36	806±41	789±45	782±37	765±28
Bones	%	11,96	12,03	11,79	11,73	11,14
	g	552±29	563±21	547±19	553±31	529±29
Inter-muscular fat	%	11,07	12,93	12,15	12,16	11,75
	g	511±18	605±13	564±22	573±26	558±11
Loss	%	0,58	0,49	0,54	0,46	0,42
	g	27	23	21	22	20

Tabelul 5. Morphological structure of the chest

Lot		I	II	III	IV	V
Weight	g	3907±112	3926±87	4142±93	4196±119	4281±54
Muscles	%	45,07	47,60	51,40	51,83	55,17
	g	1761±34	1869±22	2129±54	2175±37	2362±41
Skin and underskin fat	%	23,82	22,82	21,07	20,68	19,08
	g	931±26	896±15	873±33	868±17	817±28
Bones	%	7,62	7,53	7,55	7,65	7,14
	g	298±15	296±13	313±19	321±24	306±23
Intermuscular fat	%	22,42	21,11	19,12	18,99	17,82
	g	876±31	829±44	792±29	797±32	763±27
Loss	%	1,04	0,91	0,84	0,83	0,77
	g	41	36	35	35	33

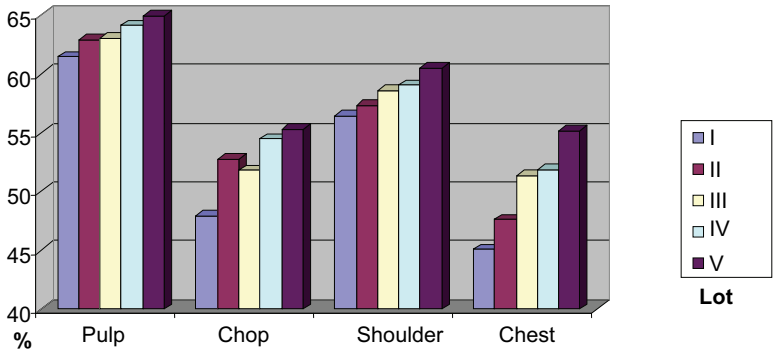

Fig. 1. Lean meat content in the dissection parts

Table 6. Percentage of muscular mass in experimental piglet's carcasses

Lot	E.U method.	Z.P Method	Class
I	49,08	49,37	R
II	51,32	50,85	U
III	51,87	52,08	U
IV	54,29	55,12	E
V	54,08	54,78	U

CONCLUSIONS

1. The swine genotype has a big impact over the growth energy and the productive capacity of the commercial hybrids used for crossbreeding.
2. The muscular mass assessment confirmed that meat percentage varied inside of each lot and had the highest overall value by using hogs of Pietrain X Landrace breed and Pietran pure breed hogs.
3. Carcases with the highest percentage of muscular tissue were recorded in the lot #4 with 55.12% E grade, followed by piglet carcases from the lot #5 with 54.78% U-grade.

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EFFECT OF USE OF PREMIX IN DAIRY COWS

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Abstract

The aim of our study was to analyze the effect of using premix on milk production and reproduction indicators. The study was conducted for a period of 12 months, in 10 small (up to 4 cows) and medium farms (about 6 cows), which are breeding Holshtein breed, in Lushnja and Fier districts. The data are showing that, cows that have received throughout lactation premix have produced 162 liters of milk more than those not treated with premix. Also, the days open period was 21 days shorter than cows not treated with premix and the insemination index was improved. Farmers note that cows consume premix are vital and have better appetite than the group of cows that do not take premix. Statistical data processing was done with Statgraphics Centurion XVI.

Key words: *Milk production, days open, insemination index, breed, management.*

INTRODUCTION

A successful nutrition program combines a proper balance of energy, protein, vitamins, and minerals [1]. Most milk producers in Albania are providing to their cows' proper levels of energy and protein however, often they overlook the minerals and vitamins. In general, the role of minerals and vitamins tends to be underestimated, because of the small proportion compared to other nutrients, and the false assumption that "micro" means less important than "macro"[3]. In addition, producers often do not see the clinical signs of trace mineral & vitamins deficiency described in text books. In many herds trace mineral levels are not deficient enough to cause these outward signs – but it is the sub-clinical deficiencies that may be the most costly to the producer because they often go unnoticed since they do not have an immediate impact on milk yield or growth and may take several months before reduced reproductive performance or impaired health appears [5]. Thirty years ago, balancing rations for dairy cows to meet maintenance and production requirements as far as energy, protein, vitamins, minerals and water was considered enough to prevent any deficiency or nutrient imbalance that could impact reproduction [2, 4]. In recent years, the relationship between nutrition and reproduction is a topic of increasing importance and concern among dairy producers, veterinarians, feed dealers and extension workers. Even though minerals have been an important component of a dairy cow ration, little is known about marginal effects of mineral deficiencies, imbalances orexcessive intakes.

MATERIAL AND METHOD

In this article, we'll explore the role of minerals and vitamins, and their deficiencies; especially trace minerals, focusing on milk production and reproduction only.

The difference in feeding between the control group (without premix) and experimental group (with premix) consisted that animals from experimental group in addition to the basic diet received 100 gr/daily of mineral and vitamin premix (or a proportion of 2-3% from the concentrate feed). Accelerated Genetics premixes are formulated to meet all the mineral and vitamin needs of dairy cattle. They can conveniently be added to any dairy ration or added along with any supplemental protein-grain mix. Dairy premixes contain the highest quality minerals and vitamins needed for optimal animal performance.

At formation of groups of cows were considered the following: performance for the

previous lactation, fat contents in milk, age of animals, and state of health.

Data collection: During 12 months were collected data on:

- Milk Production
- Open Days (Uterine repose)
- Insemination Index

Open Days represents the time interval, in days, from calving until the fecund insemination.

The Insemination Index represents the mean number of artificial inseminations performed in order to obtain a pregnancy.

The data from 10 small (up to 4 cows) and medium farms (about 6 cows), in total 59 cows which are breeding Holshtein breed, in Lushnja and Fier districts were processed.

Data statistic processing was carried out with Statgraphics Centurion XV.

RESULTS AND DISCUSSIONS

Milk production: The data against the type of farms are showing in the table below:

Table 1: Data on milk production, days open and insemination index

Farms		Control group			Experimental group		
		Yield (kg)	Day open	Ins. Index	Yield (kg)	Day open	Ins. Index
Small (20 cows)	Mean	4070	110	1,6	4220	89,7	1,3
	Range	3545-4568	82-157	1-3	3751-4654	65-124	1-2
Medium (39 cows)	Mean	4274	108,8	1,65	4449	87,8	1,43
	Range	3630-4700	79-165	1-3	3620-5445	67-127	1-3
Total (average)		4173	109,2	1,64	4335	88,4	1,39

Table 2: Summary statistics on milk yield

	Control group Milk yield--all cows	*Exp. Group Milk yield-all cows*
Count	28	31
Average	4201.43	4374.9
Standard deviation	338.26	393.079
Coeff. of variation	8.05108%	8.98487%
Minimum	3550.0	3620.0
Maximum	4700.0	5450.0
Range	1150.0	1830.0
Stnd. skewness	-0.887787	0.604215
Stnd. kurtosis	-1.06244	0.845615

This table shows summary statistics for the two samples of data. Of particular interest here are the standardized skewness and standardized kurtosis, which can be used to determine whether the samples come from normal distributions. Values of these statistics outside the range of -2 to +2 indicate significant departures from normality, which would tend to invalidate the tests which compare the standard deviations. In this case, both standardized skewness values are within the range expected. Both standardized kurtosis values are within the range expected.

Use of this premix for the cows' feeding showed positive influence on the balance of the ration as for contents of micro elements resulted to the increasing of the animals' milk.

While milk yield in control group of cows during the experimental period was 4173 kg per cow (4070 kg for the small farms and 4274 kg for the medium farms), in the experimental group it amounted 4335 kg milk per cow (4220 kg for the small farms and 4449 kg for the medium farms) or 162 kg more (3.9%) .

Photo 1. View from a Dairy Farm using premix in Lushnja district, Albania.

Reproduction

(a) Days open. The relationship between the level of milk production and reproduction is currently the subject of much debate. Data from some university research herds suggests that high producing cows do not have poorer reproductive performance than their lower producing herdmates [4].

The high incidence of the reproduction disorders in dairy cows is influencing the main reproductive parameters and causing important economic losses by not accomplishing the desired milk production. An increase in the number of days between

calving and conception is typically associated with reduced profitability in dairy cows. This reduction is partly caused by factors such as increased breeding cost, increased risk of culling and replacement costs, and reduced milk production.

This table below shows summary statistics for the two samples of data (days open for the cows with and without premix).

Table 3: Summary statistics on Days open

Of particular interest here are the standardized skewness and standardized kurtosis, which can be used to determine whether the samples come from normal distributions. Values of these statistics outside the range of -2 to +2 indicate significant departures from normality, which would tend to invalidate the tests which compare the standard deviations. In this case, both standardized skewness values are within the range expected. Both standardized kurtosis values are within the range expected.

(b) Insemination index. As is shown in Table 1 the insemination index for the cows treated without premix is 1,64 (range 1,6-1,65) while for the cows treated with premix is 1,39 (range 1,3-1,43).

According to **Smith and Chase,** higher producing cows tend to receive their first service later after calving, require more services per conception and have more days open. However, the heritability of reproductive traits is very low. Thus, it appears that we must look to the areas of physiology, nutrition and management rather than genetics for solutions to the reproductive problems encountered in today's high producing, intensively managed dairy herds.

Relation - Days open vs. Milk yield-no premix. The output shows the results of fitting a linear model to describe the relationship between Days open and Milk yield-no premix (total). The equation of the fitted model is

Days open = -21.942 + 0.0310415*Milk yield-no premix (total)

Since the P-value in the ANOVA table is less than 0.05, there is a statistically significant relationship between Days open and Milk yield-no premix (total) at the 95.0% confidence level. The R-Squared statistic indicates that the model as fitted explains 18.9446% of the variability in Days open. The correlation coefficient equals 0.435253, indicating a relatively weak relationship between the variables.

	Control group Day open	*Exp. Group Day open*
Count	28	31
Average	109.214	88.4516
Standard deviation	24.6762	18.5056
Coeff. of variation	22.5943%	20.9217%
Minimum	79.0	65.0
Maximum	165.0	127.0
Range	86.0	62.0
Stnd. skewness	1.7886	1.39982
Stnd. kurtosis	-0.455694	-0.722833

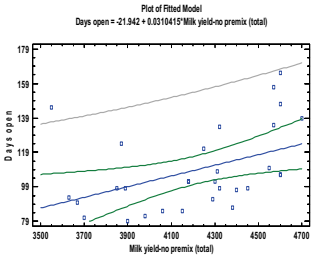

Fig 1. Relation between Days open vs. Milk yield-no premix-no premix

Relation - Days open vs. Milk yield-with premix. The output shows the results of fitting a linear model to describe the relationship between Days open and Milk yield-with premix (total). The equation of the fitted model is

Days open = -32.9244 + 0.0279533*Milk yield-with premix (total)

Since the P-value in the ANOVA table is less than 0.05, there is a statistically significant relationship between Days open and Milk yield-with premix (total) at the 95.0% confidence level. The R-Squared statistic indicates that the model as fitted explains 34.3823% of the variability in Days open. The correlation coefficient equals 0.586364, indicating a moderately strong relationship between the variables

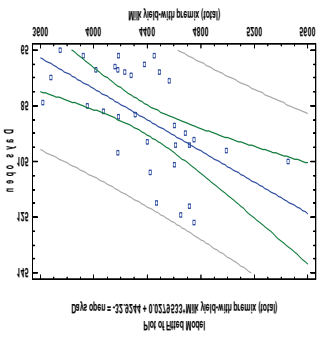

Fig 2. Relation between Days open vs. Milk yield-with premix

Relation - Insemination index vs. Milk yield-no premix. Since the P-value in the ANOVA table is greater or equal to 0.05, there is not a statistically significant relationship between Insemination index and Milk yield--all cows no premix at the 95.0% or higher confidence level. The R-Squared statistic indicates that the model as fitted explains 3.70027% of the variability in Insemination index. The correlation coefficient equals 0.192361, indicating a relatively weak relationship between the variables.

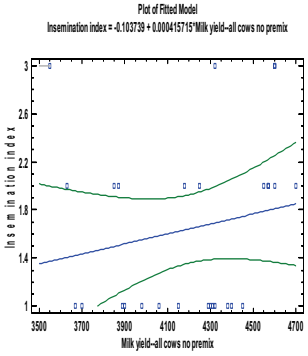

Fig 3. Relation - Insemination index vs. Milk yield-no premix

Relation - Insemination index vs. Milk yield-with premix. Since the P-value in the ANOVA table is less than 0.05, there is a statistically significant relationship between Insemination index and Milk yield--all cows with premix at the 95.0% confidence level. The R-Squared statistic indicates that the model as fitted explains 56.664% of the variability in Insemination index. The correlation coefficient equals 0.752755, indicating a moderately strong relationship between the variables.

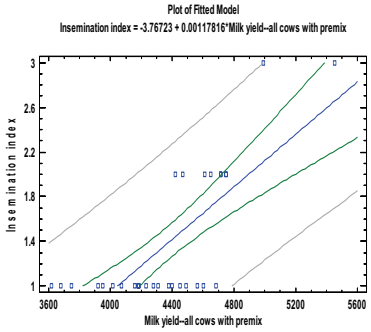

Fig 4. Relation between Insemination Index vs. Milk yield-with premix

CONCLUSIONS

- The experimental group (using premix in their diet) produced 162 kg more milk than the control group. In addition the experimental group had 21 days open shorter than control group and the insemination index was 1, 39 while in control group 1,64.
- The farmers' attention should be focused on close monitoring the pregnant cows, feeding and proper attendance of the cows during the dry period, calving assistance, the control of the puerperal period, heat detection and artificial insemination at optimal time.
- Producers should avoid the mineral and vitamin deficiencies and overfeeding. If a little bit is enough, twice as much will not be better and may in fact cause problems.
- Proper vitamin and mineral balance must be provided in dry cow rations when feed intake is restricted and (or) low quality forage is fed to control or reduce body condition. To ensure adequate intake, vitamins and minerals should be fed in small amounts of low energy concentrates or mixed in a complete dry cow ration.

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ACHIEVEMENT, TESTING AND EVALUATION A MATHEMATICAL MODEL TO OPTIMIZE SWINE NUTRITION

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Abstract

The paper presents a mathematical model for calculating the rules of energy and protein in growing pigs and fattening. Were used functions and parameters in the literature (Whittemore, Kyriazakis and Emmans), including our results. Based on this model is shown a procedure for calculating the feed rations. The method of mathematical modelling of the metabolic processes offers the possibility to assess feed allowances in their evolution, related both to the growth rate and to carcass quality. This paper presents an approach of this issue. Because of a given weight gain the quality of this gain is included in an interval ($\min\frac{Lr}{Pr} \le \frac{Lr}{Pr} \le \max\frac{Lr}{Pr}$, where Lr = daily retained lipids and Pr = daily retained protein), it results that feed allowance will be expressed as intervals of the form [$norm_{min}$, $norm_{max}$]. We obtain thus an infinity of possible values for the feed allowance. The choice of one possible value or of another one depends on the used raising technology, on the purpose of growing expressed in economic terms and/or in terms of a human-friendly food. In the present paper, besides the modality of calculating the intervals for the energy and protein allowance we shall also present formulas which, in our opinion, characterise a physiological evolution of the weight gain and of the lipid/protein ratio of the carcass.

***Key words*:** energy requirement, mathematical modelling, pig metabolic processes, protein requirement

MATERIAL AND METHOD

The following parameters should be taken into consideration when calculating the energy and protein requirements:
- body weight (W, g);
- intended final body weight (ΔW, g);
- ratio between the lean gain (protein + water) and the retained protein (α);
- ratio between the daily retained fat and protein ($\frac{Lr}{Pr} = \beta$);
- ratio between the daily gain of ash and protein ($\frac{Ashr}{Pr} = \gamma$);
- biological value of the diet (BV);
- environmental temperature (Ta);
- digestible energy per kg DM of diet (MJ/kg DM).

The body weight [kg], function of the age, is calculated with a Gompertz - type equation:

$$G = A \times e^{e^{B(t-t^x)}} \quad [kg] \quad (1)$$

where:

A = body weight at maturity
B = growth coefficient
t = age in days
t^x = inflexion point, i.e. the time in days when maximum gain is achieved

The net weight, *Gn* can be estimated with the formula:

$$Gn = G/1{,}05 \quad [kg] \quad (2)$$

and the net weight gain *ΔGn* is the sum of *Pr (retained protein), Lr (retrained lipids), Cen_r (retained ash)* and *Ar (retained water)*.

The values of *Pr*, were calculated with the following formula:

$$Pr = B \times Pt \times \ln\left(\frac{Pt}{Pt}\right) \quad [kg] \quad (3)$$

where *Pt*, kg is given by the formula:

$$Pt = \hat{Pt} \times e^{e^{B(t-t^x)}} \quad [kg] \quad (4)$$

Table 1. The following parameters

Type	Sex	B	Pt [Kg]	Pr [Kg]	(Lr/Pr) min
Familiar	M	0,0105	37,5	0,145	0,9
	F	0,0100	35,0	0,120	1,1
	C	0,0095	34,5	0,114	1,2
Comercial	M	0,0115	42,5	0,180	0,7
	F	0,0110	40,0	0,162	0,9
	C	0,0105	37,5	0,145	1,0
Elite	M	0,0125	47,5	0,218	0,5
	F	0,0120	45,0	0,199	0,7
	C	0,0115	42,5	0,180	0,8
Superelite	M	0,0135	52,5	0,261	0,4
	F	0,0130	50,0	0,239	0,5
	C	0,0125	47,5	0,218	0,6

The daily lipid gain *Lr* was calculated from *Lr/Pr* ratio.

$$Lr = Lr/Pr \times Pr \text{ [kg]} \quad (5)$$

where *Lr/Pr* has been calculated differently for the males, females and castrated pigs, according to their age (Burlacu et al., 1996):

For growing boars**:**

$$Lr/Pr = e^{-0,935 - 0,0288t + 0,000826t^2 - 0,00000616\ t^3 + 0,00000001\ 5t^4} \quad (6)$$

For gilts**:**

$$Lr/Pr = e^{-2,633+0,08t - 0,00142t^2+0,0000154t^3 - 0,0000000793t^4+0,00000000001513t^5} \quad (7)$$

For the castrated pigs**:**

$$Lr/Pr = e^{-2,074+0,06364t - 0,001317t^2+0,0000168t^3 + 0,00000009\ 77t^4+0,000000000206t^5} \quad (8)$$

The daily retained water and ash (Ar + Cenr) are calculated with the formula:

$$Ar + Cenr = \frac{Ar + Cenr}{Pr} \times Pr \quad \text{[kg]} \quad (9)$$

where $\frac{Ar + Cenr}{Pr}$ has the following values:

For growing boars and castrated pigs**:**

$$\frac{Ar + Cenr}{Pr} = e^{2,739 - 0,0434t + 0,000421t^2 - 0,000001325t^3} \quad (10)$$

For gilts**:**

$$\frac{Ar + Cenr}{Pr} = 37,423 \times e^{-\frac{t}{10,703}} + 4,154 \times e^{-\frac{t}{744,84}} \quad (11)$$

The net weight *Gn*, ([Kg] at the age *t* + 1) = Gn + (ΔGn at moment *t)*, where initial *t* = 35 days, and initial *Gn* = 9.5 Kg for all sexes and categories.

B) Estimation of *EM* norms

EM = EMm + EPr + ELr + Q' [MJ/day] (12)

For the calculation of:

EMm = requirement of metabolisable energy for maintenance [MJ/day]
EPr = requirement of metabolisable energy for body protein synthesis [MJ/ day]
ELr = requirement of metabolisable energy for body lipids synthesis [MJ/ day]

The following formulas are to be used:

$EMm = 1,75 \times Pt^{0,75}$ [MJ/ day] (13)

$EPr = 54,6 \times Pr$ [MJ/ day] (14)

$ELr = 53,3 \times Lr,$ [MJ/ day] (15)

where: Lr (lipid gain in kg.) = 1.1 $Pr^{0.07} \times$ Pr

C) Estimation of the norms for available protein and limiting amino acids

PA = Pm + Pr : 0.813 [kg] (16)
where:

Pm (net protein for maintenance)

$Pm = 0,04 \times Pt$, [kg] (17)

P_r = gain of body protein [kg] 0.813 is the output of PA use for Pr

- requirement of lysine = PA x 70, [g] (18)
- requirement of met. + cys. = PA x 40, [g] (19)
- requirement of triptophan = PA x 15, [g] (20)
- requirement of threonine = PA x 45, [g] (21)

RESULTS AND DISCUSSIONS

THE DIETS CALCULATION

We are often confronted in practice with situations when feeding is limited. In this situation (restricted feeding) we use a different way of calculation; following is the procedure for energy and amino acid requirement calculation:

Input data: Body age: *G* [kg]
Average daily gain: *ΔG* [kg]
Age: *t* [days]

Parameters: B, Pt, Pr , t^* - with the values and significance shown above.

Stage I: Calculation of the requirement for metabolisable energy and protein corresponding to the minimal *Lr/Pr* ratio.

The value of $\frac{Lr}{Pr}\min$ ratio was calculated on the basis of the experimental results:

$$\frac{Lr}{Pr}\min = a + \frac{b}{1+e^{-\frac{t-c}{d}}} \quad (22)$$

where for the castrated pigs we used the values:

a = 0.677; b = 1.95; c = 148; d = 23.63

The amount of retained protein is given in this case by:

$$Pr = \frac{\Delta G}{1{,}05\left(1+\frac{Lr}{Pr}\min+\frac{Ar+Cenr}{Pr}\right)} \quad [kg]$$

where:

ΔG - average daily gain [kg]

The above formulas are to be used for $\frac{Ar+Cenr}{Pr}$ Pt, Pm, PA, EMm, EPr, Lr, ELr, EM, LizD, M+CD, TRID, TREONINAD.

REMARK 1: Obviously, the important measures in determining the requirement of energy and protein, with this system of calculation, are: the metabolisable energy *EM* and the available protein *PA*.

The two stages of calculation presented above show one more fact, maybe striking at first sight, but perfectly justified physiologically: for restricted feeding and weight gains lower than the maximal ones the existence of variable values for *Lr/Pr* ratio.

$$\frac{Lr}{Pr} \in \left[\frac{Lr}{Pr}\min; \frac{Lr}{Pr}\max\right]$$

Involves the existence of norms belonging to intervals:

$$EM \in [EM_{min};\ EM_{max}]$$
$$PA \in [PA_{min}; PA_{max}]$$

In other words, for a fixed daily weight gain, for each value of $\frac{Lr}{Pr}$ there is a distinct norm of energy and protein.

The graphic presentation of Observation 1 is as follows:

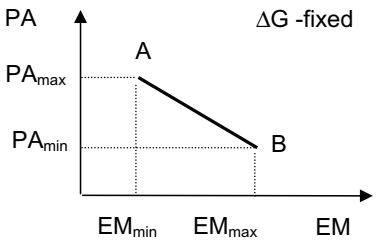

Fig. 1. The graphic presentation of Observation 1

Any pair *EM, PA* from segment *AB* represent pertinent values allowing achieving the set weight gain.

Obviously, each time the quality indicator given by the *Lr/Pr* ratio will be different.

REMARK 2: It may be readily observed that the protein norms evaluated with the system presented here eliminate the value of the digestible protein. However, diet optimization involves the essential use of an equation with *PBD* (digestible crude protein).

The connection between *PBD* and *PA* is given by the biological value of the diet:

$$VB = \frac{PA}{PBD} \Rightarrow PBD = \frac{PA}{VB}, \quad 0 < VB < 1$$

As *VB* can not be known beforehand, it results that the norm of *PBD* depends on the nature and structure of the raw diet ingredients; since the value of *PBD* is no longer unique, it can no longer be used traditionally in the "tables of norms" even though diet optimisation is still done at the level of the digestible nutrients.

Stage II. Calculation of the requirement for metabolisable energy and protein, corresponding to the maximal *Lr/Pr* ratio.

We calculate the maximum intake of metabolisable energy:

$$EM_{max} = 44\left(1 - e^{-0{,}0204\ G}\right) \quad [MJ]$$

We calculate the maximum amount of retained protein with the formula:

$$Pr_{max} = B \cdot Pt \cdot \ln\frac{Pt}{Pt}$$

With the formula

$$EPr = 54{,}6 \times Pr \quad [MJ/day]$$

We compute the energy required to retain the protein corresponding to *Pr* max.

We calculate the energy required to retain the lipids:

$$ELr = EM_{max} - EM_m - EPr - Q, \quad [MJ]$$

Hence the maximal amount of retained lipids:

$$Lr_{max} = \frac{ELr}{53,3} \qquad [kg]$$

Thus, we obtained the maximal ratio retained lipids to retained protein:

$$\frac{Lr}{Pr}max = \frac{Lr_{max}}{Pr_{max}}$$

Further, we use the same procedure as in stage I starting with the calculation of *Pr* inclusive.

Figure 2 shows the dependence of *PBD* requirement function of the biological value of the diet.

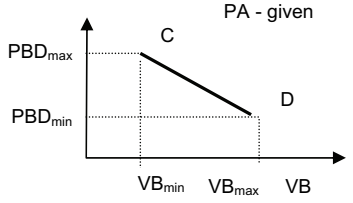

Fig. 2 The dependence of *PBD* requirement function of the biological value of the diet

Figure 3 shows the dependence of *PBD* requirement function of the available protein.

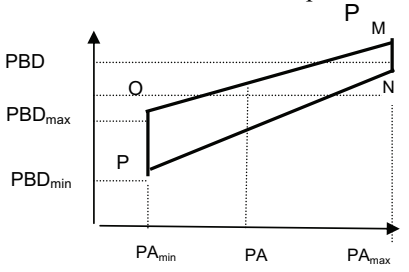

Fig. 3. The dependence of *PBD* requirement function of the available protein

Figure 4 shows the relation between *EM* and *PBD*.

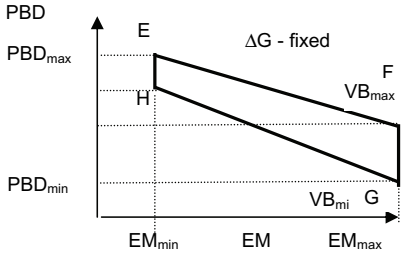

Fig. 4. The relation between *EM* and *PBD*

Any point on *EFGH* trapezium is a norm expressed in *EM, PBD* for the set ΔG.

The existence of an area *EFGH* for the requirement of *EM* and *PBD* is due to the two parameters $\frac{Lr}{Pr} \in \left[Lr/_{Pr}min, \frac{Lr}{Pr}max \right]$ and VB $\in$ [VBmin, VBmax].

REMARK 3: For simplification, the tables may show the average values for *EM* and *PA* (and therefore for the amino acids too).

$$EM_{tabel} = \frac{EM_{max} + EM_{min}}{2}$$

$$PA_{tabel} = \frac{PA_{max} + PA_{min}}{2}$$

CONCLUSIONS

The method of mathematical modelling of the metabolic processes offers the possibility to assess feed allowances in their evolution, related both to the growth rate and to carcass quality. This paper presents an approach of this issue. Because of a given weight gain the quality of this gain is included in an interval ($\min\frac{Lr}{Pr} \le \frac{Lr}{Pr} \le \max\frac{Lr}{Pr}$, where Lr = daily retained lipids and Pr = daily retained protein), it results that feed allowance will be expressed as intervals of the form [$norm_{min}$, $norm_{max}$]. We obtain thus an infinity of possible values for the feed allowance. The choice of one possible value or of another one depends on the used raising technology, on the purpose of growing expressed in economic terms and/or in terms of a human-friendly food. In the present paper, besides the modality of calculating the intervals for the energy and protein allowance we shall also present formulas which, in our opinion, characterise a physiological evolution of the weight gain and of the lipid/protein ratio of the carcass.

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GROWTH AND DEVELOPMENT OF BROILER CHICKENS UNDER THE USE OF THE ADSORBENTS "PRIMIX-ALFASORB" AND THE PROBIOTICS "PRIMIX-BIONORM-K" IN MIXED FODDERS

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Abstract

The research undertaken in order to determine the optimal dose and efficacy of the use of additives "Primix-Alfasorb" and "Primix-Bionorm-K", was conducted on broiler chickens of the meat hybrid COBB-500, in the laboratory of the Department of General Animal Husbandry of the State Agrarian University of Moldova, in the period from 11.18.2011 to 30.12.2011. To conduct the experiment on the basis of analogies, five groups of clinically healthy one day-old chickens of the Cobb 500 hybrid, 21 head each, were formed. The first group served as the control group, and received the basic diet; in the fodder for the second and the third groups, the experimental ones, the enterosorbent "Primix-Alfasorb" was added – 0.2 and 0.4 kg/t respectively; in the fodder for the fourth group the probiotic preparation "Primix- Bionorm-K" in quantities of 0.2 kg/t was added, and the fifth group received both probiotic and the adsorbent in quantities of 0.2 kg/t. In the trial the average live weight of the broiler chickens, which received the supplement "Primix-Alfasorb" in quantities of 0.2 and 0.4 kg per 1 t in EG_1 and EG_2, amounted to 1995.62 g and 2060.10 g respectively, with an average daily gain of 46.37 and 47.89 g, which was higher than in the control group by 2.20 and 5.55%. The supplementation of the fodder for the broiler chickens with the additive "Primix-Bionorm-K" at the level of 0.2 kg/t, increased the live weight of the broiler chickens in the EG_3 by 10.40% ($P \leq 0.01$), the average daily gain by 10.62%, while the cost of the fodder per 1 kg of gain decreased by 7,94% compared with the control group. When both "Primix-Alfasorb" and "Primix-Bionorm-K" were added, the broiler chickens' live weight in the EG_4 in the scientific and economic trial, was of 1967.95 g, which was higher than the same index in the control group by 0.70 %. It was determined, that the optimal rate of enterosorbent "Primix-Alfasorb" input in the diets for broiler chickens was of 0.2 kg/t, and of the "Primix-Bionorm-K" of 0.2 kg/t.

Key words**:** *broiler chicken, productivity, probiotic, adsorbent, fodder additives*

INTRODUCTION

In the present conditions of the industrial poultry breeding, the veterinary load on the body of poultry significantly increases under the development of intensive production technologies. Due to the fact, that international organizations prohibit the use of antibiotics in animal husbandry, there emerges the necessity of studying and use of analogues, which do not produce harmful effects on animals and humans.

Nowadays, of all the fodder additives, probiotics are the most important. They are biological preparations, consisting of living organisms or their products of fermentation, with antagonistic activity against pathogenic and unwanted microflora in the animals' intestines [4].

The mechanism of their action is based on the species specificity of bacteria and their composition. There are probiotics composed of one type of bacteria (monoprobiotics), or associations of several strains - "associate probiotics," or "simbiotics", which derives from the word symbiosis, representing either a liquid suspension or dry powder.

It is known that the use of microbial drugs in animal husbandry improves fodder efficiency, accelerates the growth of animals, their productivity, and reduces production costs and the number of cases of illness and death [10, 11, 14, 15, 7, 8, 16].

Currently, probiotic preparations may replace antibiotics in mixed fodders for young poultry, in order to improve digestion, to speed up the adaptation of animals to diets, to increase the

efficiency of fodder use and productivity of animals, and to prevent and treat gastrointestinal diseases.

The new probiotic fodder additive "Primix-Bionorm-K" was produced by Ltd "Ariadne" (Ukraine) on the basis of freeze-dried cells, specially selected for resistance to antibiotics, with a pronounced antagonism to pathogenic microflora - 14 strains of lacto- and bifidobacteria with an activity of $1x10^6$CFU/g contains a prebiotic - fructooligosaccharides, B vitamins, pectin and natural acidifier. The drug has no analogue, as the microorganisms which compound it are protected from the effects of gastric juice and bile acids.

When fodders and other agricultural products were analyzed, a high pollution of microscopic fungi (80-100%) was identified; in the majority of cases (40-60%) they were toxigenic [5].

Studies show, that animal husbandry supports serious economic losses, because of the reduced productivity and the reproduction of farm animals, arising from mycotoxicosis [2].

Nowadays, more than three hundred mycotoxins are known, and most of them have toxic effects on animals and poultry. Because of their accumulation, mycotoxins gradually destroy the animals and poultry's immune system. This effect is specific to almost all mycotoxins, but its detection is practically impossible without the use of special methods.

Due to the fact, that it is practically impossible to completely prevent the contamination of fodders with microscopic fungi and mycotoxins, a promising way of the improvement of the usefulness of animal fodders is the addition of drugs with adsorbent action to the fodders [13].

Adsorbents are among the drugs that contaminate the mycotoxins and eliminate their waste products, preventing their deleterious effects on the body. Chelators a new generation of drugs for the prevention and treatment of mikotoksycosis, with sorption and detoxifying properties, are based on the principle of complete binding and removal of mycotoxin by the instrumentality of complex components that have different mechanisms of action, and are directed against different groups of toxins.

The enterosorbent "Primix-Alfasorb" has sorption properties, it is a fodder additive (manufactured at the Ltd "Ariadne", Ukraine) which contains cellulose, hemicelluloses, lignin and pectin, and has a high activity. It is a complex of activated biopolymers, which has been deeply processed and activated.

Since lignin is a natural polymer having an irregular structure, being covalently bound to cellulose and hemicellulose, it forms the cell walls of plants. The greatest amount of lignin is found in the stems of grasses and grain surface membranes, especially buckwheat [3].

Probiotics "Monosporin" and "Batsell" are also used to reduce the harmful effects of mycotoxins, to prevent and treat infectious diseases in poultry, as well as to improve the nutritional value of fodders [17].

According to [9], the addition of probiotic Subtilis MK to the mixed fodder increased the safekeeping of chicken stock by 7.6% and the live weight by 1%.

At the same time, in the literature there are practically no data on the use of drugs of sorption nature in combination with probiotic complexes, in order to improve the implementation of the biological resources of broiler chickens. Such a complex application would contribute to the reduction of the acid-binding ability of fodders, to their disposal of mycotoxins, to the improvement of gastro-intestinal microflora, and, consequently, to the reduction of the toxicological load on the body, improving safekeeping, poultry productivity, as well as the quality and ecological purity of the production.

MATERIAL AND METHOD

The scientific research into the determination of the optimal dose and efficacy of the use of additives "Primix-Alfasorb" and "Primix-Bionorm-K" were conducted using broiler chickens of the meat cross COBB-500 in the laboratory of the department of General Animal Husbandry of the Agrarian State University of Moldova, in the period from 18.11.2011 to 30.12.2011. For the trial on the basis of analogues (by the method of [1]) five groups of one day-old chickens, twenty-one head each, were formed (Table 1).

Table 1. Scheme of the trial on broiler chickens

Group	Number of heads	Feeding properties
CG	21	Main mixed fodder (MF)
EG_1	21	MF + 0.2 kg/t Primix-Alfasorb
EG_2	21	MF + 0.4 kg/t Primix-Alfasorb
EG_3	21	MF + 0.2 kg/t Primix-Bionorm-K
EG_4	21	MF + 0.2 kg/t Primix-Bionorm-K + 0.2 kg/t Primix-Alfasorb

The chickens in the control group received the basic diet; the broilers in the experimental groups received the probiotic or enterosorbent in addition to the basis diet. The feeding was carried out using complete and balanced mixed fodders, in accordance with the accepted feeding standards [6].

For the chickens in the control and experimental groups the same conditions of keeping were created, in accordance with zootechnical requirements.

Throughout the entire trial the poultry were weighed individually every week. A daily record of the consumed fodder was made.

The obtained data were statistically processed [12].

RESULTS AND DISCUSSIONS

During the trial on the determination of the effectiveness of the influence of the adsorbent "Primix-Alfasorb" and probiotic "Primix-Bionorm-K" on the productive quality of broilers, and the identification of the optimal dose of supplementation, the fodders recommended for Cobb-500 cross were used, depending on the age of chickens, and that were balanced in all nutrients (Table 2).

Table 2. Nutritional value of the mixed fodders

Indices	Starter	Growth	Finisher
Fattening period in days	0 - 10	11 - 22	23 - 42
Energy, Kcal/kg	2988	3083	3176
Crude protein, %	21.00	19.00	18.00
Lysine, %	1.20	1.10	1.05
Digestible lysine, %	1.08	0.99	0.95
Metionin, %	0.46	0.44	0.43
Digestible metionin, %	0.41	0.40	0.39
Methionine + cystine, %	0.89	0.84	0.82
Methionine + digestible cystine, %	0.80	0.75	0.74
Triptofan, %	0.20	0.19	0.19
Treonin, %	0.79	0.74	0.72
Arginin, %	1.26	1.17	1.13
Calcium, %	1.00	0.96	0.90
Digestible phosphorus, %	0.50	0.48	0.45
Sodium, %	0.20	0.17	0.16
Chlorine, %	0.20	0.20	0.20
Linoleic acid, %	1.25	1.25	1.00

According to the results of weekly control weighing, it was found out that the used drugs had an effect on the productive qualities of the broiler chickens.

The indices of the dynamics of live weight showed a positive effect of the adsorbent "Primix-Alfasorb" and the probiotic preparation "Primix-Bionorm-K" on the growth of broiler chickens (Table 3, fig. 1).

Photo 1. Keeping of broiler chickens in the trial

Photo 2. Individual weighing

Table 3. Dynamics of the live weight of broiler chickens in the scientific and economic trial

Groups	The live weight of broiler chickens, g						
	at the beginning of the trial	7 days	14 days	21 days	28 days	35 days	at the end of the trial
CG	48.62±0.800	111.29 ± 3.993	304.95 ± 12.369	612.38 ± 16.487	1055.95 ± 25.639	1434.00 ± 31.410	1954.24 ± 46.842
EG_1	48.05±0.927	121.52 ± 3.907	317.71 ± 17.047	641.38 ± 24.824	1051.57 ± 35.982	1448.62 ± 46.712	1995.62 ± 57.034
EG_2	48.81±0.764	131.24 ± 3.632	331.24 ± 12.525	637.43 ± 19.536	1024.05 ± 35.701	1503.91 ± 47.739	2060.10 ± 60.210
EG_3	49.48±1.018	130.33 ± 3.704	326.57 ± 10.695	647.38 ± 12.382	1123.05 ± 20.713	1582.62 ± 34.565	2157.52 ± 34.263
EG_4	48.76±0.532	115.95 ± 2.662	298.24 ± 9.486	587.71 ± 13.532	998.52 ± 19.760	1460.67 ± 30.779	1967.95 ± 33.686

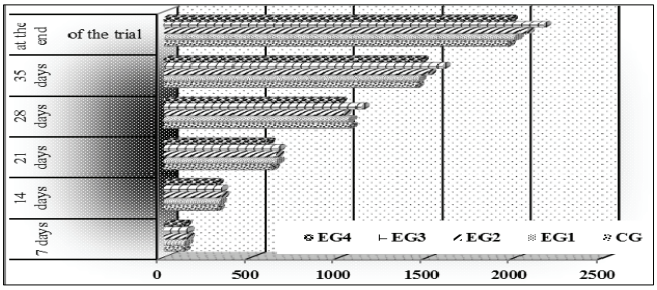

Fig.1. The live weight of broiler chickens by periods of growth

When the investigational adsorbent was added to the mixed fodder for the chickens in the experimental groups (EG_1 and EG_2), an increase in the live weight of chickens was observed, in comparison with the control group, respectively by 41.38 and 105.86 g. The highest rate of growth was in the poultry in the experimental group EG_2, whose mixed fodder was supplemented with the adsorbent "Primix-Alfasorb" at the level of 0.4 kg/t. The chickens in this group surpassed the chickens in the control group concerning the increase of live weight by 5.42%. The chickens in the group EG_3, which received the probiotic "Primix Bionorm-K" at the level of 0.2 kg/t surpassed their analogues in the control group concerning the average daily gain on the 7, 14, 21, 28, 35 and 42-th day, respectively by 19.98, 1.33, 4.35, 7.24, 21.56 and 10.51%, and at the end of the growth period the indices on live weight in this group were higher compared with the control group by 10.4%. When the preparations of probiotic and adsorbent were used together (EG_4), no significant changes in the dynamics of live weight of chickens in the trial were observed, and the daily gain of broilers on the 14, 21 and 28-th days was lower in comparison with the analogues in other experimental

groups, as well as in comparison with the control group, and the differences were, respectively, by 5.88, 5.84, and 7.39% (Table 4, fig. 2). During the whole trial, the daily gain of chickens in this group was slightly higher compared with the control group (0.71%).

Table 4. The dynamics of the absolute and average daily weight gain chickens - broilers in the trial

Groups Data	The average live weight gain							
	25.11.2011	02.12.2011	09.12.2011	16.12.2011	23.12.2011	30.12.2011	the whole for the experience	
	average daily live weight gain						absolute	average daily live weight gain
CG	9.571	27.667	43.918	63.367	54.007	74.320	1905.619	45.372
EG_1	11.204	28.027	46.238	58.599	56.721	78.143	1947.571	46.371
EG_2	11.306	28.571	43.741	55.231	68.551	79.456	2011.286	47.888
EG_3	11.483	28.034	45.830	67.952	65.653	82.129	2108.048	50.192
EG_4	10.082	26.041	41.354	58.687	66.020	72.469	1919.190	45.695

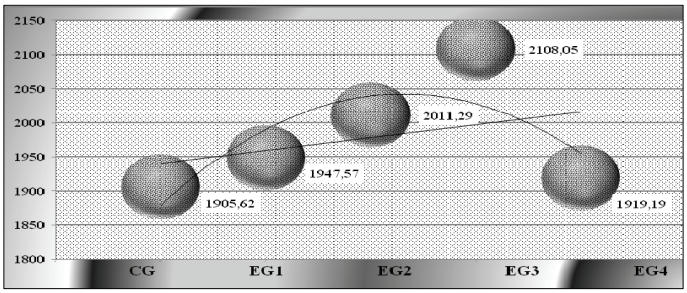

Fig.2. The dynamics of the absolute weight gain per one chicken during the trial, g

The supplementation of the mixed fodder for broiler chickens with the drugs "Primix-Alfasorb" and "Primix-Bionorm-K" within 42 days, contributed to the decrease of their fodder consumption (Table 5, fig. 3), at the same time the fodder consumption per 1 kg of live weight gain of the experimental chickens was lower, because they used the fodders more effectively in comparison with the control group (Table 6).

Table 5. Fodder consumption by broiler chickens in the trial, g

Weeks	Groups				
	CG	EG_1	EG_2	EG_3	EG_4
1	4045	4103	3671	3725	3276
2	10073	9458	9953	10762	8416
3	11850	11099	10304	11138	11299
4	13710	13489	13873	13597	11987
5	17003	17218	18332	18046	17608
6	20480	21737	21446	21316	21605
During the trial, g	77161.000	77104.000	77579.000	78584.000	74191.000
During the trial, %	100.00	99.93	100.54	101.84	96.15
Difference, %	100.00	-0.07	+0.54	+1.84	-3.84

Table 6. Fodder consumption per 1 kg of weight gain of chickens in the trial

Indices	Groups				
	CG	EG_1	EG_2	EG_3	EG_4
Increase in body weight during the trial, g	1905.62	1947.57	2011.29	2108.05	1919.19
Fodder consumption per 1 kg of weight gain, kg	1.93	1.89	1.84	1.78	1.84
The difference in fodder consumption for weight gain, kg	-	-0.04	-0.09	-0.15	-0.09
Fodder consumption for weight gain,%	100	97.93	95.34	92.23	95.34

The best results on fodder conversion were observed in EG_3, which received Primix-Bionorm-K" at the level of 0.2 kg/t.

Cost-effectiveness of the use of the adsorbent "Primix-Alfasorb" at the level of 0.4 kg/t (EG_2), and the probiotic preparation "Primix-

Bionorm-K" at the level of 0.2 kg/t (EG_3) in the mixed fodders for broiler chickens per group compared with the control group during the trial was 3.04 and 5.11 dollars respectively (Table 7, fig. 4).

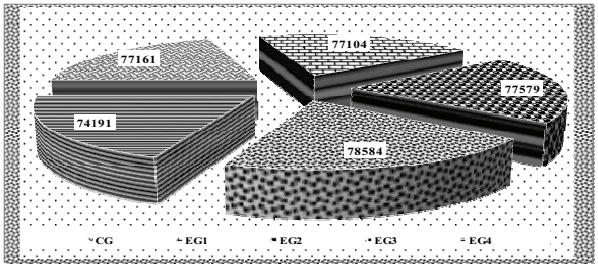

Fig.3. Fodder consumption by broiler chickens by growth periods, g

Table 7. Cost-effectiveness of the use of additives in the mixed fodders for broiler chickens

Indices	Groups				
	CG	EG_1	EG_2	EG_3	EG_4
Absolute weight gain per one chicken during the trial, g	1905.62	1947.57	2011.29	2108.05	1919.19
Absolute weight gain of the group during the trial, kg	40.02	40.90	42.24	44.27	40.30
Selling price of 1 kg of live weight of broiler chickens, dollars	1.75				
Fodder consumption per group during the entire period, kg	77.16	77.10	77.58	78.58	74.19
The price of 1 kg of fodder (on average), dollars	0,62				
The cost of the mixed fodder consumed in the trial, dollars	47.84	47.80	48.10	48.72	46.00
The quantity of the preparation consumed in the trial, kg	-	0.015	0.031	0.016	0.015/0.015
Price of the preparation used in the trial, dollars	-	18.75		90.00	18.75/90.00
Nominal income per group during the trial, dollars	22.20	23.50	25.24	27.31	22.90
The difference in the conditional income per group compared with the control group during the trial, dollars	-	1.30	3.04	5.11	0.70

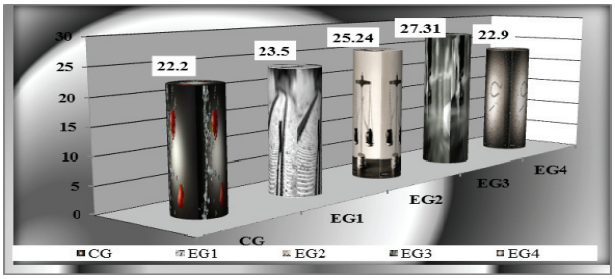

Fig.4. Nominal income per group during the trial, dollars

CONCLUSIONS

- In the scientific and economic trial the average live weight of broiler chickens in EG_1 and EG_2, which received the additive "Primix-Afasorb" in an amount of 0.2 and 0.4 kg per 1t, was respectively of 1995.62 and 2060.10 g, with the average daily gain of 46.37 and 47.89 g, which was higher than in the control group by 2.20 and 5.55%.
- The fodder consumption per 1 kg of live weight gain of broilers in the scientific and economic trial, when using fodders containing "Primix-Alfasorb" in an amount of 0.2 and 0.4 kg/t, was of 1.89 and 1.84 kg vs. 1.93 kg in the control group.
- In the scientific and economic trial it was established that the optimal input rate of the enterosorbent "Primix-Alfasorb" into the diets for broiler chickens was of 0.2 kg per 1 ton of fodder.
- The supplementation of the fodder for broiler chickens with the additives "Primix-Bionorm-K" at the level of 0.2 kg/t increased the live

weight of broiler chickens by 10.40%, the average daily gain by 10.62% ($P \leq 0.01$), while reducing the fodder consumption per one kg of growth by 7.94% compared with the control group.

- When both "Primix-Alfasorb" and "Primix Bionorm-K" were added (in the quantity of, respectively, 0.2 kg per 1 ton), the average live weight of broiler chickens in EG_4 was of 1967.95 g, which was higher than the same index in the control group by 0.70%.

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ASSESSMENT NUTRITIONAL VALUE AND EFFICIENCY FOR USE OF A NEW SOURCE OF VEGETABLE FODDER

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Abstract

Fodder sources for livestock sector diversification is always present a problem in Moldova. Viticulture industry in our country occupies an important place in agriculture and annually the result of processing of grapes obtained large quantities of such waste as: grape marc, peel grapes, wine lees and others. These wastes can serve as a very precious source of feed for farming animals. Currently, waste from processing of grapes is used in other fields - medicine, food industry. As a result of these processing products are obtained comparative new and less studied, such as cake or macuhul (cake) made of grape seeds, shell beans and others. The aim of our research was to studied nutritional value and the possibility use macuhului (cake) made of grape seed in nutrition of young cattle and appreciation the optimal level of inclusion of this product in the composition combined fodder. Macuhul (cake) made of grape seeds is a precious waste of processing industry can be successfully used in rations of farming animals because it contains a sufficient level of protein (10.5%), lipids (5.2), sugar (9.8%), macro-and microelements. Taking into consideration the productivity of animals, ingestion level of feeds, blood indices, we believe that the optimal level of inclusion of the macuhului (cake) made of grape seeds in the composition combined fodder for young cattle is 5 to 10%.

Key words: *diversification fodder sources, cake of grape, combined fodder.*

INTRODUCTION

The diversification of fodder sources Fodder for livestock sector is always actual problem in Moldova. Industry of viticulture in our country occupies an important place in agriculture and in results of processing of grapes annually obtained large quantities of such waste as: grape marc, peel grapes, wine lees and others. These wastes can serve as a very precious source of feed for farm animals because, for example, the nutritional value of 1kg of dry pomace is 0,5 to 0.6 A, 11-13% crude protein, 9-10% fat gross and 28-30% crude fiber. Of mineral substances in a kg of pomace contained: 9,7 g calcium, 2,5 g phosphorus, 250-300 mg iron, 0,15 to 0,17 mg cobalt, 19,3 to 21 mg copper, 39, 5 to 42,0 mg of manganese, 32,5 to 34,2 mg of zinc [4, 5, 6, 7].
At present the waste from grapes are used in other fields - medicine, food industry. As a result of these processing are obtained new and less studied secondary products such as cake or macuhul (cake) from grape seed, peel beans and others [1].
The aim of our research was to study the nutritional value and the possibility to use the macuh (cake) from grape seed in nutrition of young cattle and appreciation the optimal level of inclusion of this product in the composition combined fodder.

MATERIAL AND METHOD

The experiment was conducted according to the cattle breeding farm of the Scientific and Practical Institute by Biotechnology in Animal Husbandry and Veterinary Medicine on three lots of calves, each of 5 heads each age 4-6 months.
In the selection of animals for experience were taken into consideration the following indices: date of birth, body weight, sex, health status.

After these factors were fairly homogeneous groups, calves in the first group having the average age the 122,6 days in second group 125,5 and 123,4 days in the third group, body weight was, appropriately, 111,4; 110,4, and 110,6 kg.

Voluminous fodder (hay, silage) were distributed in the equal amounts in all groups, evidence of feed are made daily with weighing in the medium in groups. Particularities of nutrition is in fact the animals in group I (control) received in addition to the basic fodder ration after a standard recipe, in second group (experimental) combined fodder containing 5% macuh (cake) from grape seed, and in group III (experimental) this figure increased to 10%.

Evidence of Productivity was made by weighing individual calves - monthly. The blood biochemical and morphological indices were studied twice during the experience.

RESULTS AND DISCUSSION

For carrying out of the experience was prepared the required amount of standard combined fodder include traditional ingredients for Moldova and vitamin-mineral premix "Bujorel".

The receipt of combined fodder differed between groups only after content of macuh (cake) from grape seeds (Table 1)

Table 1. Recipes combined feed for young cattle aged 4-6 months, with the inclusion of macuh (cake) from grape seeds, used in the experiment (%)

Ingredients	**Combined fodder**		
	no.1 control	no.2 experimental	no.3 experimental
Barley	58	53	48
Corn	5	5	5
Wheat	10	10	10
Peas	10	10	10
Macuh from sunflower	15	15	15
Macuh from grape seeds	-	5	10
Premix „Bujorel”	1	1	1
Kitchen salt	1	1	1

The difference between these recipes consists only in using in recipe No.2 of the 5% macuh (cake) from grape seeds and 10% in the recipe No 3. Also in the same proportion decreased content of barley, so in the combined experimental fodder barley No.2 and No.3 in the proportion of 5:10% was replaced with macuh from grape seeds. This was opportune the contents and fairly high in protein, lipids and carbohydrates of the waste, extremely important nutrients during the growing young cattle (Table 2).

Table 2. The chemical composition of combined fodder and raw materials

Indices	Combined fodder			barley	macuh from grape seeds
	no. 1 0% macuh from grape seeds	no. 2 5% macuh from grape seeds	no. 3 10% macuh from grape seeds		
Humidity, %	10,2	10,1	10,1	9,0	9,1
Lipids,%	4,5	4,54	4,7	2,34	5,2
Protein, %	12,6	13,0	13,5	9,6	10,5
Sugar ,%	4,4	4,9	5,3	2,2	9,8
Starch,%	53,3	52,98	53,04	53,65	2,12
Crude ash,%	4,02	4,14	4,27	2,43	2,95
Crude fiber,%	5,41	6,83	7,53	5,38	26,5

Data of Table 2 shows that protein content increases from 12,6% in combined fodder witness to 13,0% in combined fodder No.2 and up to 13,5% in combined fodder No.3. The differences are caused by the fact that No. 2 and No.3 combined fodder the barley was replaced at a rate of 5 and 10% respectively with macuh (cake) from grape seed has a higher protein content – 10,5% compared to 9,6% in barley.

For the same reasons in combined experimental fodder sugar content increases (from 4,4 to 5,3%, crude fiber (from 5,4 to 7,53%) and crude ash (from 4,02 to 4,27 %).

Were used during the experimental rations identical in all groups, which included kg / head / day: combined fodder, 2,0, vetch hay + oat – 0,7-0,8, alfaalfa hay – 1,6 to 1,7, green alfaalfa mass + corn -2,7 to 2,9.

After rations nutrient content of all groups were balance and meet the requirements of the food standards of these animals.

One of the basic indices was studied during experimental animals was productivity (Fig. 1).

The individual weighing of calves effectuate for three times during the of the experience

show a gradual increase in productivity in group I control from 560 g average daily gain in the first weighing up to 785,2 g in the second and third weighing 1076,9 g. In two experimental groups these indices were, corresponding: 550; 748,1 and 1230,8 g. Similar results were obtained in the third group – 590,0; 837,0; 1046,2 g

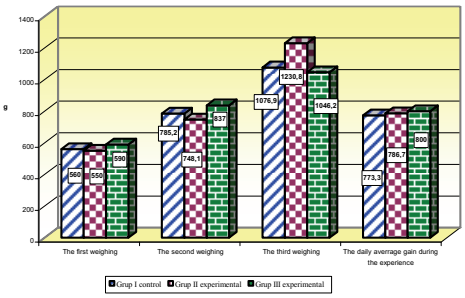

Fig.1. Dynamics of average daily gain, g

In medium in during the experimental control group was obtained the productivity to 773,3 ± 64,18 g daily growth, II experimental 786,7 ± 42,25 g and III experimental group – 800,0 ± 50,0 g

So, no essential differences in animal productivity occurred between lots the indifferent recipes of combined feed used.

At the beginning and end were studied of the experience some blood indices of animals involved in the study (Table 3).

We mention that the results demonstrate good health of animals in all groups, because all indices studied were within physiological norms as both the beginning and the end of the experience.

Essential differences between the groups studied indexes were not detected, although it may be noted a tendency to increase in the controls at the end of the experience so indicates that hematocrit 37.8% compared to 35 - 35.2% in experimental groups. Hemoglobin also has a higher index - 124.6 g / l in the control group from 115.2 to 116.9 in experimental groups.

After the albumin, calcium and phosphorus, essential differences between groups were not found, instead of glucose content was little higher - 3.9 to 4.0 mmol / l in experimental groups compared with 3.8 mmol / l in the controls.

CONCLUSIONS

1. Macuh (cake) from grape seeds is a waste of precious processing of industry can be successfully used in rations of farm animal because it contains a sufficient level the protein (10.5%), lipids (5.2), sugar (9.8%), macro-and micronutrients.
2. Inclusion of macuh (cake) from grape seeds in composition of combined fodder in the amount of 5-10% has a negative impact on young cattle productivity. Average daily gain of experimental animals was 786.7 to 800.0 g compared to 773.3 g in the control group, 1.7 to 3.5% or more.
3. Study of blood biochemical indices at young cattle has not found a negative influence use of macuh (cake) grape premixtures on animal health status, data parameters situated in physiological norms for healthy animals.
4. Taking into account the productivity of animals, feed intake level, blood evidence, we believe that the best macuh (cake) include *(cake)* from grape seeds in composition of combined fodder for young cattle is 5 - 10%.

Table 3. The results of biochemical analysis of blood samples taken from young cattle

Group	Ht,%	Hb, g/l	Protein, total (g/L)	Albumin (g/L)	Calcium, mmol/L	Phosphorus, mmol/L	Glucose, mmol/L	AST (U/L)	ALT (U/L)
STANDARD	**35-45**	**90-120**	**61-82**	**27-39**	**2,1-3,8**	**1,4-2,5**	**2,3-4,1**	**43-127**	**6,9-35,3**
At the beginning of the experiences									
I	41,1	108,8	61,1	31,5	-	1,79	3,63	59,3	21,4
II	40,5	109,4	59,4	30,2	-	1,64	3,19	52,0	17,0
III	37,9	107,7	57,6	30,9	-	1,79	3,3	42,8	17,9
At the end of the experiences									
I	37,8	124,6	65,6	37,4	2,9	2,2	3,8	62,8	23,9
II	35,0	115,2	64,0	36,3	2,7	1,95	3,9	54,4	18,6
II	35,2	116,9	64,4	37,9	2,7	2,2	4,0	55,3	21,0

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DETERMINATION OF ROMANIAN ALFALFA CRUDE PROTEIN AND CRUDE FIBER CONTENTS AS WELL AS *IN VITRO* ORGANIC MATTER DIGESTIBILITY BY NIR SPECTROMETRY

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Abstract

Alfalfa (Medicago sativa L.) is a high quality forage which has been used worldwide. The superiority of alfalfa lies in its high yield, high protein content and high digestibility. The aim of this study was to develop a simple, fast and non-destructive method, named Near Infrared Spectroscopy (NIRS) to determinate alfalfa quality. To realize this study, alfalfa samples were obtained from Mănăştur Experimental Station – Farm Cojocna in 2008–2009, in one experiment carried out using randomization blocks design with two experimental factors (mineral fertilization and period of harvest). Alfalfa quality was first determinated on 48 samples by classical analyses: crude protein CP (AOAC, 1990), crude fiber CF (Fiber Cap, FOSS, DK) and in vitro organic matter digestibility OMDrt (DeBoever, 1986). Then the samples were scanned by NIRS. Calibration models were performed by PerkinElmer Spectrum Quant + 4.21 program (USA) on the 48 samples determinated by classical analysis. The results showed fully confirmed by acceptable coefficients of determination and standard error of cross-validation (R2=0.96 for CP, 0.94 for CF, 0.98 for OMDrt and SECV=0.77 for CP, 1.35 for CF, 1.13 for OMDrt). Successful results for prediction of other 176 alfalfa samples were then obtained using these calibration models: SEP=0.869 for CP, 1.058 for CF, 1.058 for OMDrt). The highest CP and OMDrt values of alfalfa were obtained in bud stage (22.0% and 66.0% respectively). While for CF, the highest content was registered in the seed formation stage (46.0%). The NIRS technique offers us the possibility to determinate rapidly and easily Romanian alfalfa important parameters, but the system could also be used for the determination of other constituents.

Keywords**:** *alfalfa, NIRS, crude protein, crude fiber, in vitro organic matter digestibility*

INTRODUCTION

In current agriculture, forage production, obtained from permanent grasslands, temporary grasslands and forage crops is an integral part of agricultural land management [1]. A correct appreciation of feed quality requires a set of general analyses such as: botanical composition, organic and mineral contents, palatability and organic matter digestibility, as well as dry matter intake [2].

Alfalfa (*Medicago sativa* L.) is a high quality forage used worldwide. The superiority of alfalfa lies in its high yield, high protein content and high digestibility. It is considered by researchers as the 'queen of forages' [3]. Archaeological informations and antic scientific papers pointed out that alfalfa was cultivated first in South-West of Asia already on 4000 years before Christ [4]. In Romania, alfalfa was first cultivated in the last part of XVIIIth century in Transylvania and Banat

Country [4]. On the agrobiologic point of view alfalfa presents some particularities such as: water resistance, low temperature resistance, good utilization of irrigation water, high capacity of regeneration after harvest period and high competitive supply [3]. Alfalfa has also a high economic importance, manifested by its high ecological plasticity and high productivity. In natural conditions, in Romania alfalfa can offers more than 50 t/ha of green mass on 3 harvests/year and in irrigation conditions more than 80 t/ha of green mass on 4 harvests/year [3]. Jarrige, [5] pointed out that alfalfa has lower proportion of leaves in the first stage of vegetation in comparison with gramineae. But advancing in vegetation stages, alfalfa crude protein content and digestibility value decrease while cell wall content increases. The main components of cell walls are fibers. The organic matter digestibility of the feed depends mainly on the fiber content and its digestibility. Digestibility decreases with the proportion of cell walls and their level of lignification in the plant. Organic matter digestibility of the feed during the first cycle of vegetation depends almost exclusively on the stage of development. Organic matter digestibility of the feed (~60%) at accrued harvest stages has the same significant high value, no matter the place, year and the level of nitrogen fertilization [5]. Routine analyses of forages are necessary to evaluate their nutritive values and calculate optimal and well balanced diets for ruminants. NIRS is a non-destructive method of analysis based on diffuse reflectance of ground samples. NIRS was applied first in the agricultural sector by Norris in the 1960's to determine moisture content in soybeans [6]. More recently, Dale et al. [7] reviewed a lot of studies regarding the use of NIRS and NIR-HSI. This technology was used particularly for fast determination of the nutrients' concentration and feeding value of dried and fresh crop materials [8-11]. NIRS technique has become a 'clean technology' very used in sustainable agriculture [10] because it is using a reduced amount of chemical substances (4, 12-13). The aim of this study was to develop a simple, fast and non-destructive method to determine alfalfa quality (crude protein CP, crude fiber CF and *in vitro* organic matter digestibility OMDrt), based on Near Infrared Spectroscopy (NIRS system).

MATERIAL AND METHOD

Alfalfa samples were obtained from Mănăştur Experimental Station – Farm Cojocna in 2008–2009, in one experiment carried out using block randomization design with two experimental factors (mineral fertilization and period of harvest). The experiment started in spring 2007, with the seeding of alfalfa. The mineral fertilization was applied each year in early spring in different levels of graduation: unfertilized or fertilized with N_{50}, $N_{50}P_{50}$, $N_{50}P_{50}K_{50}$, N_{100}, $N_{100}P_{75}$ or $N_{100}P_{75}K_{75}$. The samples were collected directly in the field in the harvest period (plant height 30 cm, bud, flowering and seed formation), then were dried at 60ºC in oven 2 days. In the next step, the samples were milled using a 5 mm screen (Grindomix GM 200, Retsch, Haan, Germany) and then milled through a 1 mm sieve (Cyclotec™ 1093, Tecator, Sweden).

Alfalfa quality was determinated on 48 samples by classical analyses: crude protein (CP) (Kjeldhal method [14]), crude fiber (CF) (Weende Scheme - Fiber Cap, FOSS, DK) and *in vitro* organic matter digestibility (OMDrt) (DeBoever method [15]). The same 48 samples were scanned for NIRS analyses to perform calibration model (36 samples) and validation model (12 samples); other 176 samples from the same experiment were then scanned for prediction. Spectra were collected over the wavelengths range 10000 to 4000 cm^{-1} (1000-2500 nm) at 8 cm^{-1} resolution with 2 cm^{-1} step, in two repetitions. Each spectrum is a mean of 32 scans of the same sample.

For the mathematical models, the program PerkinElmer Spectrum Quant + 4.21 was used. The algorithm used for calibration models was Partial Least Squares (PLS). To perform a robust calibration model, spectra were treated by following mathematical treatment: 2nd Derivative (order: 3, window: 15 pt) and a cross validation was also used: leave one out, using 18 PLS factors.

RESULTS AND DISCUSSIONS

The CP, CF and OMDrt contents of alfalfa determinated by classical methods are presented in table 1. The results were similar to results showed by Varga [12], Vintilă [16] and Vîntu et al., [13]. And it can be pointed out those higher levels of crude protein content in the first and second harvested stages where obtained because of a great proportion of leaves than stems in these two stages compared to flowering and seed formation [17].

Table 1. Crude protein (CP), crude fiber (CF) and in vitro organic matter digestibility (OMDrt) of alfalfa harvested at different stages determinated by classical methods

Harvest stage	CP	CF	OMDrt
Plant height 30 cm	14.23% - 19.79%	27.92% - 34.37%	57.30% - 63.66%
Bud	14.38% - 17.64%	30.33% - 33.75%	63.39% - 65.83%
Flowering	12.87% - 17.44%	32.03% - 39.82%	52.95% - 59.86%
Seed formation	12.53% - 15.19%	38.05% - 44.03%	38.05% - 44.03%

The PLS algorithm for calibration model was performed on the spectra with the help of data obtained by classical analyses. The characteristics of alfalfa calibration models for CP, CF and OMDrt are presented in table 2. The report SD/SECV allows a comparison of the equations developed, which are independent of units and have been used by other authors. The ratio SD/SECV of 2.5-3.0 has been considered appropriate for measuring samples quality, but to ensure the models robustness, values of at least 3.0-5.0 are necessary [18]. The SD/SECV ratio was 4.18, 3.97 6.70 for CP, CF and OMDrt respectively. The ratios for CP and CF were in the limits presented by Williams and Sobering, [18]. But the ratio for OMDrt was higher than the limits; this means that it will be necessary to perform more classical analyses for the OMDrt model. The results obtained for calibration and validation models were similar to those of other authors for the same type of biological material (table 3).

Table 2. Characteristics of alfalfa calibration models

Content	CP	CF	OMDrt
N	48	48	48
Mean	15.95	27.67	64.7
SEC	0.72	1.32	0.99
R^2	0.96	0.94	0.98
SECV	0.77	1.35	1.13
SEP	0.869	1.058	1.058
SD	3.22	5.37	7.58
SD/SECV	4.18	3.97	6.70

Legend: N: samples taken in calibration model, Mean: the mean value, SEC: the standard error of calibration, R2: the coefficient of determinations, SECV: the standard error for cross validation, SEP: the standard error of prediction, SD: the standard deviation.

Table 3. The coefficient of multiple determinations (R2) and the standard error for cross validation (SECV) of alfalfa calibration models

Calibration models for	R^2	SECV	Reference
CP	0.96	0.96	Sheaffer, et al, [19];
	0.95	0.94	Velasco, et al, [20];
	0.92	0.91	Iantcheva et al., [21];
	0.95	0.94	Brogna et al., [22].
CF	0.87	-	Brogna et al., [22];
	-	3.12.	Iantcheva et al., [21].
OMDrt	-	2.97	Iantcheva et al.,[21];
	0.87	3.70	Brogna et al., [22].

Successful results for prediction of other 176 alfalfa samples were also obtained by NIRS analysis (SEP=0.869 for CP, 1.058 for CF, 1.058 for OMDrt – table 1). This system offers us the possibility of using NIRS technique for alfalfa organic substances determination. The accuracy and reliability of the prediction of e.g. the CP, CF or OMDrt of a sample by this NIRS technique has been totally dependent on the

accuracy and reliability of the classical determination of CP, CF or OMDrt. It is the reason why it is absolutely necessary to perform accurate analyses by classical techniques.

Table 4. Crude protein (CP), crude fiber (CF) and *in vitro* organic matter digestibility (OMDrt) of alfalfa harvested at different harvest stage determinated by NIR spectrometery

Harvest stage	CP	CF	OMDrt
Plant height 30 cm	12.83% - 20.61%	15.96% - 33.61%	50.07% - 63.57%
Bud	14.32% - 22.04%,	19.53% - 35.06%	46.46% - 65.99%
Flowering	12.06% - 20.14%	17.56% - 38.47%	45.07% - 61.58%
Seed formation	11.47% - 16.83%	18.33%- 46.06%	33.16% - 45.14%.

The *in vitro* OMDrt values of alfalfa in harvest stage were indirectly related to the CF content and directly proportional to the CP content. During the years of the experience, 2008 and 2009, CP levels and OMDrt were the highest at bud stage. A lower OMDrt and a highest CF content were obtained in seed formation stage, showing the role of CF in the OMDrt; the higher the crude fiber content is, the lower the digestibility is. It can be seen in table 5 that our results obtained by NIRS method were similar to different results obtained by classical or NIRS analysis. The results are presented in function of the harvest stage (bud, flowering and seed formation).

Table 5. Crude protein (CP), crude fiber (CF) and *in vitro* organic matter digestibility (OMDrt) of alfalfa harvested at different stages

Harvest stage	CP	CF	OMDrt
Bud	20.07% [12]; 24.05% [13]; 19.80% [16]; 22.60% [19]; 19.90% [23]; 16.00% [24]; 23.03% [25].	28.70% [12]; 21.40% [13]; 33.40% [24]; 25.49% [25].	50.09% [23]; 63.00% [24].
Flowering	18.47% [12]; 21.17% [13]; 18.10% [16]; 19.20% [19]; 19.40% [23]; 13.20% [24]; 33.09% [25].	31.03% [12]; 28.50% [13]; 35.70% [24]; 29.66% [25].	51.20% [23]; 62.00% [24].
Seed formation	15.92% [12]; 16.45% [13]; 17.50% [16]; 16.40% [19]; 17.40% [23]; 12.30% [24]; 16.37% [25].	34,46% [12]; 32.00% [13]; 33.60% [24]; 40.18% [25].	45.40% [23]; 60.00% [24].

In the first year of experiment a lower content of crude protein was obtained compared to second year for the different harvest stages, because in the first year of the experiment the alfalfa is installing in the yield and the nitrogen is used for this [3]. The same effect was pointed out by Decruyenaere et al., [11] and Stanacev et al., [26]. According to the harvest stage it was observed a reduction of CP and an increase in CF in the latest harvest which could be explained by to the evolution of stems and leaves [17] leaves containing more CP and less CF than stems. Moreover Heinriches [27] and Babinec et al. [28] pointed out that losses of leaves are important because the protein concentration was higher in leaves than in stems. The crude protein content and the crude fiber contents vary between very wide limits, depending largely on the development stage of alfalfa [1].

The relative contribution of qualitative parameters mentioned above helps to lift organic matter digestibility up to 60%, contributing equally to protein and fiber content. From this it can be concluded that the digestibility and crude protein content decrease quickly, while crude fiber increase slightly and then remain constant. Demarquilly and Andrieu [17] noted that the whole plant digestibility of alfalfa has been correlated to the proportion of leaves and stems, because leaves are more digestible than stems.

CONCLUSIONS

Based on the samples supplied, it has been shown that NIRS and PLS can be used to determine CP, CF and *in vitro* OMDrt of alfalfa.

This preliminary study proves that NIRS is an extremely reliable, non-destructive and rapid technique for the prediction of quantitative chemical and physical properties of alfalfa from Romania.

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THE EFFECT OF KOMBUCHA TEA ON EGG FAT AND MEAT FAT IN LAYING QUAILS (*CORTUNIX CORTUNIX JAPONICA*)

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Abstract

Kombucha has been known as traditional medicine that can cured various disease.Kombuchatea is produced by fermented sweetened tea using symbiotic growth of fungus and bacteria.Functional properties of kombucha related to metabolite that has been produced glucoronic, catechin, epicatechin and niacin that have been reported to possess various biological activities. Eighty laying quails were used in the study to determine the egg fat and meat fat level in quails. Research using Randomized Block Design (RBD) with five treatments of Kombucha tea (0,10, 15, 20 and 25 %), and four replications. All treatments would be tested for the ability to decline the level of egg fat and meat fat, and the eggs was collected every week (0,1,2,3,4 weeks).The results clearly demonstrated that the laying quails meat fat, that have been consumed with the ration contain 20% kombucha tea for 3 weeks, has significantly (P<0.05)effect;and also decreased themeat fat compared to control, andby using kombucha tea for 3 or 4 weeks has no significantly (P<0.05) decreased egg fats. but egg fats has a tendency to decline compare to control. It can be concluded that Kombucha tea could be decreases the body's synthesis of lipid in general include egg fats and meat fats.

Keywords: *kombucha tea, egg fat, meat fat, quails*

INTRODUCTION

Fats are triesters of glycerol and fatty acids. Fats, and may be either solid or liquid, though sometimesis a heterogeneous bond which is soluble in organic solvents and does not dissolve in water. Grease is organic material that neededin body, because fat acts as a source of energy, water resources, the metabolic vitamins A, D, E, K, essential fatty acids, carier pads to protect organs and as a Shaper steroid hormone. According to [2] and [5] blood fat, cholesterol free, consist of cholesterol esters, triglycerides, phospholipids and free fatty acids. In order transportation by the blood, then should be bound to proteins, called lipoproteins. Lipoprotein is a form of complex consisting of fat with weight hight molecule (cholesterol, triglycerides, phosphor-lipids) where one or more protein (apoprotein) specific are soluble in water. So this is a functional unit of bonding for the transport of fats in the blood [2]. The composition of the fat consumed will accumulate fat composition (stored as triglycerides) in fatty tissue. Fat is synthesized by the selluler anabolic process, namely lipogenesis through acid, and hydro-lysed into fatty acids and glycerol. Emulsified fats and bile salt is broken down into acid monoglycerides and fatty acids [4]. Many health benefits have been reported by users of Kombucha Tea. The benefits arederived at due to its cleansing by detoxifying and aiding the liver and kidneysto flush the toxins from the body. The health benefits of this living beverage are varied. Many health benefits that have been reported by others from drinking Kombucha Tea.

Efforts to reduce levels of meat and egg fat in Cortunix cortunix japonica could be done with a drink kombucha tea. Fermented kombucha tea could be consumed as a food supplement that offers the required compounds in stabilizing the body's metabolism. According to [15], yeast ferments contained in kombucha tea is Candida albicans, sacharomyces, and Pichiaxylinium, Gluconicum bacteria, Acetobacterketogenum. The suspension are glucoronic acid, gluconic acid, lactic acid, oxalic acid, lactic acid, butyric acid and natural antibiotics material. In addition to producing some organic acids also produce variouskinds of vitamins such as vitamin B1, B2, B3, B6, B12, B15, Vitamin C, minerals, folicacid and enzymes [9]. This acid of glucuronic in Kombucha tea is ametabolite that is produced by a healthy liver and aids in the

detoxification of the body. By drinking kombucha tea daily will help prevent our body tissues from absorbing all the toxins found in our industrial environment that can lead to illness.

Kombucha tea contains most polyfenol, including flavonoids. One of the flavonoids is catechin derivatives, the compound is antio-xidant with the power 100 times higherthan vitamin C and 25 times vitamin E, which is also a powerful antioxidant.The Kombucha colonies used in thisinvestigation had a tendency to produce about 3.3% total acid, 0.7% acetic acid, 4.8%glucose, and 0.6% ethanol after a nine-dayfermentation. There was no lactic acidproduced by these colonies (verified with HPLC; 9). The average pH of the fermented samples tested was 2.5.

Supplementation kombucha tea on 0,5% level of the total drinking water, have a lower tendency on abdominal fatty broiler meat[15], also [16], that the total cholesterol and LDL decrease on supplementation 12,5 and 25% levels of the total drinking water, while by adding kombucha tea 25% levels of the total drinking water can increase the HDL in theblood serum.

MATERIAL AND METHODS

The research used 80 quails, with averagebody weigh 160,10 gram and the variable coefficient 3,67%, age 10 weeks. The ration consist19,16% protein and Metabolize energy 2728 kcal/kg.

The feed materials used yellow corn, rice bran, fishmeat, soybean flour, bone flour.

The formula rations were:

R0 Control diet

R1 Diet contain 10% of kombucha (5 mL)

R2 Diet contain 15% of kombucha (7,5 mL

R3 Diet contain 20% of kombucha (10 mL)

R4 Diet contain 25% of kombucha (12,5 mL)

In preliminary studies, the water consumption of quail is 50 mL/day.

Sample preparation: Kombucha tea was prepared by adding 100 g/L (10%) weight/volume sucrose and tea leaves ofdesired dry weight to boiling water. Normal drinkable tea of 4.4 g/L (0.44%) weight of dry tea per volume of boiled water, and increasedlevels of 8.7 g/L, 17 g/L, 35 g/L, and 70 g/Lwere prepared in duplicate. The fermentation time averaged twelve days at 25°C.

Randomized block design used in this study with five treatment, The treatment was repeated 4 times, and 4 replication. Quail were fed ad libitum. The data for meat fat collection at the third weeks, and fourth weeks, while egg fat collection at the first, second, third and fourth weeks. The end of this experiment is 4 weeks. Variables measured are fat meat and fat egg levels.

RESULTS AND DISCUSSIONS

Variables		W1	W2	W3	W4
Fat quail meat (g)	P1	-	-	11,38^{b}	10,97
	P2	-	-	9,67^{ab}	10,02
	P3	-	-	10,15^{b}	10,29
	P4	-	-	8,78^{a}	9,43
	P5	-	-	8,32^{a}	9,09
Fat quail egg (g)	P1	14,83	14,57	13,88	13,97
	P2	14,90	15,01	13,67	14,02
	P3	14,71	14,29	14,15	13,45
	P4	13,95	13,9	13,78	13,43
	P5	13,32	13,42	13,52	13,50

W1 = first week
W2= second week
W3= third week
W4= fourth week

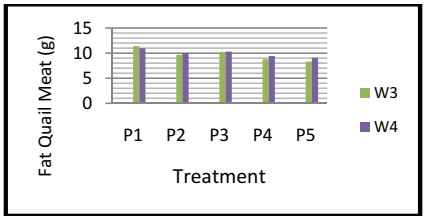

Fig. 1.Level Fat Meat of tested Animal, x=treatment(level of kombucha tea), y=level fat meat (g)

1.The effect of treatment on Fat quail Meat

Based on Figure 1, in 3 weeks after consumed Kombucha tea, showed that the level of quail meat fat is lower than the control and the quails consumed kombucha tea for 4 weeks. Consuming kombucha tea for 3 weeks, at level 20% hassignificantly effect ($P<0.05$) on the decrease the meat fat than the control (0%),10 and 15% Kombucha tea.

The percentage of theaverage fat levels of quail meat pertreatment decreased when thelevel ofKombucha tea increase. Consuming 25% of Kombucha tea couldbe reduced the fat meat

level, whileby using 10 and 15 % Kombucha tea, has no significantly (P>0.05) effect thancontrol, becausethe substance activity of kombucha tea lower which can not optimally reduced the meatfat. The effect of 20% Kombucha tea on quail meat fat, may be due to non-starchpolysaccharide component which is thefraction of water-soluble fiber[12]. The Kombucha fermented product is largely asoluble fiber fraction. By using Kombucha tea in broiler chickens drinking water can help the catabolism process of fat into energy so that the buildup of fat in the adipose tissue declined, which is also followed by a decrease in cholesterol levels. The decreasing in cholesterol where is associated some organic acids on kombucha will activated the high mobility lipoprotein (HDL: high density lipoptotein). HDL, known as which serves both as a means to transport cholesterol particles of muscle tissue and flesh to mitochondria in the liver organ would be burned into energy [6]. So, in the presence of Kombucha tea, HDLfunction increase intensively, where cholesterol and meat fat will decrease [7].

2. Effect Treatment on Fat quail Egg

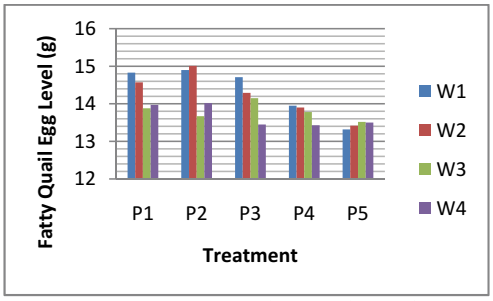

Fig. 2. Meat Fat Level of tested animal.
Notes: x = treatment(level ofkombucha tea),
Y = meat fat level (g)

Based on figure 2., by using Kombucha tea for 1, 2, 3, and 4 weeks showed the egg fat content, almost the same , and showed not significantly effect (P>0.05) on egg fat decreasing.

Yolk lipid and protein are synthesized in the liver under the influence of estrogen and progesteron and are transfered through the blood to the ovarian folicles. Lipid in the yolk are two main types lipoprotein and vitellogenins. Vitellogenin is a protein synthezised by the liver of laying female that complexes with phospholipids and cholesterol. About 90% of the energy requirement of the developing chicks is supplied by yolk lipids [10]. Although the results of statistical analysisshowed the average egg fathas no significantly, there is a tendency decline the fat egg using Kombucha tea 20, 25% for 3 and 4 weeks. The level of fat can decrease by consume Kombucha tea. According to [15], and [16], that supplementation of Kombucha tea at 25% can reduce the fat level, because it can burn the body fat. So, it can reduce the fat tissue, which will inhibit fat absorbsion in the digestive system, because the conyugate fraction of fiber derivate in Kombucha. It also could increase the excresion of fat, bile acids and decreased estrogen level, and fat deposit. It's all as precursor to egg fats, so the egg fats will be reduce [10]. Glukoronat content in Kombucha tea can conyugates excess fat in the liver enzimatically, and the coenzyme sacharolactone UDP-glukoroni transferase will bring into the path of excretion [3]. The last research that, using 25% Kombucha tea on duct, can reduce the egg fats up to 22,63% [8].

CONCLUSIONS

The results clearly demonstrated that laying quails fat dietary Kombucha tea 20% , for 3 weeks consume, showed the lowestmeat fat (8,78 gr). and egg fat has a tendency to decline compare to control. It could be concluded that Kombucha tea could be decreased the body's synthesis of lipid in general include egg fat and meat fat.

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EFFECT OF FEED ON THE BASIS OF SOYBEAN IN PIG NUTRITION

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Abstract

This paper is a review of summarizing the results of researchers who have studied the effect of soybean meal and soybean grits in the diet of certain categories of pigs. As for the sows and fattening pigs soybean meal may be the only protein feed in the diet, which meets the needs of these groups in proteins, typically without correction of amino acids with synthetic amino acids. If the meal is properly heat treated, diet of fattening pigs is possible without the use of fish meal, and when that does not deteriorate the basic parameters of production. Soybean meal in addition to high quality protein and contains significant amounts of fat, which is of particular importance in the nutrition of young pigs that have not yet developed a system for efficient enzymatic digestion of cereals, the only source of energy in the diet. The use of soybean grits increased daily gain of piglets and improves feed efficiency compared to the group in which was used powdered milk. The maximum level of soybean grits in the diet of piglets is 30% and soybean meal in the diet of fattening pigs and sows 30%.

Key words**:** *Soybean meal, soybean grits, pigs*

INTRODUCTION

Soybean *(Glycine hispida)* as the most widespread oilseed is mentioned in ancient China five thousand years before our era. At that time it was used as an ornamental plant and as a medicine plant, and only the forties of the twentieth century became an important factor in crop production and an important source of food in world [17].

Soybean is the most important vegetable protein feed used in the feeding of all types and categories of domestic animals. It can be used whole grain in the form of full-fat soybean, soybean meal and then as a by product of food industry and many other products made from modern technology.

Its importance is growing in the period when the indigenous livestock do not leave productive breeds of pigs and poultry hybrids and moves to more productive animals, which have a higher content of meat and less fat. In such conditions, increasing needs of animals in which soy protein gains importance as a crop that has the highest and best protein quality [12, 20].

Nutritional valuable raw soybeans is relatively low, what more raw soybeans may adversely affect the health of animals as a result of the presence of biologically active compounds with anti nutritious effect, ie. compounds that reduce the nutritional value affecting the availability and metabolism of nutrients.

A prerequisite for high quality soy protein and efficient use in non ruminant nutrition is adequate processing of grain, with the inactivated antinutritive factors with methods based on the thermal processing of nutrients. In soybean there are different concentrations of inhibitors that exert a significant negative effect in nutrition. The best known and most important of them is trypsin inhibitor and urease enzyme, hymotripsin and then haemaglutinin lypoksigenazes, saponins and other compounds.

Inactivating these compounds which have the structure of proteins is usually done by applying high temperature, which depends on particle size, moisture content and duration of

heat treatment. It is important to note that it is harmful as too lenient and too harsh thermal treatment. Short time of treatment and low temperature will inactivate inhibitors and they will reduce the protein digestibility. Too high temperature and long duration destroys inhibitors, but the protein is already present in the grain. In addition, high temperature reduces the solubility of proteins and can lead to other changes that will reduce their efficiency. Determination of the quality of treated soybeans is done by physical and chemical properties and biological test methods with domestic animals, with the following production parameters and animal health [16, 11, 7, 8, 10, 15, 12, 2, 22].

Feedstuffs as a result of soybeans processing

Soybean meal is the residue after extraction of oil from soybeans that during the production process exfoliates, thermal processing and extraction with organic solvents extract of oil. At the end of the process, previously pulverous toasted grain husk can be restored, or not. In modern facilities, processing of soybeans is computerized and allows continuous production, which provides almost complete separation of oil and getting a meal which contain easily digestible proteins, a reduced content of inhibitors in optimal limits range [19, 6, 7]. Soybean meal is the best known and most widely used protein feed. In the process of refining, grain is grounded into flakes, separating the shell and extracts the oil. Depending on whether the shell is returned to the meal or not, there is „standard" meal with about 44% protein, which contains more crude fibre because of the added shell, and high protein meal with about 48-50% protein, which do not contain shell and because of it contains less crude fibre and higher energy (Table 1). Meal with 44% protein is used in the diet of all categories of pigs, while those with 50% protein is intended primarily for feeding piglets, but it can be used in feed of other categories [13, 15]. The use of soybean meal in the diet of pigs that are not accustomed to eating coarse food, which dominate plant nutrients, can cause serious and long delays and disorders in the digestive tract, especially in younger piglets with a lower body mass. Therefore, the use of this meal for piglets is limited. According to some researchers, soybean meal is not suitable for feeding early weaned piglets. A problem creates the complex of protein and carbohydrates that are not cleaved in the process of heat treatment of meal. This complex causes in piglets, in the first 14 days, serious damage to the lining of the digestive tract and thickening of the intestinal villi, which reduces the capacity of the digestive tract [11].Therefore, the products of soy protein concentrate, which contains about 60-65% protein and a significant reduction of carbohydrates, and is used exclusively in the diet of piglets. Soy protein isolate is the feed from which most of the carbohydrates is extracted, so that the protein content is very high and ranges from 80-85% (Table 1) according to Boatright and Hattiarchchy [4].

Table 1. The content of nutrients in the feed from soybean

Nutrient	Soybean meal		Concentrate protein	Protein isolate	Soybeans
	With shell	Without the shell			
Dry matter, %	89	90	90	94	89
Crude protein, %	43.8	47.5	64.0	88.5	37
Fat, %	1.5	3.0	3.0	1.5	18.0
Crude fibre, %	7.4	3.4	-	1.5	6.0
SE, kcal/kg	3430	3570		4940	4200
ME, kcal/kg	3180	3235	3500	4350	3945
NE, kcal/kg	1935	2020	2000	2000	2880
NDF, %	3.13	8.9	-	-	12.0
ADF, %	9.4	5.4	-	-	8.4
Mineral substances					
- Calcium, %	0.32	0.34	0.35	0.19	0.25
- Phosphorus, %	0.65	0.69	0.81	0.62	0.57
- Manganese, %	0.27	0.30	0.32	0.14	0.29
Amino Acids					
- Lysine, %	2.83	3.02	4.20	5.26	2.35
- Met. + Cystine, %	1.31	1.41	1.90	2.20	1.15
- Threonine, %	1.73	1.85	2.80	3.17	1.44
- Tryptophan, %	0.61	0.65	0.90	1.08	0.48
- Histidine, %	1.17	1.28	1.8	2.25	0.96

Feed is primarily intended for the production of milk as a replacer for calves and for the production of compound feeds prestarter in early weaned piglets according to Boatright and Hattiarchchy [5]. Soybean grits is processed soybeans from which oil is not extracted. Because of this protein content in the soybean grits is lower in comparison with soybean meal (35-38%), but the level of oil (18%) and digestible energy (17.5 MJ) is significantly higher [13]. Soybean grits is a nutrient that can be used in the diet of all categories of pigs, but not as the only source of protein, because of high oil content. Primarily is used in the diet of piglets, due to the fact that piglets can effectively use oil as an energy source. In sows nutrition soybean grits is also recommended because oils in feeds has led to increased milk and fat content in milk of sows [12, 14, 15]. In fattening pigs nutrition soybean grits can be used in large quantities only in the first period of fattening while the use of this nutrient in the second stage of fattening may lead to the deposit of oil reserves in the fat, which negatively affects the quality and firmness of bacon [13].

Effect of soy based feeds in the pigs diet

Soybean meal in the diet of certain categories of pigs

Soybean meal is the most used protein feed in the diet of all categories of pigs. As for the sows and fattening pigs soybean meal may be the only protein feed in the ration [12, 21]. Inevitably it is the most used protein feed which quality can fulfil needs of these animals in proteins, typically without correction amino acid contents with synthetic ones. If the meal is properly heat treated, diet of fattening pigs is possible without the use of fish meal, and that does not deteriorate the basic parameters of in kind production (Table 2).

Table 2. Production of pigs in experiment with soybean meal [12]

Group	I	II
Source of protein	Soybean meal	Soybean meal + Fish meal
Number of pigs	80	80
Body weight, kg		
- At the beginning of the experiment	23.55	23.74
- At the end of the experiment	97.25	99.5
Daily gain, g	599	617
Feed consumption, kg		
- Per kg of gain	3.41	3.48
- Per hr. day	2.04	2.14
Indexes, %		
- Daily gain	100.00	103.00
- Conversion	100.00	102.05
- Consumption	100.00	104.94

However, if soybean meal is not properly heat treated, can lead to a large decrease in daily gain and feed conversion rates, which extends the fattening period and causes great economic losses (Table 3). Such condition was present at the time of the start of the domestic soybean processing plant, which where competed with all other mills that are processed primarily sunflower, and were not equipped for the efficient processing of soybeans.

Table 3. The effect of soybean meal from different oils facilities on the production of fattening pigs [12]

	Origin of soybean meal				
	"Soy Protein" Bečej	A	B	C	D
Number of pigs	48	48	48	48	48
Body weight of piglets, kg					
- At the beginning of the experiment	23.52	23.58	23.88	23.99	23.82
- At the end of the experiment	96.85	97.65	80.10	78.81	86.55
Daily gain, g	594	603	454	445	504
Feed consumption, kg					
- Per kg of gain	3.41	3.41	3.98	3.89	3.70
- Per feed day	2.02	2.05	1.49	1.42	1.86
Indexes, %					
- Daily gain	100	101.51	76.43	74.92	84.89
- Conversion	100	100.00	116.42	114.08	108.50
- Consumption	100	101.49	88.61	85.51	92.08

Table 4. The effect of soybean meal from different oils facilities on production of weaned piglets, [12]

	Origin of soybean meal				
	"Soy Protein" Bečej	A	B	C	D
Number of piglets	46	46	46	46	46
Weight of piglets, kg					
- At the beginning of the experiment	6.4	6.3	6.3	6.4	6 .3
- At the end of the experiment	7.22	7.22	7.17	2.21	8.21
Daily gain, g	360	349	237	319	330
Feed consumption, kg					
- Per kg of gain	2.26	2.12	3.14	2.37	2.5
- Per feed day	0.79	0.74	0.66	0.75	0.80
Indexes, %					
- Daily gain	100	96.9	65.8	88.6	91.7
- Conversion	100	93.8	138.9	104.9	101.6
- Consumption	100	93.7	83.5	94.9	101.3

In the case of piglets feeding soybean meal is an important and high quality protein source, when is properly heat treated, only if pigs were accustomed to feed consumption before weaning and efficient use of feed as a source of protein which is dominated by soybean meal (Table 4). In terms of tests, whose results are shown in Table 4, next to soybean, 6% of fish meal was included in mixture. However, this could not compensate for poor quality of soybean meal used in some groups of piglets [22].

Soybean meal in the piglets diet

Fullfat soybean grits is thermal processed soybeans from which oil is not extracted. Grits is the name given because, as the grinding of grain during the processing is rough, however fine grinding can create manageable problems in the manipulation of the feed. Soybean grits in addition to high quality protein contains significant amounts of fat. This is of particular importance in the nutrition of young pigs which are not able to efficiently digest carbohydrates from grains, as the only source of energy in diet. The presence of oil also is important for nutrition of sows [8, 9, 3]. Thermal treatment of grains can be made by toasting or extrusion [2, 18, 16]. In terms of inactivation of inhibitors present both methods are effective, but extrusion have some advantages because of the high pressure and mechanical friction, which causes destruction of cell membranes, and thus to increase the digestibility of nutrient substances.Table 5 shows the results of the production of piglets fed toasted soy grits [12].

Table 5. Production of pigs in the experiment with fullfat toasted soy grits in the diet [12]

Group	I	II	III	IV
Source of protein	Control	Milk powder	Toasted soy grits	
		10%	5%	10%
Daily gain, g				
- Averages	373	381	398	400
- Index, %	100.00	102.14	106.70	107.24
Feed conversion, kg				
- Averages	2.05	2.03	1.94	1.92
- Index, %	100.00	99.02	94.63	93.66
Feed consumption, kg				
- Averages	0.76	0.76	0.76	0.77
- Index, %	100	100	100	101.32

The use of this feed increases the daily weight gain of piglets and improves feed efficiency (Table 5). The results indicate that the soybean grits led to an increase in daily gain and reduced feed consumption compared to the control group in which soybean meal was used, but also in the experimental group in which powdered milk was used. Extruded soybean grits is a feed containing 38% protein, 18% fat and 3% crude fibre, ash and up to 6% and 8% moisture. Nutrient content of toasted soybean meal is the same as extruded [15]. The difference between toasted and extruded soybean grits is in the way of dehulled seed thermal processing. Toasting is the thermal treatment of a strictly controlled temperature, so not to damage the protein, and to inactivation trypsin inhibitor [10]. This method has no effect on carbohydrate complex. Unlike the toasting process, extrusion is a combination of temperature, pressure and moisture. In addition to inactivation of inhibitor materials, extrusion has an impact on the structure of proteins and carbohydrates. Extrusion leads to gelatinization of starch in which large

molecules of polysaccharides are split and thus increases the utilization. Table 6 shows the effect of toasted and extruded soybean grits on the production of weaned piglets [12].

Table 6. Effect of toasted and extruded soybean grits on the production of weaned piglets [12]

Group	I	II	III	IV
Treatments	Control	Skim milk	Soybean grits	
			Toasted	Extruded
Number of piglets	24	24	24	24
Body weight, *kg*				
- At the beginning	6.08	5.92	6.08	6.06
- At the end	25.08	27.09	27.91	28.50
Daily gain	347	385	397	408
Feed consumption, *kg*				
- Per feed day	0.72	0.72	0.73	0.73
- Per kg of gain	2.07	1.87	1.84	1.76
Indexes, %				
Daily gain	100.00	110.95	114.41	117.58
Feed consumption	100.00	100.00	101.39	100.00
Feed conversion	100.00	90.34	88.89	85.02

The maximum level of feedstuffs in the pigs diet

The nutritive value of certain feedstuffs is different and affected by the content of basic nutrients, their structure and digestibility, as well as the presence of antinutritive substances or substances that provide nutrients unfavourable or unpleasant taste and odor. All this has an impact on the convenience of use of nutrients in the diet of certain types and categories of animals [20]. Of course this has an impact on the cost of feed. Because there was a need to define the maximum participation of nutrients in the mixtures of certain categories of pigs, so as to prevent an excessive share of less valuable and less expensive feed, which would impact on the reduction of gain and deterioration in feed conversion, and in some cases it could cause health disorders [1]. Of course, the main objective is optimization of compound structure to find the cheapest feed in the circumstances, but it must be a mixture that will enable the efficient production of pigs. Limiting participation less valuable nutrients in the mixtures is necessary [9]. Table 7 shows maximum participation of some feedstuffs in the diet of different categories of pigs.

Table 7. The maximum participation of soybean, rapeseed and sunflower meal in the diet of certain categories of pigs, % [1, 9]

Feed	Starter	Pigs for Fattening	Sows	
			Pregnant	Lactating
Austin (2000)				
- Soybean meal	15	25	15	20
- Soy protein concentrate	20	0	0	0
- Soy Protein Isolate	10	0	0	0
- Soybean grits	0	20	10	10
- Rapeseed meal	0	15	15	15
- Sunflower meal	0	20	10	0
Christiansen (2005)				
- Soybean meal	15 (30)	30	30	30
- Soybean grits	15 (30)	15	20	20

CONCLUSIONS

Based on the summarized results of the use of soybean meal and soybean grits in the diet of different categories of pigs the following conclusions can be drawn. If properly heat treated, soybean meal can be used as the only protein feed in pig fattening that meets the needs of the animal in protein. Maximum level of soybean meal in the diet of fattening pigs and sows is 30% and in piglets. Use of soybean grits in the diet of piglets, improved production results when it is compared to the milk powder and the maximum level of soybean grits is 30%. In the diet of fattening pigs amount of soybean grits is 15% and 20% in sows diet.

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EFFECT SOF FEEDING DIFFERENT LEVELS OF GUAR MEAL ON PERFORMANCE AND BLOOD METABOLITES IN HOLSTEIN LACTATING COWS

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Abstract

A study was carried out to determine the effects of using different levels of Guar meal on performance and blood metabolites in Holstein lactating cows. Sixteen lactating Holstein cows(DIM=95±10)were used in Latin square design with 4 block and 4 repeats. Animal in each group fed 1 of 4 experimental rations. Diets contain 0, 50, 75 and 100 percentage cottonseed meal were replaced with gaur meal. Diets were similar as NE_L and crude protein (Iso caloric and iso nitrogenous) on dry basis. Cows were fed with total mixed ration individually. Dry matter intake and milk yields were higher for cows fed with 0% guar meal and lowest for 100 cottonseed meal replaced by guar meal, but no significant difference were found among FCM 4%.Milk fat and protein percentage and yields were highest for 50 % Guar meal, but no significant difference was found between milk lactose and calcium. Milk Urea Nitrogen and blood urea nitrogen were not significantly affected by experimental diets.

Key word: Lactating Holstein cows, Guar meal, Performance, Blood Metabolites

INTRODUCTION

As a primary product of a fodder plant called guar (Cyamopsis tetragonoloba), guar gum is extensively used as emulsifier, thickener, and stabilizer in food and oil industries. After the gum is extracted, guar meal is processed by toasting the guar seeds at high temperature to remove the natural trypsin inhibitor, thus enhancing its nutritive value and digestibility.

Guar meal is a relatively inexpensive high protein meal produced as a by-product of guar gum manufacture.

The protein content of guar meal ranges between 33 to 45% depending on fraction type (3, 6, 14, and 20). Guar meal results from combinations of two fractions, the germ and hull. The germ and hull constitute approximately 44 and 21% of the guar bean, respectively. However, the germ and hull proportions of the guar bean are not consistent with the relative amounts of the fractions mixed in guar meal (11).

Also, the degree of contamination of the germ and hull fractions with guar gum is not equivalent within these proportions when mixed into commercial guar

Guar meal can also be used as a binding agent in feed formulation. It is characterized as free flowing, has light greenish color and coarse texture, 100% non-transgenic, and contains a minimum of 45% oil and albuminoid. More importantly, guar meal is free from salmonella, E. coli, and aflatoxin (14, 11).

Data show (13) guar meal is comparable to soybean meal in terms of nutritional content. For instance, the minimum crude protein percentage of guar meal is rated at 50% compared to 48% of soy bean meal.

Its crude fiber is at 6.8% maximum, while that of soybean meal is at 3%; it has a minimum crude fat content of 5% versus 1% of soybean meal, and has a higher protein solubility of 89% than soybean meal with 78%.

Analysis for amino acids also showed that guar meal has 3.22% lysine, 0.79% cystine, 1.94% threonine, 3.62% arginine, 3.7% leucine, 0.73% methionine, 1.51% meth+cystine, 0.68% tryptophan, 2.31% isoleucine, and 2.35% valine (8).

Raw guar meal can constitute up to 25% of cattle rations. Processed meal can be used as the sole protein component of cattle diets (7).

Few nutritive values have been determined: N degradability for expanded guar meal is in the 65-75% range and is influenced by the amount of heat treatment. N degradability for unprocessed meal was 85% (12). The only reported OM digestibilities are 76% and 71% for the processed and unprocessed meal respectively (10, 23).

In dairy cows, palatability problems have been reported when more than 5% guar meal was included in the diet. However, dairy cows and heifers fed rations containing 10-15% guar meal got acquainted to its odor and taste after a few days. Intake remained lower than with the control diet (cottonseed meal) but dairy performances were not affected. In growing dairy calves, flavoured guar meal and toasted guar meal gave slightly better rates of intake and gain than raw guar meal during the first month (16).

British feeders (15) have reported palatability problems when five per cent or more of guar Meal was used in concentrate rations for lactating dairy cows, but Conrad and Neal (5) Observed no palatability problems when beef cattle were group-fed 2.3 kg of guar meal per animal daily, distributed over sorghum silage. Favorable results have been obtained with cattle rations containing up to 50% guar meal when the percentage in the diet was increased gradually (9).

Conrad (4) obtained total gains of 144, 154 and 155 kg during a 150-day growing period with crossbred steer calves fed 2.3 kg daily of raw guar, processed guar and cottonseed meals, respectively, plus a full feeding of sorghum silage. Similar results were obtained during the fattening period.

Returns per head, dressing percentage and carcass grades tended to be higher in animals fed the processed guarand cottonseed meals.

The objectives of our study were to evaluate effects of using different levels of guar meal on performance and blood metabolites in Holstein lactating cows.

MATERIAL AND METHOD

Experimental data:twelve multiparous (n = 3, DIM=100±15) lactating Holstein cows were used in a 4 × 4 Latin square design to determine effects of using different levels of guar meal on performance and blood metabolites in Holstein lactating cows.

Treatments contain 0, 50, 75 and 100 percentage cottonseed meal were replaced with gaur meal. Periods were 21-d, the first 14-d were used an adaptation, and last 7-d were used for sampling. Composition of experimental diets is shown in table 1 and 2. All diets were similar as $NE_{L,}$ crude protein and Acid Detergent fiber on dry base (1.59, 15.4% and 25.2 respectively). Cows were fed TMR in two separate feeding at 0800 and 1700 prepared daily by hand mixing in the manger and milked twice daily prior to feeding with milk yield measured and recorded at each milking. Milk samples were taken during d 15 and 17 and analyzed for milk fat, milk protein, milk lactose and milk SNF by Milko scan (Milko scan 4500, Denmark). Feed offered and refused were recorded daily to adjust feed offered for 10% refusal. Cows were housed in tie stall barn. Stall mattresses were to prevent ingestion of bedding. Body weights were recorded in beginning and final of each period after a.m. milking.

Laboratory Analysis: Weekly samples of feed offered were dried in a forced air oven at $100^{\circ C}$ to determine DMI. Samples were ground through a 2-mm screen indweller Mill (TOSHIBA, IRAN). Air dried samples were analyzed for DM, ash, OM, crude protein and ether extract according to methods of AOAC (1), Neutral Detergent Fiber, NDF, was determined according to the procedure Van soest (21) . Blood samples were obtained on d 20 of each period from coccigial vein. Samples were obtained between 3h after morning feeding with vein inject tubes (pars Darrow, Iran).

Blood samples were placed in ice immediately after collection, kept out of light, and transported to the laboratory within 5 min

where samples were centrifuged at 3000 × g for 10 min and the plasma was frozen at -20°C . Samples were analyzed for plasma glucose, BUN, Ca, P.

Table 1.Ingredient composition of diets fed to cows during experimental periods diets

Ingredient	Diet[1]			
	control	B	C	D
Alfalfa hay	18.15	18.15	18.15	18.15
Corn silage	20.88	20.88	20.88	21.28
Wheat hay	5.51	5.56	5.51	4.97
Barley	20.13	23.37	23.62	26.85
Wheat bran	9.94	7.45	7.45	6.71
Sugar beet pulp	4.97	6.31	7.40	8.80
Guar meal	-	3.48	5.86	11.43
Cottonseed meal	18.8	14.17	9.44	-
Calcium carbonate	0.99	0.84	0.74	0.54
DCP	0.04	0.29	0.44	0.79
Sodium bicarbonate	0.5	0.5	0.5	0.5
Vitamins A, D and E[2]	0.24	0.24	0.24	0.24
Nacl salt	0.19	0.19	0.19	0.19

[1] control = diet without Guar meal, B = diet containing 50 %, C = diet containing 75 %, D = diet containing 100 % cottonseed meal replaced by guar meal. [2] contained 20,000 IU of vitamin A/kg, 6,235 IU of vitamin D/kg, and 98 IU of vitamin E/kg.

Statistical Analysis

Data were analyzed by ANOVA including period, treatment, square and cow with in square using the general linear model procedures of SAS . Type III sums of squares were used and the residual served as error term for all tests. The experimental design was a Latin Square design. Within treatment, cows were blocked according to DIM and milk production.

The following model was used:

$$Y_{ijkl} = \mu + T_i + P_j + B_k + B(k)_L + E_{ijkl}$$

Where Yijkl = observation, μ = overall mean, Ti = treatment effect, Pj = period effect, Bk = square effect, B(k)l = cow with in square and Eijkl = residual effect

All means are least square means and differences were reported as significant when $P < 0.05$. Differences among treatment mean for significant Effects were determined using the Duncan multiple range test .

Table 2. Chemical composition of diets fed to cows during experimental periods

Item	Diet			
	control	B	C	D
NE_L, Mcal/kg of DM	1.59	1.59	1.59	1.59
CP	15.4	15.4	15.4	15.4
By pass protein(% of CP)	39.1	37.8	36.4	33.7
NDF	42.4	42.2	42.4	42.2
ADF	25.1	25.4	25.4	25.4
NFC[1]	32.5	33.0	33.2	33.0
Ca	0.83	0.83	0.83	0.83
P	0.53	0.52	0.52	0.52

[1] Nonfiber carbohydrate calculated by difference 100 – (%NDF + %CP + %Ash +%EE).

RESULTS AND DISCUSSIONS

The mean of different traits compartment are shown table 3.Diets were formulated to be isonitrogenous with 15.4%CP, isoenergetic with 1/59 Mcal $NE_{L.}$

Treatments effect on DMI (kg/d) was significant ($P > 0.05$). So that DMI for control

diet was highest (17.90 kg/d) and for D diet was lowest (15.22 kg/d); in the other word, increasing DMI was associated with decreasing guar meal percentage diets. In dairy cows, palatability problems have been reported when more than 5% guar meal was included in the diet. However, dairy cows and heifers fed rations containing 10-15% guar meal got acquainted to its odor and taste after a few days. Intake remained lower than with the control diet (cottonseed meal) but dairy performances were not affected. In growing dairy calves, flavoured guar meal and toasted guar meal gave slightly better rates of intake and gain than raw guar meal during the first month (16,19).

The results of study were different with previous studies (22,16).

Increasing levels of undegradable protein in rumen can be increase DMI (10)

Milk production was increased for cows fed B (containing 50 % guar) compared with other treatments (25.06, 26.03, 22.71 and 21.39, respectively) ($P < 0.05$).

Milk fat (%) was increased for diets contain guar meals compared with control diet ($P < 0.05$), but had no difference between B,C and D ($P > 0.05$). Milk fat concentration was highest for second diet and was different significantly ($P < 0.05$) among the diets. Milk protein was influenced by treatments ($P > 0.05$). Milk protein concentration was lowest for C diet and was different significantly ($P < 0.05$) among the diets suggesting that Guar meal at B(50%) diet was adequate substituting for cotton seed meal.

Milk urea nitrogen did not differ among treatments (Table 3), Results of this experiment agreement with studies of Sigh (19) and Schepher (17).

Table 3.Least square mean of performances of cows which were fed experimental diets

Item	Diet				SEM
	control	B	C	D	
DMI, kg/d	17.90[a]	16.53[b]	16.42[b]	15.22[c]	0.19
Milk, kg/d	25.06[a]	26.03[a]	22.71[b]	21.39[b]	0.80
Milk [1],kg/d	23.24[a]	26.80[b]	20.38[c]	21.31[c]	1.85
Milk fat,%	3.53[b]	4.07[a]	3.72ab	3.98[a]	0.58
Milk fat yield, kg/d	0.88[b]	1.06[a]	0.84[b]	0.85[b]	0.011
Milk protein,%	2.80[ab]	2.92[a]	2.67[b]	2.96[a]	0.014
Milk protein yield, kg/d	0.70[a]	0.76[b]	0.60[c]	0.63[c]	0.015
Milk lactose,%	4.67[ab]	4.72[a]	4.61[ab]	4.58[b]	0.25
Milk lactose yield,kg/d	1.17[a]	1.23[a]	1.05[b]	0.98[b]	0.016
Milk Ca ,mg/dl	10.44	10.21	10.90	10.97	0.28

a,b,c Means in row with different superscripts differ (p< 0.05).
n.s. = non significant[1] Fat corrected milk for 4% fat(FCM)

Plasma metabolites concentrations hasn't significantly difference exception of BUN (table 3) decreasing BUN with increasing guar meal percentage of diets may have affected low rumen degradable CP of guar meal, concentration ammonia in rumen or decrease microbial protein synthesis. High degradability CP of diets and low fermentable organic matter was caused increasing BUN in this experiment whereby suffusion energy for microbial N did not supply (18).

CONCLUSIONS

It was concluded that substitution 50% of cottonseed meal with guar meal had the best effect on performance.

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CONTRIBUTION OF BOTH SOLUBLE AND INSOLUBLE FRACTIONS OF UNTREATED AND TREATED *ACACIA SALIGNA* AND *LEUCAENA LEUCOCEPHALA* WITH DIFFERENT LEVELS OF UREA TO RUMEN FERMENTATION, *IN VITRO*

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Abstract

The objectives of this work are to characterise the in vitro fermentation contribution of both soluble and insoluble fractions, and the effects of ensiling of acacia (Acacia saligna, AC) and leucaena (Leucaena leucocephala, LE) leaves with different levels of urea (U, 0, 1, 3 or 5%) on gas production, energy value and organic matter digestibility (OMD%) of AC and LE. The acacia and leucaena were ensiled for 35 days. Ground samples (200 mg DM) of the ensiled materials from the eight treatments were incubated in glass syringes with rumen fluid obtained from fistulated sheep fed berseem hay and commercial concentrate mixture twice a day. Cumulative gas production was recorded at 3, 6, 9, 12, 24, 48, 72 and 96 h of incubation and the kinetics of gas production was described by using the equation: Gas (Y) = a + b (1-exp^{-ct}).Ensiling of AC and LE leaves with U increased crude protein and ash, while the contents of tested samples of total phenol (TP), total tannins (TT) and condensed tannins (CT) were decreased. Also, ensiling of AC and LE leaves with U significantly (P <0.05) decreased gas production. All washed samples showed losses of soluble material. The gas production was, in general, significantly (P<0.05) higher for the unwashed substrates. Leucaena gave the highest values of gas production compared with acacia. The gas production volume was significantly (P <0.05) higher for ensiled AC and LE leaves without U than ensiled AC with 5%U or ensiled LE with 3%U. The maximum rate of gas production increased after ensiling AC and LE leaves with 5% and 3% U, respectively. The calculated values of metabolizable energy (ME) and net energy (NE) were significantly (P<0.05) increased for ensiled AC with 3 and 5%U, while ensiled LE with U was not significantly affected. The organic matter digestibility (OMD %) and microbial protein production were significantly (P<0.05) higher for ensiled AC and LE with U, while short chain fatty acids (SCFA) were significantly (P<0.05) decreased. The concentrations of TP, TT and CT were strongly correlated (p<0.01). The TP, TT and CT were negatively related (p<0.01) with neutral detergent fiber (NDF) and acid detergent fiber (ADF), but not with hemicelluloses (HEMI). The crude protein was strongly correlated (p<0.01) with NDF, ADF and CT and negatively related (p<0.01) with TF and TT, but not with HEMI. In conclusion, there were negative effects on the in vitro gas production occurring more consistently when AC and LE were ensiled with different levels of U, while OMD% and microbial proteins were significantly (P<0.05) increased. The in vitro digestibility and gas production parameters were significantly correlated with chemical composition of shrubs. Finally, it is generally more appropriate to measure the degradation of organic matter as usual dry matter can give problems of interpretation of the significance of the soluble fraction.

Key words**:** *Acacia saligna*, energy value, gas production*, in vitro,* insoluble residual*, Leucaena leucocephala*.

MATERIAL AND METHOD

Samples and site description

Two tropical plants used in this study: *Acacia saligna* and *Leucaena leucocephala* leaves (whole leaves: rachis plus leaflets) have been sampled from the Experimental Farm, Faculty of Agriculture, Alexandria University, Alexandria, Egypt. Duplicate 100 g samples of AC and LE leaves were treated with water (20 ml/100 g fresh leaves) or with urea solutions at increasing levels of 1, 3 or 5%. Each sample of treated leaves was stored in a plastic bag which was closed with adhesive rubber to create anaerobic conditions. Storage time was 35 days. Bags containing treated leaves were stored in a big black plastic bag which was also closed with adhesive rubber. After storage, treated leaves were dried at 65 ^{o}C for 48 h and then ground to pass through a 1 mm screen.

Procedure for removal of soluble compounds

All forages were analysed in duplicate in three different runs according to the procedure described by Pedraza (1998). Samples of

known dry matter content (0.5 g, 1-mm screen) were soaked in 150 ml distilled water at room temperature during 1 h and 45 min while shaken intermittently. Samples were then filtered through a Whatman No.1 filter paper and washed with water until approximately 500 mL of filtrate was recovered. The filter paper containing the insoluble residue was dried at 60°C until constant weight.

The filter papers were transferred to previously tared plastic bags and sealed. Weighing was carried out after 1h, to calculate the filter paper insoluble residue. The soluble fraction was calculated by difference between initial weight of feedstuff and insoluble residue.

Chemical Analyses

Representative samples of ensiled AC and LE with different levels of urea (0, 1, 3 or 5%) were subjected to dry matter (DM), organic matter (OM), ether extract (EE), crude fiber (CF) and ash determinations following the procedure of AOAC (1990). Nitrogen (N) content was measured by the Kjeldahl method (AOAC 1990). Crude protein (CP) was calculated as N X 6.25. The neutral detergent fiber (NDF), acid detergent fiber (ADF) and hemicelluloses (HEMI) were determined according to Van Soest et al. (1991). Total phenols (TP), total tannins (TT) and condensed tannins (CT) were determined according to Makkar (1999). All chemical analyses were carried out in duplicate samples.

Measurement of in vitro gas production

In vitro gas production was undertaken according to Menke and Steingass (1988). Rumen fluid was collected before morning feeding from fistulated sheep fed berseem hay and commercial concentrate mixture twice a day. The rumen fluid was filtered through four layers of cheese–cloth and flushed with CO_2. The CO_2-flushed rumen fluid was added (1:2, v/v) to the buffered mineral solution (Onodera and Henderson, 1980), which was maintained in a water bath at 39^{o}C, and combined.

All laboratory handling of rumen fluid was carried out under a continuous flow of CO_2. Samples (200 mg) of ensiled AC and LE and insoluble residue were accurately weighed into glass syringes fitted with plungers. The syringes were pre-warmed at 39^{o}C before injecting 30 ml rumen fluid-buffer mixture into each syringe and excess gas was released. The syringes were incubated in a water bath at 39 ^{o}C. Two blank syringes containing 30 ml of the medium only were also included.

All the syringes were gently shaken 30 min after the start of incubation and every one hour for the first 12 h of incubation, thereafter five times daily. The gas production was recorded at 3, 6, 9, 12, 24, 48, 72 and 96 hours of incubation. Total gas values were corrected for blank incubation which contained only rumen fluid. Cumulative gas production (Y) at time (t) was fitted to the exponential model of Ørskov and McDonald (1979) as follows: Gas (Y) = a + b (1-exp-ct), where; a = gas production from the immediately soluble fraction, b = gas production from the insoluble fraction, c = gas production rate constant for the insoluble fraction (b), t = incubation time.

Determination of degradation of dry matter in situ

Degradation of dry matter in situ at 24 h of incubation was undertaken according to osuji et al (1993).

Estimation of energy values, organic matter digestibility, short chain fatty acids and microbial proteins

The energy values and the percentages of organic matter digestibility of forages can be calculated from the gas produced on incubation of 200 mg feed dry matter after 24 h of incubation with the levels of crude protein, ash and crude fat (Menke et al., 1979 and Menke and Steingass, 1988) as follows:

$$ME\ (MJ/kg\ DM) = 2.20 + 0.136\ GP + 0.057\ CP + 0.0029\ CP^2$$

$$OMD\ (\%) = 14.88 + 0.889\ GP + 0.45\ CP + 0.0651A$$

Where: ME is the metabolizable energy, OMD (%) is the percentage of organic matter digestibility, GP is the 24 h net gas production (ml/200 mg DM) after 24 h of incubation. CP, crude protein (%) and A, ash content (%).

$$NE\ (Mcal/Ib) = [2.2 + (0.0272 \times Gas) + (0.057 \times CP) + (0.149 \times CF)] / 14.64$$

Where: NE is the net energy; Gas, the net gas production in ml from one-gram dry sample after 24 h of incubation; CP, crude protein (%); CF, crude fat (%) then, net energy unit converted to be MJ/kg DM.
Short chain fatty acids (SCFA) were calculated according to Getachew et al. (2005) as follows:

$$SCFA = (-0.00425 + 0.0222\ GP) \times 100$$

Where: GP is 24 h net gas production (ml/200 mg DM).
Microbial protein was calculated as 19.3 g microbial nitrogen per kg OMD according to Czerkawski (1986).

Statistical Analysis
Data were subjected to analysis of variance (ANOVA) using the General Linear Model (GLM). Significant differences between individual means were identified using least significance difference (LSD) multiple range test (SAS, 2000). A simple correlation analysis was used to establish the relationship between chemical compositions and polyphenolic concentrations and *in vitro* gas production according to Steel and Torrie (1980).

RESULTS AND DISCUSSIONS

Chemical Composition
The chemical composition of ensiled AC and LE leaves with different levels of U (0, 1, 3 and 5% U) for 35 days are presented in Table 1. Results indicated great variations between the tested samples in their contents of CP, NDF, ADF, TF, TT and CT contents. The CP values ranged from 181 to 369.4 g/kg DM and were higher in LE compared with AC. These results are in agreement with those of Kumar and D'Mello (1995). The present data show that U treatments of AC and LE increased their CP contents. Also, NDF and ADF content were increased after ensiling with U for 35 days. El-Serafy et al. (1983) reported that urea addition changed the chemical composition of water hyacinth, total nitrogen increased and crude fiber (CF), NDF and ADF decreased as the period of ensiling increased. For all the samples the ADF fraction was a large proportion of the NDF, indicating high content of cellulose and lignin and low levels of hemicellulose. These results are in agreement with those of Abdulrazak et al. (2000). The values of TP, TT and CT concentration ranged between 12.3-112.2, 11.9-96.6 (eq. g tannic acid/kg DM) and 00.8-24.0 eq. g leucocyanidin/kg DM), respectively. Ensiling of AC and LE leaves without U had the highest values of all of them, while AC and LE with 5% of urea had the lowest values. The results of AC are consistent with values reported in the literature (Akkari et al., 2008). Ash content in the samples ranged from 84.7-134.7 g/kg DM and was the lowest in ensiled AC and LE without U.

In Vitro *Gas Production*
Gas production for the means of the ensiled LE and AC with different levels of urea (0, 1, 3, 5 %) is presented in Fig.1 and 2 and Tables 2 and 3. The cumulative volume of gas production increased with increasing time of incubation, and the differences in gas production occurring during the early hours indicated little differences in total gas produced at 24 h. There were significant ($P<0.05$) differences among the tested samples in terms of total gas production and parameters.
The produced gas at 96 h ranged from 25 -36.7 ml/200 mg DM. Total gas produced at 96 h of incubation was significantly ($P < 0.05$) higher for the ensiled AC and LE without U than in AC with 5% U. The highest GAS24, GAS96 and b were observed for the ensiled AC and LE leaves without U. Hadi et al. (2003) suggested that interactions between NDF, CP, ADL and ash contents influenced the kinetics of gas production. Kamalak et al. (2005) noted considerable variations among alfalfa varieties in terms of gas production at all incubation times resulting to the differences in the chemical composition of the varieties of alfalfa. The present data showed that all washed samples showed losses of soluble material. In general, leucaena leaves had the greatest washing loss compared with acacia leaves (Table 2).
The gas production was, in general, higher for the unwashed treated or non-treated acacia leaves, while the results of leucaena were on the opposite side may be leucaena contains some rumen microflora antagonistic substances which are removed by washing.

The results are agreement with Arhab et al, 2007 who showed that the gas production of the insoluble fraction of vetch-oat hay was higher than that of the *Aristida pungens* and date palm leaves.

Estimated gas production rate (c) varied from 0.028 ml/h in insoluble fraction of untreated acacia to 0.053 ml/h in unwashed treated LE with 3% of urea. The values of (c) increased after ensiling with urea. The rate degradation of the all washed samples is lower than its unwashed ones.

The results are agreement with Arhab et al, 2007. The intake of a feed is mostly explained by the rate of gas production (c) which affects the passage rate of feed through the rumen, whereas the potential gas production (a +b), is associated with degradability of feed (Khazaal et al. 1995). Although the present study showed that the ensiling AC and LE leaves with urea decreased the values of produced gas, (a) and (b), the value of (c) was significantly ($P<0.05$) increased compared with ensiled samples without U.

Determination of degradation of dry matter in situ

Degradation of dry matter *in situ* in untreated and treated leuceana and acacia with different levels of urea and unsoluble fractions (0, 1, 3, 5%) are presented in Table 4.

The present data showed that the range values of undegradable DM were from 39.37 to 91.11. The lowest value was 39.37 for unwashed and untreated leucaena with U, while the highest value was 91.11 for washed and treated acacia with 5% U. Treatment of leucaena and acacia with urea caused increase undegradable DM. This indicates that this feedstuff contains some rumen microflora antagonistic substances, tannins which are decreased by urea treatment (Table 1).

The same trand of values were obsorbed by washing of leuceana and acacia (Table 4).

Energy contents, organic matter digestibility, short chain fatty acids and microbial protein

The predicted metabolizable energy (ME, MJ/kg DM), net energy (NE, MJ/kg DM), organic matter digestibility (OMD, %), short chain fatty acids (SCFA, mM) and microbial protein (MP, mg/kg DM) of ensiled AC and LE leaves with 0, 1, 3 and 5% U are presented in Table 3. The present data show that the ME and NE were higher ($P<0.05$) for ensiled AC leaves with 3% U than for ensiled AC leaves without U, while no significant differences were detected between LE samples. Khazaal et al. (1993) correlated the chemical composition (i.e. CP, NDF, ADF or ADL) with the in vitro two-stage digestibility, in sacco degradability and gas production with voluntary intake.

The calculated organic matter digestibility from gas production values at 24 h was subsequently highest in ensiled AC and LE leaves with 3% U (489.2 and 536.9 g/kg DM, respectively) and lowest in ensiled AC and LE leaves without U (455 and 517 g/kg DM, respectively) (Table 3). Condensed tannin concentrations were significantly correlated with the in vitro dry matter digestibility ($r=0.77$, $P=0.043$), extent of degradation ($r=0.829$, $P=0.021$) and cumulative gas production at 24 h ($r=0.798$, $P=0.032$) (Khazaal et al., 1993).

Microbial proteins and SCFA ranged from 54.89-64.77 g/kg DOM and 40.15-57.91 mM, respectively. Microbial proteins were significantly ($P<0.05$) increased after ensiling with urea, while SCFA were decreased. Blümmel et al. (1997) noted an inverse relationship between in vitro gas production and microbial biomass yield.

The relationship between the concentration of phenolic compounds, crude protein and cell-wall component of the untreated and urea treated of AC and LE

Table 1. Proximate analysis (g/kg DM) of ensiled acacia and leucaena leaves with different levels of urea (0, 1, 3, or 5% U) for 35 days

Items	CP	EE	Ash	NDF	ADF	HEMI	TF	TT	CT
AC (0%)	181.0	18.1	84.7	410.9	318.4	92.5	112.2	96.6	27.6
AC (1% U)	222.1	16.6	98.9	419.5	324.5	95.0	69.3	68.8	6.3
AC (3% U)	268.4	16.1	96.7	472.0	347.5	124.5	46.1	45.7	1.4
AC (5% U)	322.0	15.2	91.8	486.5	374.5	112	17.6	17.2	1.0
LE (0%)	251.6	53.1	93.2	428.8	361.2	67.6	88.3	59.9	4.9
LE (1% U)	350.4	53.8	134.7	473.9	412.4	61.5	23.1	22.7	1.4
LE (3% U)	369.4	45.7	131.3	489.1	418.6	70.5	13.4	13.0	1.1
LE (5% U)	368.9	34.9	108.2	559.5	457.4	102.1	12.3	11.9	0.8

AC, acacia; LE, leucaena; AC(0%, 1%U, 3%U, 5%U), acacia with 0%, 1%, 3% and 5% of urea; LE (0%, 1%U,3%U, 5%U), leuceana with 0%, 1%, 3%, 5% of urea; CP, crude protein; EE, ether extract; CF, crude fiber; NDF, nutrant detergent fiber; ADF, acid detergent fiber; HEMI, hemicellulose; TF, total phenol (eq-g tannic acid/kg DM); TT, total tannins (eq-g tannic acid/kg DM); CT, condensed tannin (eq-g leucocyanidin/kg DM).

Table 2. Cumulative gas production (ml/200 mg DM) after 12, 24, 48, 72, 96 h of incubation and gas production parameters in ensiled leuceana leaves with different levels of urea (0, 1, 3, or 5% U) for 35 days

	12	24	48	72	96	a	b	c
Leuceana (raw)	18.0	28.0	34.7	36.0	36.7	6.156	31.522	0.044
Leuceana (raw), Insoluble	12.0	24.0	32.7	32.7	35.3	0.000	37.183	0.044
Leuceana (1%)	15.0	24.0	30.0	30.0	30.0	3.459	27.517	0.052
Leuceana (1%), Insoluble	10.0	23.0	30.0	32.0	33.0	0.000	35.264	0.041
Leuceana (3%)	14.0	24.0	27.3	28.0	29.3	3.590	25.735	0.053
Leuceana (3%), Insoluble	9.0	22.0	30.0	30.0	32.0	0.000	34.542	0.042
Leuceana (5%)	15.0	24.0	31.0	31.0	31.0	2.854	29.352	0.049
Leuceana (5%), Insoluble	10.0	20.0	28.0	30.0	32.0	0.000	35.259	0.041

Table 3. Cumulative gas production (ml/200 mg DM) after 12, 24, 48, 72, 96 h of incubation and gas production parameters in ensiled acacia leaves with different levels of urea (0, 1, 3, or 5% U) for 35 days

Items	**12**	**24**	**48**	**72**	**96**	**a**	**b**	**c**
Acacia (raw)	16	25	33	34	34	3.532	31.746	0.046
Acacia (raw), insoluble	9	17	29	29	31	0.852	33.034	0.028
Acacia (1% U)	16	24	32	32	33	3.639	30.147	0.046
Acacia (1%U),insoluble	11	20	30	32	32	1.329	33.374	0.034
Acacia (3%U)	15	24	31	32	33	3.026	30.808	0.046
Acacia (3%U),insoluble	9	18	26	26	28	0.0	29.148	0.037
Acacia (5%U)	12	20	26	26	27	0.749	26.651	0.051
Acacia (5%U),insoluble	8	16	23	24	25	0.0	26.435	0.037

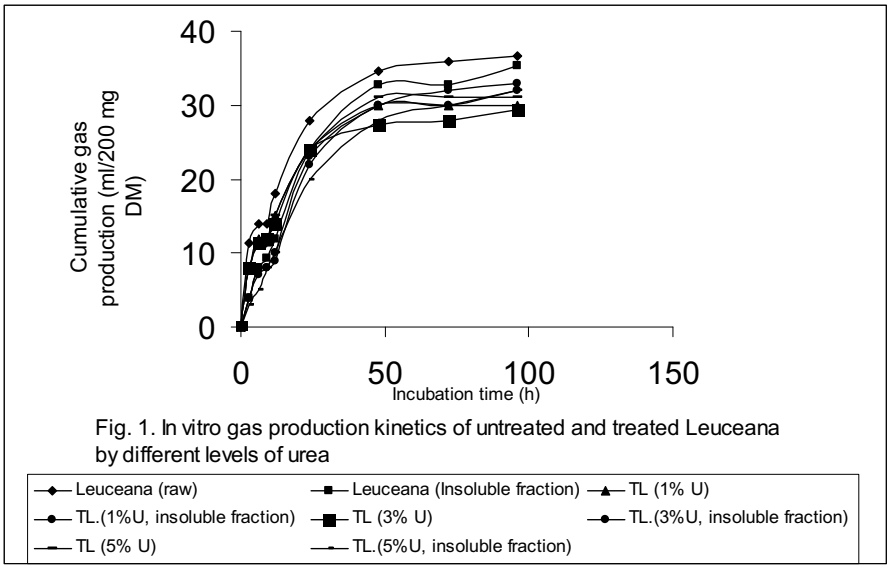

Fig. 1. In vitro gas production kinetics of untreated and treated Leuceana by different levels of urea

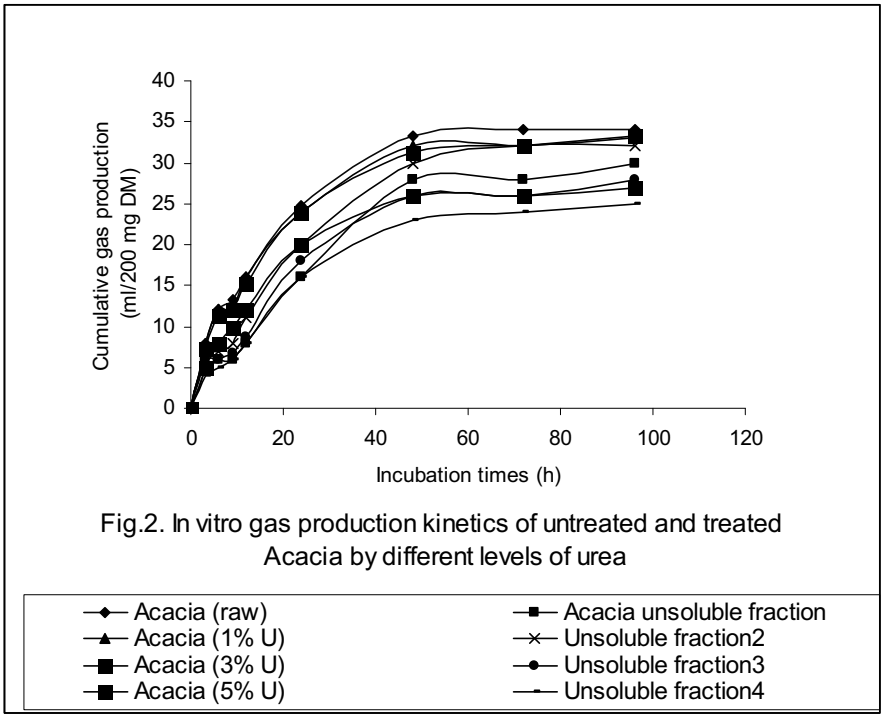

Fig.2. In vitro gas production kinetics of untreated and treated Acacia by different levels of urea

Table 4. Undegradable dry matter and disappearance materials in untreated and treated leuceana and acacia with different levels of urea and unsoluble fractions

Sample type (S)	Undegradable DM (%)	Disappearance %
Leucaena, raw	39.37	60.63
Unsoluble Leu, raw	64.68	35.32
Leucaena,1% U	48.28	51.72
Unsoluble Leu, 1% U	72.25	27.75
Leucaena, 3% U	53.46	46.54
Unsoluble Leu, 3% U	68.94	31.06
leucaena, 5% U	58.88	41.12
Unsoluble Leu, 5% U	73.64	26.36
Acacia, raw	48.24	51.76
Unsoluble Ac, raw	94.73	5.27
Acacia, 1% U	62.00	38.00
Unsoluble Ac, 1%U	86.49	13.51
Acacia, 3% U	69.72	30.28
Unsoluble Ac, 3%U	79.66	20.34
Acacia, 5% U	71.98	28.02
Unsoluble Ac, 5%U	91.11	8.89

Table 5. Metabolizable energy (ME), net energy (NE), organic matter digestibility (OMD), short chain fatty acids (SCFA) and microbial protein (MP) synthesis prediction in untreated and treated acacia and leuceana with different levels of urea

	ME (MJ/kg DM)	OMD (%)	SCFA	MP
Acacia	6.60^{c}	45.50^{e}	50.51^{b}	54.89^{e}
Acacia 1%	6.74^{c}	46.85^{d}	49.03^{b}	56.52^{d}
Acacia 3%	7.00^{b}	48.92^{c}	49.03^{b}	59.02^{c}
Acacia 5%	6.76^{c}	47.75^{cd}	40.15^{c}	57.60^{cd}
leucaena	7.52^{a}	51.70^{b}	57.91^{a}	62.36^{b}
Leucaena 1%	7.55^{a}	52.86^{ab}	49.03^{b}	63.76^{ab}
Leucaena 3%	7.63^{a}	53.69^{a}	49.03^{b}	64.77^{a}
Leucaena 5%	7.60^{a}	53.52^{a}	49.03^{b}	64.56^{a}

ME, metabolizable energy (MJ/kg DM); NE, net energy (MJ/kg DM); OMD, organic matter digestibility (%); SCFA, short chain fatty acids (mM), MP, microbial protein (g/kg DOM).
a,b,c,d,e means within the same column with differing superscript are significantly different.

CONCLUSIONS

Ensiling of AC and LE leaves with U increased crude protein and ash, while the contents of tested samples of total phenol (TP), total tannins (TT) and condensed tannins (CT) were decreased. Also, ensiling of AC and LE leaves with U significantly ($P < 0.05$) decreased gas production. All washed samples showed losses of soluble material. The gas production was, in general, significantly ($P<0.05$) higher for the unwashed substrates. Leucaena gave the highest values of gas production compared with acacia. The gas production volume was significantly ($P < 0.05$) higher for ensiled AC and LE leaves without U than ensiled AC with 5%U or ensiled LE with 3%U. The maximum rate of gas production increased after ensiling AC and LE leaves with 5% and 3% U, respectively.

The calculated values of metabolizable energy (ME) and net energy (NE) were significantly ($P<0.05$) increased for ensiled AC with 3 and 5%U, while ensiled LE with U was not significantly affected. The organic matter digestibility (OMD %) and microbial protein production were significantly ($P<0.05$) higher for ensiled AC and LE with U, while short chain fatty acids (SCFA) were significantly ($P<0.05$) decreased.

The concentrations of TP, TT and CT were strongly correlated ($p<0.01$). The TP, TT and CT were negatively related ($p<0.01$) with neutral detergent fiber (NDF) and acid detergent fiber (ADF), but not with hemicelluloses (HEMI). The crude protein was strongly correlated ($p<0.01$) with NDF, ADF and CT and negatively related ($p<0.01$) with TF and TT, but not with HEMI. In conclusion, there were negative effects on the *in vitro* gas production occurring more consistently when AC and LE were ensiled with different levels of U, while OMD% and microbial proteins were significantly ($P<0.05$) increased. The *in vitro* digestibility and gas production parameters were significantly correlated with chemical composition of shrubs.

Finally, it is generally more appropriate to measure the degradation of organic matter as usual dry matter can give problems of interpretation of the significance of the soluble fraction.

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OCHRATOXINS - FOODER CONTAMINANTS AN IMPACT ON ANIMALS AND HUMAN HEALTH

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Abstract

Under certain conditions Aspergillus, Penicillium can produce and release secondary metabolites in feed type: Ochratoxin A (OTA). Ochratoxin A (OTA) has been shown to be highly nephrotoxic compounds, hepatotoxic and teratogenic. Ochratoxin affect animal health and can be found in animal products (meat, eggs, milk) presenting a potential risk to human health. Strategies to control OTA in feed and food requires early identification and removal of contaminated products in the food chain.Toxicity of mycotoxins depends on their source and dose, duration of exposure and composition. This paper aims to address this type OTA mycotoxins in feedingstuffs and possible risk you may present it on animals and man.

Key words: *ochratoxin A, mycotoxins, feed, human toxicity, animals toxicity*

INTRODUCTION

Ochratoxin A (*OTA*) was discovered in 1965 as a secondary metabolite of a strain of *Aspergillus ochraceus. OTA* is one of the highly dangerous mycotoxins for human and animals health [3].
Ochratoxins are produced by several species of the fungal genera *Aspergillus* and *Penicillium.* In genus *Penicillium*- OTA is produced by *P. verrucosum* and *P. nordicum* and in genus *Aspergillus* by *A. ochraceus*, *A. melleus*, *A. auricomus*, *A. ostianus*, *A. petrakii*, *A. sclerotiorum*, and *A. sulphurous*[7,14].
In recent years the analyses of some food products and fodder demonstrated that, P. *viridicatum*, P.*griseofulvum* and possibly P. *solitum* also produced ochratoxins. From genus *Aspergillus*: *A niger* and A.*carbonarius* have been reported as ochratoxigenic fungi[1,10,15]
OTA contamination of food products from animals (milk, eggs, meat) or processed, are usually explained as a result of the animal's digestive absorption of feed contaminated with OTA[21] .

OTA - TOXICITY IN ANIMALS AND HUMANS

*OT*A is mutagenic, immunosuppressive in several species of animals and in humans. Ochratoxin is primarily a kidney toxin but if the concentration is sufficiently high there can be damage to the liver as well. She affects mainly the kidneys, in which it can cause both acute and chronic lesions; a neurotoxin effect has been demonstrated in all mammalian species.

Animal toxicity

Nephrotoxicity: Pigs, being most sensitive to ochratoxins, suffer from porcine nephropathy. The kidney, there is a proximal tubular atrophy, fibrosis and sclerosis glomerulara These effects were observed after feeding the feed level of OTA was between 200 to 4000 g/Kg [18].
Carcinogenesis: To date known carcinogenic effects in the renal unit at: mice and rats [2,5,8]. Pigs although OTA is metabolized

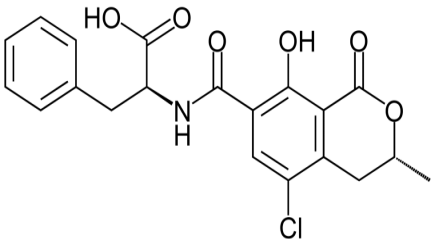
and excreted relatively quickly, there were no reported cases of kidney cancer, female, or after ingestion of two years.[6].

Human toxicity
Nephropathy: Epidemiological studies have shown that OTA can occure in humans a higher incidence of nephropathy and renal tumors.[4,18,20].
That is way the European Scientific Committee on Food indicates a lower tolerable intake, below 5 ng/kg /per day [20].
*Liver toxicity***:** Following oral administration OTA is present in blood for 35 days [11].
Carcinogenesis: After studies found that renal tumors often occur in food consumption is greater than 70 g / kg per day of OTA. [12,13,14].

TYPES OF OCHRATOXINS AND CHEMICAL STRUCTURE

Currently known three types of ochratoxin A, B, C. Depending on the degree of toxicity they are: OTA, OTC, OTB
The chemical composition of Ochratoxin A is in the figure 1 [10] .

Fig. 1. Ochratoxin A from Aspergillus sp.



MATERIAL AND METHOD

To minimize the impact of the presence of mycotoxins in feed breeding pigs with direct influence on their determinations were carried out to establish the quality of feed used. This mycotoxin OTA was evidenced by using ELISA method of working, a rapid quantitative method of screening. The determination is made based on working kit protocol used is based on the reaction of antigen - antibody.

ELISA kit (Enzyme-linked immunosorbent assay-enzyme immunoassay) contains:
- Microtiter plate consisting of 12 strips with 8 wells each, coated with antigen;
- Standards of different concentrations of mycotoxins ;
- All reagents and buffers required (Anti-body
- specifically of mycotoxin, Conjugate (with enzyme), Substrate Solution, Stop Solution , Washing buffer).

RESULTS AND DISCUSSIONS

This paper has proposed to address the presence of mycotoxins OTA in feed intended for pigs in 2010-2012. Samples were representative sample for each lot and have to comply with harvesting. If consumption of moldy feed containing secondary metabolites such as: Ochratoxin A (OTA).
Toxicity of mycotoxins depends on the source and their dose, duration of exposure and composition. Samples analyzed samples were represented by the following matrix: combined fodder for pigs, corn beans, bran, ground grain.
The results of determinations made are shown in the table below (Table 1).

Table 1

Matrices	Nr. Samples			OTA, (µg/ Kg)		
	2010	2011	2012	2010	2011	2012
Mixed fodder for pigs	3	3	4	Ned…0 ,478	Ned.	Ned.
corn beans	6	4	3	Ned.	Ned.	Ned.
bran	3	4	4	Ned.	Ned.	Ned.
ground grain	6	5	6	0,36.. 0,74	0,12.. 0,24	0,12..0 ,21

Ned.- undetectable

Values obtained from determinations were performed according to the legislation. The Commission of the European recommended guidelines for the maximum tolerable limits of different mycotoxins in feed, cereal and cereal products for animal feeding [22] (Table 2).

Table 2

Mycotoxin	Products intended for animal feed value	Maximum level (µg/kg
OTA	Feed materials (*) Cereals and cereal products; Completary and complete feedstuffs for pigs	250 50

CONCLUSIONS

- Worldwide OTA contamination of feed and food is a major concern because it is considered a nephrotoxic and carcinogenic agent,the origin of many kidney diseases;
- Mycotoxins toxicity depends on the source and their dose, composition and duratio of exposure ;
- Due to the toxic effect of mycotoxins the maximum level in fooder and food in subject to European legislation
- Since the values obtained from analyzes did not exceed the maximum permitted by law, they pose no risk to animal and human health

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RESEARCH ON THE ACTIVITY OF COMPLEX MIXES OF FOLIAR FERTILIZERS AND HERBICIDES ON THE NUTRITION VALUE OF FODDER

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Abstract

From the research of the herbicides used worldwide it is ascertained that in most of the cases mixes of products are used to obtain a wide action range. Usually mixes of herbicides and complementary action are used, thus obtaining products having a wide action range which enables their use in various fields. The use of the herbicide mixes has important technical and economic benefits: the number of crops treatments are significantly diminished, therefore of the equipment, manpower, thus obtaining important diminishment of the energy consumption.

Key words**:** *fertilizer, foliar, herbicide*

INTRODUCTION

Taking into consideration the technical and economic benefits, we studied the possibility to obtain fertilizing compositions with herbicide mixes. In Romania, mixes made up of the acid 2.4 D and Dicamba manufactured and traded under the name of Icedin Forte and Icedin Super are homologated and licensed to be used.

MATERIAL AND METHOD

To obtain a wide action range we used a mix of 2.4 D (28%) and Dicamba (35%) as dimethylamine (DMA). This systemic herbicide is absorbed by plants both by the root system and by the leaves.

The biochemical mechanism used by Icedin to destroy the weed resides in deranging the growth processes and inhibiting the development of the root system.

ICEDIN FORTE is used to fight and control weeds in the corn, wheat, rye, barley, two row barley, oat crops (when the plant has 2-5 leaves), (in the springtime during the twinning phase until the formation of the second node and the weeds are in the rosette phase, namely they have 2-6 leaves); consumption 2 l/ha for corn, wheat, barley; 1,5 l/ha for the two-row barley and barley.

The ICEDIN SUPER herbicides are used to fight and control the weeds of the straw cereals and used in the vegetation states mentioned for ICEDIN FORTE; consumption 1 l/ha of crop.

In table 1 there are the main species of weeds destroyed by the 2.4D acid and the ICEDIN type products (2.4-D and Dicamba).

From the data presented in Table 1 it clearly comes out that the ICEDIN products have a wider herbicide range than 2.4-D, therefore they were selected to make the mix of fertilizers and pesticides.

We may obtain concentrated emulsions of liquid foliar fertilizers and Icedin, using emulsifier as thickening agents and dispersing agents. We decided that we may obtain compositions of chemical fertilizers as concentrated emulsion using liquid fertilizers (including foliar) and Icedin, having the composition below:

- 100-129 g Icedin;
- 300-350 g solution of foliar liquid fertilizers;

- 20-25 g emulsifier NF-10 (as thickening agent);
- 2,0-2,5 g polyvynilic polyvynil alcohol with GH=88-92% (as dispersing agent).

Table 1. Main species of weeds destroyed by the ICEDIN type herbicide

Name of the weed		Control rate	
Common name	Scientific name	2,4-D	ICEDIN
Yarrow (milfoil)	Archillea millefplium	**	***
Corn cockle	Agrosterma githago	0	****
Mayweed / wild chamomile	Anthemis sp.	**	****
Wild bishop Heart-padded hoary-cress	Biofora radians Cardania draba	0 **	*** ****
Shepherd's-purse	Capsella bursza pastoris	***	****
Creeping thistle	Cirsium arvense	***	****
Convolvulus	Convulsis arvenis Descurania sophia	** ***	*** ****
Field (common) horsetail	Ecuisentum arvense	***	****
Cleavers	Galium aparine	0	****
Chamomile	Matricaria chamomilla	0	****
Matricaria	Matricaria inodora	0	****
Corn poppy (corn rose)	Papaver rhaeas	0	***
black-bindweed pale persicaria	Polygonum convulvulus Polygonum lapthiofolium	** **	**** ****
Sheep's (red) sorrel Austrian fieldcress	Rumex acetosella Rorippa austrica	** **	*** ***
Corn Sow thistle, (Dindle, Field Sow Thistle, Gutweed, Swine Thistle)	Sonchus arvensis Sonchus oleraceans	** **	**** ***
Common chickweed	Stellaria media	0	***
Field Penny-cress Corn (common) speedwell Veronica	Thalaspi arvense Veronica arvense Veronica hederifolia	*** 0 0	**** **** ****
Common vetch Tufted (cow) vetch Hairy (tiny) vetch Hungarian vetch Field pansy	Vicia angustifolia Vicia cracca Vicia herusta Vicia pannonica Viola arvensis	** ** ** ** **	*** *** *** *** ***

LEGEND:
0=inefficient
**= the weeds are approximately 50% destroyed
***= the weeds are approx. 75% destroyed
****=the weeds are approx. 100% destroyed

To obtain a stable in time concentrated suspensions (in which no separations or sedimentations of products as sediment occur), to the obtained compositions we added various jellifying agents.
We used jellifying agents of the polysaccharide class and polyacrylamide solutions in concentrations of 0.1-0.5% compared to the total weight of the mix.

RESULTS AND DISCUSSIONS

CPP-Bucharest tested the herbicide on experimental lots cultivated with Flamura variety wheat. The first treatment was carried out in April and the second one in May. The mix of herbicide fertilizer was sprayed using the manual pump. The observations were made 30 and 60 days after the treatment. The experiments were made in dryness conditions (high temperatures and absence of rain). As standard substance we used the Icedin Super herbicide. During the tests we also monitored the effect of the fertilizer on the way the plants develop and on the increase of the seeds production.
The results of testing the herbicide efficiency of the mix of fertilizer and Icedin Super are presented in table no. 2
The Cereals and Technical Plants Research Institute (I.C.C.P.T.) of Fundulea made the tests on the selection and efficiency of post-emergent application of the mix of foliar liquid fertilizer and Icedin Super at a dose of 5.0 l/ha for the wheat crops. The treatments was carried out when the plants had 2-3 internodes and the dicotyledonous weeds had more than 4 – 6 leaves. To apply the mix we used 400 l of water/ha. The experiments were carried out in unfavorable weather conditions: prolonged dryness, high temperatures (35 - 41°C), extremely small quantity of rain.
The testing took place in the wheat field of Flamura 85 variety and the assessment of the herbicide efficiency was carried out 14 and 28 days after the treatment.

Table 2. The herbicide efficiency of the mix of foliar fertilizer and Icedin Super in fighting the wheat crops weeds

Product	30 days after the treatment						60 days after the treatment					
	Dicot.		Monocot.		Total		Dicot.		Monocot.		Total	
	Dens.	E (%)	Dens.	E (%)	Dens.	E (%)	Dens.	E (%)	Dens.	E (%)	Dens.	E (%)
Untreated sample	10	-	8.5	-	18.5	-	8	-	5.5	-	13.5	-
Fertilizer + Icedin Super 4 l/ha	2	80	2	76.5	4	78.4	3	62.5	2	63.7	5	63
Icedin Super 1 l/ha (standard)	1	90	0	100	1	94.6	2	75	1.5	72.7	3.5	74.1
	DL 0.1(%)=7.58						DL 0.1(%)=4.32					

Dens. = number of plants /m^2
E(%) = efficiency compared to the untreated Mt
Weeds present in the wheat crop
Dicotyledonous weeds: Cirsium arvense
Convulvulus arvensis
Veronica spp
Monocotyledonous weeds: Digitaria sanguinalis
Stegaria glauca

Table 3. Results of the herbicide activity efficiency f the mix of foliar fertilizer and Icedin Super in the wheat crops (ICCPT - Fundulea)

Product	Dose l/ha	Application cleaning	ERWS grade	Efficiency		Average yield		Species of uncontrolled weed (according to the dominance)
			Sel.	14 zile	28 zile	kg/ha	(%)	
Untreated	-	-	1	0	0	3200	100	GAl., Pap., Anth., Chen., Cirs., Conv., Ver., Delph
Icedin Super (standard)	1,0	Postem.	1	90	90	3546	111	Conv., Gal.+Deph>
Icedin Super+ Foliar fertilizer	5,0	Postem.	88	90	90	3520	110	Conv., Gal.+Delph>

Variety of cultivated wheat: Flamura 85
Seeded on: 11.10.1999
Treatment date: 03.05.2000
Rain: 20 days postem.....52.3 mm
Infestation rate: 75%
Present weeds: Galium, Papaver, Anthemos, Matricarin, Cirisum, Convulsuvus, Veronica, Delphinium
DL 5%=370 kg/ha
1%=457 kg/ha
0.1%=610 kg/ha

CONCLUSIONS

After the tests made at I.C.P.P. Bucharest, the following conclusions were drawn:

a) the mix of fertilizer-Icedin Super provides a satisfactory control of the wheat crops weeds;

b) the herbicides efficiency of the composition fertilizer-Icedin Super was comparable to the one of the substance used as standard (Icedin Super); the differences related to the herbicide efficiency of the mix of fertilizer-Icedin Super and the one recorded for the standard substance are in the limits of the specific errors of the statistic calculations;

c) in the evaluation of the tests results we must take into consideration the dryness conditions of the experiments (unfavorable: high temperatures, absence of rain) etc.;

d) because of the unfavorable weather conditions the data recorded on the effects of the fertilizers on the yield increase did not enable evaluations; yet the stimulating effects of the fertilizer on the plant development during vegetation were highlighted by a more intense coloration of the leaves representing the proof of the photosynthesis processes stimulation; moreover, we also noticed as a positive effect of the fertilizer, a higher resistance of the plants to the dryness.

As a conclusion, we assert that the mix of fertilizer and Icedin Forte provides an adequate control of the weeds in the wheat crops.

After the tests carried out at I.C.C.P.T Fundulea, the following conditions were drawn:
a) the herbicide efficiency of the mix of foliar fertilizer and Icedin Super is satisfactory (88-90%), comparable to the efficiency of Icedinului Super used as standard substance 14 days after the treatment and equal 28 days after (both products had a herbicide efficiency of 90%). As well, the selectivity of the mix fertilizer + herbicide was similar to the one of the standard product;
b) in the assessment of the herbicide efficiency we must take into account two determining factors:
-b.1.) the treatment was far too late compared to the vegetative state of the weeds (the dicotyledonous weed had more than 4 – 6 leaves; the species Convulus, Galium, Papver and Delphinium were 10-15 cm tall; in this stage of weeds' vegetative development, the efficiency of the herbicides is significantly reduced;
-b.2.) the assessment of the fertilizer's influence on the production of berries was not possible because of the dryness conditions of the experiments 28 days after the treatment, on the areas treated with mix of fertilizer Icedin and those treated with Icedin Super (standard substance), the weeds totally dried out; given the circumstances, the yields increases for each separate case did not significantly differentiate (the treatment with the mix of fertilizer + herbicide recorded a 110% increase and for the lots treated with standard substance the yield increase was of 111%.
As a conclusion, we believe that the herbicide efficiency and selectivity of the mix of foliar fertilizer and Icedin Super were similar to those recorded when using Icedin Super herbicide as standard substance.

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STUDY ON THE ACTION EFFICIENCY OF THE MIXES OF HERBICIDES AND FERTILISERS IN THE CORN CROPS

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Abstract

Using mixes of herbicides and fertilizers we obtain synergic effects between the components of those compositions, which is materialized in superior crops compared to the crops obtained when these products are separately used. The mix of fertilizer – herbicide is very efficient on the mono and dicotyledonous weeds of the corn crops. As well, the toxicity of these mixes to mammals is moderate, pertaining to the toxicity group III.

Key words**:** *fertilizer, foliar, herbicide*

INTRODUCTION

The efficiency tests of the mix of fertilizer and Icedin Super in the corn crops were carried out by the scientific researchers within the Research Institute of Corn Crops (ICPP) of Bucharest. The experiments were made in conditions of high rate of weeds (the number of weds reached even 107 plants/m^2). On the grounds of the theoretic and technical – economic reasons as well as of preliminary investigations, we studied the possibility to realize compositions of foliar fertilizers and Idecin as concentrated suspensions.

MATERIAL AND METHOD

The Icedin type herbicides have a wide action range, therefore they are used to fight and control more than 200 species of annual and perennial dicotyledonous weeds, including those resistant to the action of the 2.4 D acid (in the mix two herbicides with complementary action are used, which determines a convenient widening of the action range). The Icedin products are applied post-emergent, during the vegetation when the air temperature is of minimum 7°C, tending to be higher. Liquid compositions of foliar fertilizers and Icedin are used as concentrated suspensions.

The suspension is made by inserting the Icedin, the emulsifier and the dispersing agent into the fertilizer solution, by agitation, at 30-75°C.

The obtained concentrated suspensions have been analyzed from the point of view of the stability of the active products (herbicides) they are made of. After 30 – 45 days from the making the diminution of the active products' (2.4 D acid and Dicamba) concentrations was no longer found.

As a conclusion, we may obtain concentrated suspensions of liquid fertilizers and Icedin type fertilizers with appropriate physical – chemical stability in time.

The solid mixes (as granules) made up of chemical fertilizers and Icedin type herbicides were made by depositing the herbicides in solution on the fertilizers granules and eliminating humidity by means of a warm air current.

On the grounds of the theoretical and technical – economic reasons as well as of preliminary investigations, we reached the conclusion that liquid compositions of foliar fertilizers and Icedin may be obtained as concentrated suspensions.

RESULTS AND DISCUSSIONS

The observations were made 30 and 60 days after the treatment.
The results of the tests of the herbicide efficiency of the mix of fertilizer and Icedin Super in fighting and controlling the weeds of the corn crops are presented in table 1.
As standard herbicide we used Icedin Super.

The toxicity to mammals of the ICEDIN products is moderate, the average lethal doses (DL_{50}) being of 305-320 mg/kg of live weight. These herbicides pertain to the toxicity group III. DL_{50} for mammals of 2.4-D is of 350-360 mg/kg, the amine salt of 2.4 D has DL_{50}=980-1200 mg/kg (low toxicity). For mammals, Dicamba has DL_{50}=1200-1300 mg/kg (low toxicity).

Table 1. The results of the herbicide action efficiency of the mix of fertilizer and Icedin Super in the corn crops

Product	30 days after the treatment						60 days after the treatment					
	Dicot.		Monocot.		Total		Dicot.		Monocot.		Total	
	Dens.	E (%)	Dens.	E (%)	Dens.	E (%)	Dens.	E (%)	Dens.	E (%)	Dens.	E (%)
Untreated sample	2.5	-	1.5	-	4	-	0.5	-	1	-	1.5	-
Un-weeded sample	17.5	-	80	-	97.5	-	21.5	-	88.5	-	107	-
Fertilizer +Icedin Super 4l/ha	0	100	27.2	66	27.2	72.2	5.5	74.5	22	74.3	27.5	74.3
Icedin Super 1l/ha	3.5	80	25	68.8	28.5	70.8	2.5	88.4	26	69.6	28.5	73.4

Dens.= density compared to un-weeded Mt.

Weeds present in the corn crop when the treatment was made:

Annual dicotyledonous
Amaranthus retroflexus
Chenopodium album
Galisonga parviflora
Polygonum Spp
Portulaca oleracea
Solanum nigrum
Sonchus oleraceus

Perennial dicotyledonous
Cirisium arvense
Convolvulus arvensis

Annual monocotyledonous
Setaria spp.
Echinochloa crusgalli

CONCLUSIONS

After the tests made within I.C.P.P. Bucharest, the following conclusions were drawn:
a) the efficiency of the herbicide action of the mix of fertilizer and Icedin Super was similar to the one of the product used as standard (Icedin Super); both the mix of fertilizer – herbicide as well as Icedin Super are highly efficient on the dicotyledonous and monocotyledonous weeds;
b) the assessment of the results of the herbicide efficiency of the mix of fertilizer with herbicide must take into consideration two important issues:
-b.1.) the experiments were made in a period when certain weeds were in more advanced vegetative states, when they are more resistant to the herbicide action of Icedin;
-b.2.) the testing was carried out in conditions of extremely high weed rate (107 plants/m^2);
c) the conditions of the experiments (non irrigation) did not allow definite assertions related to the effects of the fertilizers on the yield increase.
d) Icedin products have moderate toxicity in mammals.

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THE INFLUENCE OF SUPPLEMENTARY FEEDING OF LAMBS CONCERNING THEIR BODY WEIGHT AT THE END OF THE LACTATION

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Abstract

The demographic explosion recorded worldwide in the last century and also the increasing level of living had as result the constantly increasing of the meat requirements.The researches were made in a company from the city of Vaslui, on two groups of half-breed lambs obtained from the crossing of females of Merinos de Palas with males of Ile de France.At the first group the supplementary feeding started when the lambs were 10 days old, when in their food it was provided, in specially arranged places, high quality hayand concentrated forage made in the farm, containing 50 % corn, 40 % oat and 10 % pea, ad libitum.The second group was fed supplementary starting at the age of 10 days, when the lams food was provided, in specially arranged places, high quality hay and compound grain forage for lambs given on discretion. In the period of 10-40 days in the lambs food it was administrated compound grain forage "starter" which provides a level of the protein of 16 % and a minimum level of phosphorus of 0, 45 % and in the period of 40-60 days it was administrated in the lambs food compound grain forage "grower" which provides a level of the protein of 15,8 % and a minimum level of phosphorus of 0,40 %. At the age of 30 days as well at the age of 60 days the lambs from the second group had accomplished body weights higher than the lambs from the first group. The average gain in weight made by the lambs in the second group was higher than the average gain in weight of the lambs in the first group. The lambs in the second group had record in the first 30 days of life average gain of 362 g/day while the lambs in the first group recorded a average gain of 224 g/day. In the period of 30-60 days, the lambs in the second group realized an average gain of 322 g/day while the lambs in the first group realized an average gain of 202 g/day. On the whole period of lactation, 60 days, the lambs in the second group realized and average gain of 322 g/day while the lambs in the first group realized an average gain of 213 g/day.

Keywords: *meat , weight gain, forage*

INTRODUCTION

The purpose of breeding of sheep are many (milk, meat, wool, pelts), but worldwide and newer at national level, the main direction and the main purpose of breeding sheep is the production of meat. Even if in our country, the sheep meat it is still a little bit consumed, about 10-20 % of the total meat consumed, Romania can become an important producer of sheep meat for the European market, because of the big and still growing number of reproduction sheep and the wide coverage of paddocks and pastures. The purpose of the researches made is to establish the influence of supplementary food with compound grain forage on the average gain in weight of the lambs in the period of lactation.

MATERIAL AND METHOD

The researches were made in a company form the city of Vaslui, on a group of half-breed lambs obtained from the crossing of females of Merinos de Palas with males of Ile de France.
There were made two experimental groups, each one containing lambs obtained from 40 mother sheep.
The first group was composed of 55 lambs, raised in special made places. The supplementary feeding was made beginning with the age of 10 days, when the lambs received high quality hay, and concentrated

forage made in the farm, containing 50 % corn, 40 % oat and 10 % pea, given on discretion.
The second group was composed of 53 lambs, raised in special made places. The supplementary feeding was made beginning with the age of 10 days, when the lambs received high quality hayand compound grain forage.
The group of the mother sheep was fed in both the periods of mating and gestation and also the period of lactation with the same recipe of forage, made of fibred forages, succulent forages and concentrated forages.
The keeping of sheep was made for 150-160 days in stalls and 205-215 days in paddock. During stalling, the sheep were kept in shelter arranged properly and provided with paddocks. The concern was to make some conditions of comfort for the animals, providing an useful space of accommodation of 1,5 m²/head of adult sheep in shelters and 2,55 m²/head in paddock, with a feeding line of 0,5m/head of sheep.

Table 1. The prolificacy and the weightatbirth

Specification	Number of mother sheep	Number of lambs born	Prolificacy %	Weigh of the lambs at birth (Kg)
Group I	40	55	137,50	4,16
Group II	40	53	132,50	4,22

Table 2. The dynamic of the body weight

Specification	Weight of the lambs at birth (Kg) X ± Sx	Weight of the lambs at 30 days (Kg) X ± Sx	Weight of the lambs at 60 days (Kg) X ± Sx
Group I	4,16±0,08	10,88±0,15	16,94±0,24
Group II	4,22±0,08	15,02±0,12	23,54±0,22

To assure the vital functions it was provided in the sheep ratio 2,5-3 kg D.M., 1,5 - 1,6 milk nutritive units, 70-75 PDIN/PDIE, 4-5 g Ca, 2,5-3 g P, for 100 kg weight, supplementary added to these amounts 15-20 % in the period of preparing for mating and with another 25-45 % in the period of gestation and in the first 1-3 months of lactation[1]. The daily ratio administrated was balanced in mineral substances and vitamins, to prevent metabolic disorders. The requirment of vitamins was provided in the season of grazingwith green forages. The feeding in the period of stabulation was made with legume hays, 0,5-1 kg/head/day, with succulent forages, fodder beet 1,5-2 kg/head/day, with fermentedcorn 1,5-2 kg/head/day,with a mixture of concentrated forages, composed of 25-30 % barley, 50-60 % corn, 8-12 % sunflower buns, 1 % salt, 2 % chalk. The using of the cultivated paddocks was made on a large period of time, having benefits upon the health and the productive level of the animals, avoiding long and exhaustive roads.
At the paddocks it was provided water on discretion and a place to rest overlaid with clouds. The necessary of water was 3-4 times bigger than the amount of dry substance ingested, respective 3-6 l/day[2].
A special attention was given to the sheep in lactation, which were administrated forages to stimulate the dairy secretion, succulet forages, fermented corn , fodder beet and green forages[3].
In the grazing period the forage ration was completed with a supplement of fiber and concentrate forages.

RESULTS AND DISCUSSIONS

After the recording of calving, the lambs were weighed at birth, at the age of 30 days and also at the age of 60 days, the age when the weaning happened, the results being presented in the following.
From the information presented in table number 1 the conclusion can be made that the weight of the lambs at birth, but also prolificacy, records at the two groups approximately equal values. The lambs obtained in the first group had the weight at birth of 4, 16 kg, while the lambs obtained in the second group had the weigh at birth of 4, 22 kg.
At the age of 30 days and also at the age of 60 days, the lambs from the second group had the body weight higher than the lambs in the first group.
At the age of 30 days, the lambs in the second group had the body weight of 15, 02 kg while the lambs in the first group had the body weight of 10, 88 kg; at the age of 60 days the lambs in

the second group had a body weight of 23, 54 kg , while the lambs in the first group had the body weight of only 16,94 kg.

Table 3. The dynamic of the averageweight gain

Specification	Average weight gain in the first 30 days (g) X±SxV (%)	Averahe weight gain in the interval 30 -60 days (g) X±SxV(%)	Average weight gain in the first 60 days (g) X±SxV(%)
Group I	224±6 **13,60**	202±7 **14,16**	213±7 **13,16**
Group II	362±6 **12,26**	284±7 **15,86**	322±7 **14,38**

The daily average weight gain recorded by the lambs from the second group was of 362 g/day in the first month of life, 284 g/day in the second month of life, with an average of 322 g/day in the period of lactation(0-60 days), while the average weight gain of the lambs in the first group was 224 g/day in the first month of life, 202 g/day in the second month of life, with an average of 213 g/day in the period of lactation. By calculation, it was found that the differences between the genotypes are significant (The Fisher Test, P< 0,5).

The amount of combined forage, consumed by the lambs in the second group was of 5, 75 kg/head while the lambs in the first group consumed 6,20 kg/head of combined forage form the farm.

CONCLUSIONS

1.The body weight of the lambs in the second group is higher than the one of the lambs in the first group, at the age of 30 days and also at the age of 60 days. Among the lots on the significant differences in the conditions in which the time of formation of the lots were not significant differences found

2. The average weight gain of the lambs in the second group is higher than the one of the lambs in the first group, at the age of 30 days and also at the age of 60 days.

3. Even if the price of the combined grain forage is higher than the price of the combined forage made in the farm, the profit obtained by selling the 6,6 kg (the difference of the average weight of the lambs in the second group and the average weight of the lambs in the first group at the age of 60 days) is superior the supplementary costs made with the forages.

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THE YIELDING CHARACTERISTIC OF SENTUL CHICKENS FED DIET CONTAINING PAPAYA LEAVES MEAL (*Carica papaya L. Less)*

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ABSTRACT

Sentul chicken is one of the local chicken come from Ciamis, West Java-Indonesia, and a dual-purpose type that can utilized for eggs and meat production. In other way, this bird is very good for chicken meat species, because has a compact body and white skin color. One of alternative to improve the sentul chicken quality is by giving the ration which has papaya leaf meal; rich of high crude protein, contained carotene, vitamin C and high of minerals. The research aimed to find out how far the treatment on the yielding characteristics. The research used 75 day old chick and then divided into twenty five cages. The experiment conducted with Completely Randomized Design, five papaya leaves meal levels in the ration, namely: 0% (R_0), 2.5% (R_1) ,5% (R2), 7.5% (R3), and 10% (R4), repeated five times, where each replication consist of three sentul chickens. Final body weight, carcass percentage and abdominal fat percentage were parameters observed. The results showed that by using papaya leaf meal up until 7.5% gave no significant effect ($P > 0.05$) on final body weight, carcass percentage and abdominal fat percentage. But by giving 10% papaya leaf meal gave decreased effect on final body weight, carcass percentage and abdominal fat percentage ($P < 0.05$). The real conclusion of this experiment that by giving 7.5% papaya leaf meal gave the best of carcass quality

Keywords *: Yielding characteristic, papaya leaf meal, sentul chicken*

INTRODUCTION

Sentul chicken is one of the local chicken come from Ciamis, West Java-Indonesia, with grey feathers as its distinctive feature, with a variation of grey and brown yellowish feathers and orderly arranged feathers in its breast like dragon scale. Sentul chicken is a dual-purpose type that can utilized for eggs and meat production. In other way, this bird is very good for chicken meat species, because has a compact body and white skin color [15]. Sentul chicken is one of the farm products that have high nutritional value and are preferred by consumers. Effort to meet consumer demand and increased the productivity of sentul chickens, need other alternatives to improve the quality of sentul chicken.

One of the alternative way how to improve the sentul chicken quality is by giving in the ration which has papaya (*Carica papaya*) leaf meal. Papaya leaf meal supplementation in poultry rations has been proved to reduced the cost and improving profit margin, because contain alkaloids compounds and proteolytic enzymes (papain, papaya peptidase and chymopapain). Papain is an effective natural digestive aid which breaks down protein and makes digestive tract clean [13, 6]

Papain in high concentrations caused toxic effects in the form of perforated wall of the oesophagus [14]. Papain in high concentrations changed the duodenum pathophyisiology thus could be inhibit microorganism performance and disturb the digestive function resulting in decreased of body weight gain caused by the protein absorption is not perfect. Chemical process occurs in the duodenum by releasing trypsin enzyme which is useful for hydrolizing protein amino acids. [11] states that the enzyme papain is a proteolytic enzyme that

has a catalyst to break down and reduce the protein.

Papaya leaves also contain β-carotene is pro vitamin A serves as many as 18 250 IU and can be used as a source of natural Xantophyl [3]. Papaya leaves contains 140 mg vitamin C, vitamin E 136 mg, Vitamin B1 0.15, 35 grams of calcium, 63 mg phosphor and iron is 0.80 mg [17]. Beside that their have contain crude protein of 20.88 percent, 0.99 percent calcium, phosphorus 0.47 percent and Gross energy 2912 kcal / kg [3,17]

Papaya leaves have tannins that are the limiting factor and anti-nutritional substances, that bind to proteins and inhibit the protease enzymes activity, forming complexes with proteins so makes the digestibility of protein decreased [11]. Tannins in low concentrations can inhibit bacterial growth pathogen [12]. [2] reported that tannins have ability to form complexes with several molecules including carbohydrate, protein, minerals and digestive enzymes. According to [4] tannins have the ability to form complexes with proteins and digestive enzymes that interfere with the digestion of feed resulting in impaired growth of bird. The content of tannins in fresh papaya leaves of 5-6 percent [17].

Besides papaya leaves if used as poultry feed ingredients with high crude fibre content, although attendance is required as a "bulk" and prevent the clumping of food in the stomach [5] because the high crude fibre content and the presence papaya leaf tannin makes difficult in digest and will result decreased of body weight gain, so the resulting of carcass weights will be low. Therefore the use of this papaya leaf need special treatment in advance to be dried and processed them into powder is expected to reduced or even eliminated the influence of the anti-nutrient.

Several studies have been conducted the addition of until 2 percent of papaya meal in the ration had no effect on feed consumption and egg production of local chicken [10]. The addition of papaya leaves as much as 6 percent in commercial rations give the effect on feed consumption, weight gain and increased feed conversion of laying hens [1]. [16] reported that sentul chicken were offered 0, 2.5, 5.0 and 7.5 percent papaya leaf meal had similar on production and egg quality and by using 10 percent can enhance egg yolk color.

Efforts to use the papaya leaf meal as a complement material and give the positive effect on sentul chicken growth is a good idea and makes environment sustain. Based on this reason the current study was conducted to determine the effect of papaya leaf meal in the ration that produce the best final body weight, carcass weight and abdominal fat percentage of sentul chicken.

MATERIAL AND METHOD

The research used 75 day old chicks, divided into twenty five cages, each flock consisted of three birds. Round feeder and waterer, and 60 watts of hanging bulb lamp as heater at the middle of each flock were provided. The ration consisted of yellow corn-meal, fish meal, rice bran meal, soy-bean meal, papaya leaf meal, vegetable oil, bone meal, $CaCo_3$ and premix as additive feed in 17 percent protein and 2900 Kcal/kg of metabolizable energy [15]. The experiment rations were:

R_0 Ration control, without papaya leaf meal

R_1 Ration contained 2.5 percent papaya leaf meal

R_2 Ration contained 5.0 percent papaya leaf meal

R_3 Ration contained 7.5 percent papaya leaf meal

R_4 Ration contained 10.0 percent papaya leaf meal

The composition, nutrient content, metabolizable energy content of the Rations are showed in Table 1 and Table 2. Completely Randomized Design (CRD) was used with 5 treatments; each treatment was replicated 5 times. The data was analyzed by using Analysis of Variance, and the difference among treatments were tested by using Duncan‘s Multiple Range Test. The analyzed variables were final body weight, carcass weight and abdominal fat percentage.

Table 1. The Composition of the rations in percentage

Ingredients	Ration				
	R0	R1	R2	R3	R4
Yellow corn meal	57.00	57.00	57.00	57.00	57.00
Soy-bean meal	14.00	13.00	12.00	12.00	11.50
Rice bran meal	17.50	16.00	14.50	12.00	10.00
Fish meal	7.00	7.00	7.00	7.00	7.00
Papaya leaf meal	0.00	2.50	5.00	7.50	10.00
Vegetable oil	2.50	2.50	2.50	2.50	2.50
CaCo3	0.50	0.50	0.50	0.50	0.50
Grit	1.00	1.00	1.00	1.00	1.00
Premix	0.50	0.50	0.50	0.50	0.50
Total	100.00	100.00	100.00	100.00	100.00

Table 2. The Nutrients and Metabolizable Energy Content of Ration

The Nutrients	R0	R1	R2	R3	R4
Crude Protein (%)	17.14	17.05	17.12	17.10	17.20
Crude Fat (%)	7.57	7.44	7.25	7.18	7.25
Crude Fibre (%)	3.68	3.72	3.97	4.52	4.82
Calcium (%)	1.16	1.18	1.21	1.24	1.26
Phosphorus (%)	0.69	0.66	0.65	0.66	0.66
Lysine (%)	1.22	1.17	1.09	1.06	1.02
Cystine + methionine (%)	0.69	0.68	0.65	0.65	0.63
Metabolizable Energy (Kcal/kg)	2.908	2.918	2.936	2.926	2.910

RESULTS AND DISCUSSIONS

The effect of dietary treatment diets on final body weight, carcass percentage and abdominal fat percentage of sentul chicken is shown in Table 3.

Table 3. The average of Final Body Weight, Carcass Weight and Abdominal Fat Percentage

	R0	**R1**	**R2**	**R3**	**R4**
Final Body Weight (g)	867.36[a]	881.84[a]	890.92[a]	872.24[a]	703.34[b]
Carcass Weight (%)	65.40[a]	65.77[a]	64.32[a]	64.12[a]	58.97[b]
Abdominal Fat (%)	2.36[a]	1.73[a]	1.65[a]	1.60[a]	1.56[b]

Note : The similar superscript in the same row show non significant difference (P>0.05)

Final Body Weight

The final of body weight were variation from the lowest R4= 703.34 gram to the highest 890.92 gram (Fig.1). Analysis of variance showed (Table 3) that by addition of papaya leaf meal has significant effect (P< 0.05) on final body weight of broiler. Adding the papaya leaf meal until 7.5 percent in the ration of broiler still gave a good result. The result was parallel on feed and protein consumption those were also no significant different (P >0.05) among the treatment (R0, R1, R2 ,R3) and significant different to R4. Its mean that the papaya leaf meal from 2,5 percent until 7.5 percent in the ration did not influence diet palatability and chicken appetite, but have limitedness on final body weight achievement.

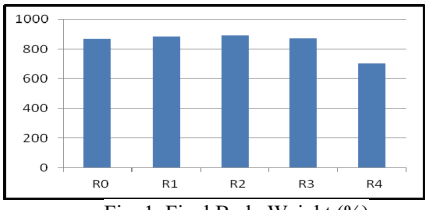

Fig. 1. Final Body Weight (%)

A decrease in final body weight of R4 (10%) due to increased levels of enzyme papain in papaya leaf meal with increasing concentrations of papaya leaf meal in the ration given to R 4 can not tolerated by the body of sentul chicken. Papain in high concentrations caused the change in the duodenum pathophysiology, thus inhibiting the performance of microorganisms and disturb the digestive function resulting of

body weight gain decreased caused by the absorption of protein is not perfect. According [14] papain in high concentration can cause toxic effects in the form of perforated wall of oesophagus. Therefore the use of papaya leaf meal should be limited given as an the active compounds. However, papaya leaves have tannins that are the limiting factor and an anti-nutritional substances can affect the function of amino acids and the use of protein. According to [4] tannins have the ability to form complexes with proteins and digestive enzymes that interfere with the digestion of feed resulting in impaired of bird growth.

Photo 1. Sentul Chicken from Ciamis, West Java-Indonesia

Carcass Percentage

The carcass percentage were variations, from the lowest R4 = 58.97% to the highest R1 = 65.77 % (Fig.2). Analysis of variance showed (Table 3), that by giving papaya leaf meal until 7.5% percent in the ration did not significantly influence (P>0.05) on carcass percentage, but has significant effect (P< 0.05) when using 10 percent in the ration on carcass percentage of sentul chicken. This result parallel to feed consumption and final body weight those were also no significantly different among the treatment groups. No differences on these parameters because of protein consumptions were relatively equal in each treatment. The function of protein is primarily to build muscle or meat. A carcass a part of chicken that contains muscle or meat. Carcass weight is proportional to the final body weight, so when calculated on a percentage of the final body weight result is relatively the same percentage [9]. R4 was the lowest produced on carcass weight and percentage. The proportion of papaya leaf in R4 was higher than those in R1, R2 and R3, therefore, the ration contain more fibre and tannin , so although the feed consumption was lower than others. Tannins have ability to form complexes with proteins and digestive enzymes that interfere to the digestion of feed resulting in impaired growth of bird, so the carcass percentage (R4) was decreased.

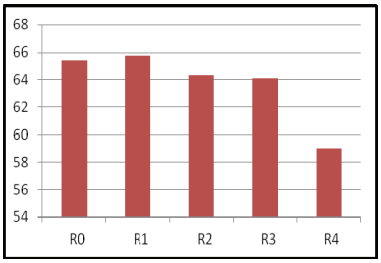

Fig. 2. Carcass Percentage (%)

According to [9] feed consumption was affected by energy contain of the feed and crude fibre, tannin and consumption protein affected the production consumption protein affected the production of carcass.

Abdominal Fat Percentage

The abdominal of fat percentage levels were variation, where R_4 by giving 10 % papaya leaf meal in the ration is most lowest 1.56% and those in R_0 without papaya leaf meal or standard ration (R_0) was the highest 2.36% (Fig. 3). Analysis of variance showed (Table 3), by treatment adding 2.5 – 7.5 % papaya meal in the ration have no significantly effect (P>0.05) on the abdominal fat percentage.

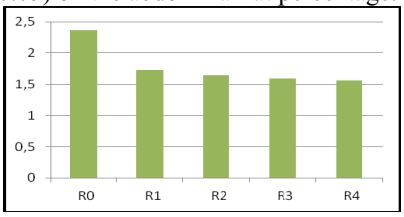

Fig. 3. Abdominal Fat Percentage (%)

By adding 10 % papaya leaf meal in the ration, there was a tendency that the level of abdominal fat percentage content going to decreased (P<0.05). In treatment R_4, proportion of papaya leaf were higher than those R1, R2 and R3. The higher fibre in ration will reduced feed consumption and intake energy is used in addition to make body balance. So the addition 10 % of papaya

leaf in ration gave significant effect on abdominal fat percentage. The low percentage of abdominal fat (1.56 – 2.36 %) results because broiler chicks on development period, much of fat is formed because the nutrients are absorbed by the body is still used for growing. According [8] that abdominal fat depend on the age of the chicken.

CONCLUSIONS

It can be concluded that by using the papaya leaf meal until 7.5 percent level in ration was still able to support a good result on broiler final body weight, carcass weight and abdominal fat percentage.

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SOME TYPICAL SYMPTOMS OF MULBERRY SILK WORM POISONING WITH THE NEONICOTINOID INSECTICIDES *CONFIDOR* AND *ACTARA*

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Abstract

An incidence of mulberry silkworm poisoning with residual amounts of the neonicotinoid chemicals Actara and Confidor is presented in the paper. The development and final outcome of toxic poisoning is described in detail. Poisoning with Actara and Confidor occurs very quickly (within 30 min for Confidor and within 1-2 h for Actara, contrary to poisoning with nicotine (within about 2-3 days). The nervous system is affected and the mulberry silkworm body changes in size, shape and structure.

Key words:*silkworm, actara, konfidor, toxic*

INTRODUCTION

In the last years the mulberry silk cocoon production in our country has been quite inconsistent. The reasons for that are the restructuring of agriculture, on the one hand, and, the competition of artificial fibers, on the other.

Quite often mass mortality of the silkworms is reported, which is accompanied by an abrupt yield decrease, worsened quality of the cocoons and the silk filament. The reasons are various, however the major one is the loss caused by silkworm poisoning with agricultural chemicals.

Mulberry silkworms, in contrast to warm-blooded animals, are highly susceptible to the effect of different chemicals such as fluorine, cadmium, zinc, lead, sulfur chemicals, carbamate and organophosphorus pesticides, insecticides, waste gases from enterprises, antibiotics, etc., even when applied at minimal rates (1, 3). The first instars are more vulnerable to the effect of the chemical substances compared to the last instars.

There are also cases of poisoning with nicotine, met in practice, although rarely. When feeding the silkworms with mulberry leaves from a plantation established near tobacco fields, the poisoning symptoms appear within 1-2 days.

The mass application of neonicotinoid chemicals coincides with the feeding stage of the larvae. The slightest negligence of the agricultural producer and not abiding by the restrictions, may lead to mass mortality of the silkworms in the region. In our daily activities in 2011 we registered such a case. That fact and the lack of data about silkworm poisoning with such chemicals motivated us to carry out a study on the effect of the chemicals Actara and Confidor on the habits, development and health status of silkworm larvae.

MATERIAL AND METHODS

Water solutions of the two chemicals were used at concentrations of the active substance 50 mg/ml for Actara and 4,6 mg/ml for Cofidor, respectively, to induce poisoing. The solutions were sprayed in the inter-row space between the mulberry trees. When the solution dried out, we collected the leaves at the periphery and the low part of the tree canopy and used them for feeding the fourth and the fifth instar larvae, which were distributed into two groups of 100 individuals for Actara and Confidor, respectively. The rest of the larvae were used as control. The studied silk worms were of the hybrid 'Super 1' x 'Hesa 2', at 4^{th}-5^{th} day of development for the respective age. In order to isolate and

prove the existence of the chemicals, we applied the thin-layer chromatography method suggested by Hristev and Ivanova (2).

Development of toxicosis

The leaves with visual traces of the chemicals, after drying out of the solution, were given to the silkworms. The first symptoms of food refusal and vomiting were observed within 15-20 minwhen the leaves treated with Confidor were offered andwithin 60-100 min after treatment of the leaves with Actara. 20-30 min later an obvious shortening of the body and swelling was established, as well as 'C' or 'S'-curving spasms of some silkworms.

Fig. 1. Normally developing silkworms

Fig. 2. A leaf with traces of the chemical

Fig. 3. Silkworms with shortened body

During feeding the silkworms got restless, uncoordinated body movements were observed, accompanied by frequent up and down shaking of the head (tics) and excretion of dark brown liquid from the mouth. The body felt softer to touch and the autopsy showed disintegration of the structure of some organs and their turning into homogeneous dark-brown mass. Within 2-3 hours all the larvae, which had consumed mulberry leaves treated with Actara and Confidor, died.

Fig. 4. Silkworms with loss of appetite and vomiting

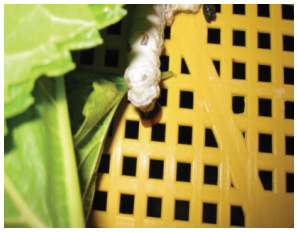

Fig. 5. A moment of vomiting

CONCLUSIONS

The results of the investigation showed that mulberry silkworms are highly susceptible to the effect of small amounts of the neonicotinoid chemicals Actara and Confidor. The conclusions of other authors that the younger instars are more susceptible to toxic substances, were confirmed. In contrast to poisoning with nicotine, when toxicos is develops within 2-3 days, poisoning with Actara and Confidor develops very quickly (within 30 minfor Confidor and within 1-2 h for Actara), affecting the nervous system and causing changes of the body in size, shape and structure.

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A COMPARISON OF DUCK AND CHICKEN EGG YOLK FOR CRYOPRESERVATION OF EGYPTIAN BUFFALO BULL SPERMATOZOA

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Abstract
Cryopreservation of domestic animal spermatozoa has been widely used for artificial insemination and egg yolk is one of the most commonly used cryoprotectants during the freezing- thawing process. The aim of the present study was to compare the effectiveness of different duck egg yolk (DEY) concentrations (10, 15 and 20% DEY) with chicken egg yolk (20% CHEY) on the cryopreservation of Egyptian buffalo spermatozoa following dilution, equilibration and freezing-thawing processes. For this purpose, one ejaculate of semen from each of three Egyptian buffalo bulls were collected twice each week for 4 weeks with artificial vagina (42ºC). Pooled ejaculates were divided into four parts and were diluted in Tric citric acid glycerol extender containing either 10 or 15 or 20% DEY or 20% CHEY at 37ºC. Extended semen was equilibrated for 4h at 5ºC and then was filled in 0.5 ml straws and frozen in liquid nitrogen. Thawing of semen was performed at 37ºC for 30s. Progressive sperm motility, live sperm % and plasma membrane integrity after different stages of cryopreservation were assayed. Values of these parameters of post-thaw semen quality were higher for extender containing 15% DEY as compared with 20% CHEY (control extender), being 59.6% vs. 47.9%, 72.3% vs. 55.0% and 80.7% vs. 69.1% for progressive motility, live sperm and plasma membrane integrity, respectively. Using a post-thawing semen containing 15% DEY yielded comparatively highest conception rate (65.8%) followed by 20% DEY (59.3%), 20% CHEY (58.6%) and 10% DEY (58.1%). In conclusion, DEY compared to chicken egg yolk in extender improves the frozen-thawed quality of Egyptian buffalo bull spermatozoa and fertility rate.

***Key words**: Cryopreservation, Duck egg yolk, Buffalo bull semen, Fertility.*

INTRODUCTION

Currently, egg yolk is a common component of most semen cryo-preservation extenders for domestic animals. It has been shown to have a beneficial effect on sperm cryopreservation as a protectant of the plasma membrane and acrosome against cooled shock [1]. Recently several investigators have substituted chicken egg yolk with egg yolk from other a vain species as a component of media used for the cryopreservation of spermatozoa livestock [2,3,4,5,6,7] Results obtained from these trials have been conflicting. Egg yolk from the duck proved superior to that of chicken for the cryopreservation of both stallion and buffalo spermatozoa[3,4]. In contrast opposite results, [8] obtained when a similar study was conducted using boar spermatozoa. In stallion, [9] reported that chukar (***Alectoris chukar***) egg yolk yielded higher post-thaw motility than chicken yolk for cryopreservation of stallion spermatozoa. The objective of the current study was to determine if substitution of chicken egg yolk with different concentration of duck egg yolk would improve post-thaw motility, percentage of plasma membrane integrity and fertility of Egyptian buffalo bull spermatozoa.

MATERIALS AND METHODS

1-Animals and semen collection

The experiment was carried out at the international livestock Management Training Center (ILMTC), Sakha Station belonging to the Animal Production Research Institute, Ministry of Agriculture, Egypt. Semen was collected from three adult and healthy Egyptian buffalo bulls (*B. bubalis*) of similar age group maintained under uniform managemental conditions. Semen was collected by artificial vagina at 42ºC twice weekly for a period of 4 weeks (2 ejaculates x 3 bulls x 4weeks,

replicates; n = 8). Ejaculates possessing more than 70% visual motility on each day collection were pooled in order to have sufficient semen for a replicate and to eliminate the bull effect. The semen was given a holding time of 10 min at 37ºC in water bath before dilution.

The pooled semen was diluted with the Tric extender concerting 3.025 Tris (hydroxymethyl amino methane), 1.675 g citric acid, 0.75 g glucose, 7 ml glycerol, 0.005g streptomycin, 0.25g lincospectin and 20 ml chicken egg yolk or 10; 15, 20 ml duck egg yolk and completed with bi-distilled water up to 100 ml. after dilution, the semen was cooled and equilibrated for 4h at 5ºC.

Semen was then filled in 0.25 ml French straws using a semen filling machine. Straws were than plunged into liquid nitrogen (-196ºC) and stored. After 24h storage, semen straws were thawed at 37ºC for at least 30s in water bath and then incubated at same temperature for 6 h to assess post thaw quality.

2- Semen Evaluation

Sperm motility, live spermatozoa and plasma membrane integrity were determined after different stages of cryopreservation (post-dilution, post equilibration and post-thaw). Plasma membrane integrity of buffalo bull spermatozoa assay is described by [10]

3- Fertility trail

A group of 58 buffalo-cows were artificially inseminated with randomly frozen- thawed semen extended with Tris 20% EY and 62, 76 and 54 buffalo-cows were randomly AI with frozen semen extended with 10%, 15% and 20% DY, respectively. Each female was inseminated with a single frozen-thawed straw at detected estrus using the recto-vaginal technique. Pregnancy rate was performed per-rectum at two months after AI.

4- Statistical analysis

Results were statistically analyzed according to SAS system (1985). The differences among means were tested using Duncan's new multiple range test [11]. The data on in vivo fertility rates were analyzed using Chi-square test.

RESULTS AND DISCUSSION

1- Progressive sperm motility (%)

Mean values (±SE) of progressive sperm motility for four experimental extenders at different stags of cryopreservation are given in Table 1. Post dilution sperm motility did not differ among extender containing 20% CHEY (control) and extenders containing 10% DEY and 15% DEY, the values being 76.7± 1.12, 74.6± 0.74 and 77.1± 0.14, respectively. This shows that DEY has no beneficial effects over CHEY immediately after dilution of semen. Moreover, increasing DEY to 20% significantly decreased sperm motility to 72.1± 0.97 when compared with other extenders. Similarly, post equilibration percentage of sperm motility did not differ among extenders containing 20% CHEY, 10%. DEY and 20% DEY, the values being 67.6%, 65.8% and 66.7%, respectively, while extender containing 15% DEY had a higher ($P < 0.05$) percentage of sperm motility, being 73.8 ± 0.65.

However, when post-thaw sperm motility after 24h storage in liquid nitrogen was considered, all extenders containing DEY showed significantly higher values (52.1, 59.6 and 52.5% for 10, 15 and 20% DEY, respectively) compared to 47.9% for control extender containing 20% CHEY ($P < 0.05$). We attempted to optimize the concentration of duck egg yolk in extender. The progressive sperm motility showed that the 15% DEY in extender was the best concentration to provide the best cryoprotective action for Egyptian buffalo sperm among other three concentrations of tested. These findings are supported by those [3] and [12], who recorded improved sperm motility parameters when the stallion and Nili-Ravi buffalo semen were frozen in extenders containing DEY as compared to CEY. Also, [4] found the highest forward motility of buffalo bull spermatozoa at 6 h post-thaw in DEY extender compared to those having egg yolk from other avian species including guinea fowl, indigenous hen and commercial chicken.

Table 1. Effect of different concentrations from duck egg yolk and 20% hen egg yolk in extenders on motility, live and plasma membrane integrity of buffalo bull spermatozoa at different stage of cryopreservation

Test	D.S.	CEY	DEY		
		20%	10%	15%	20%
Motility Sperm %	PD	$76.7^{A} \pm 1.12$	$74.6^{AB} \pm 0.74$	$77.1^{A} \pm 0.14$	$72.1^{B} \pm 0.97$
	PE	$67.9^{B} \pm 0.97$	$65.8^{B} \pm 0.83$	$73.8^{A} \pm 0.65$	$66.7^{B} \pm 0.71$
	PT	$47.9^{C} \pm 0.96$	$52.1^{B} \pm 0.97$	$59.6^{A} \pm 0.42$	$52.5^{B} \pm 0.75$
Live Sperm %	PD	$81.0^{A} \pm 1.24$	$76.3^{AB} \pm 2.1$	$82.3^{A} \pm 4.0$	$70.9^{B} \pm 3.4$
	PE	$70.7^{B} \pm 1.15$	$70.9^{B} \pm 1.69$	$78.4^{A} \pm 1.03$	$65.8^{B} \pm 3.4$
	PT	$55.0^{C} \pm 0.95$	$61.5^{B} \pm 0.89$	$72.3^{A} \pm 0.50$	$58.7^{BC} \pm 2.46$
Plasma Membrane%	PD	$88.4^{A} \pm 0.82$	$84.0^{B} \pm 1.35$	$89.0^{A} \pm 0.67$	$82.5^{B} \pm 1.75$
	PE	$79.8^{B} \pm 1.31$	$81.4^{B} \pm 1.13$	$85.8^{A} \pm 0.78$	$77.8^{B} \pm 1.84$
	PT	$69.1^{C} \pm 0.70$	$73.8^{B} \pm 1.30$	$80.7^{A} \pm 0.54$	$71.2^{BC} \pm 1.82$

- A, B, C: Means within the same row with different superscripts are significantly different at P<0.01.
- DEY: Duck Egg Yolk, CEY: Chicken Egg Yolk, DS: Different Stage PD: Post-dilution, PE: Post Equilibration, PT: Post Frozen –thawing

The improvement in the post-thaw semen motility of Egyptian buffalo bull due to replacement of 20% CHEY with different concentration of DEY in the extender can be attributed to differences in the composition of egg yolk from the two a vain species [12].

2- Live spermatozoa and plasma membrane integrity.

According to the results, 15% DEY had the best cryoprotective effect in terms of the highest live spermatozoa and plasma membrane integrity compared to the other three extenders evaluated at different stages of cryopreservation (Table, 1). Post extension live sperm percentage and plasma membrane integrity did not differ between extender containing 15% DEY and control (20% CHEY) extender. The values being 82.3 vs. 81.0% and 89.0 vs. 88.41%, respectively. However, Post equilibration and post-thaw live sperm and plasma membrane integrity were significantly ($P < 0.05$) higher in 15% DEY extender as compared with extender containing 20% CHEY, the values being 78.4 vs. 70.7% and 85.8 vs. 79.8%, respectively, Table (1). The present findings are supported by [12], who recorded improved post-thaw livability when the Nili-Ravi buffalo semen was frozen in extender containing DEY as compared to CEY, the values were 7.03 ± 0.10 and 6.5 ± 0.15h, respectively ($P < 0.05$). This supports the idea that low density lipoproteins present in the egg yolk stick to the sperm plasma membrane during freeze-thaw process, preventing loss of phospholipids through improving membrane tolerance for the freezing process [13].

According to [2], the basic components of the yolks from chicken and duck eggs did not differ, but the ratios of fatty acids and phospholipids classes were different. Yolk from duck eggs had more monounsaturated fatty acid than yolk from chicken eggs. Moreover, yolk from duck eggs contained more phosphotidylionsitol than CEY. [6] found that karayaka ram frozen semen extended in chicken egg yolk recorder lower percentages regarding sperm motility (35 ± 1.6 vs. $47 \pm 2.5\%$), viability (50 ± 3.4 vs. $58 \pm 2.6\%$) and membrane integrity (44 ± 3.3 vs. $49.2 \pm 5.0\%$) as compared to DEY extender.

On contrast, [8] found that frozen-thawed bull sperm progressive motility and sperm viability were significantly higher in extender containing chicken egg yolk as compared to DEY, the values being 48.9 vs. 37.1 and 53.3 vs. 42.6%, respectively. It is suggested that the improvement or decline in post-thaw quality of mammalian spermatozoa with EY of different avian species in freezing extender is attributed to the differences in biochemical composition of the yolk [14,2,15].

3- Fertility of frozen buffalo Semen.

A total of 58, 62, 76 and 54 buffalo females were artificially inseminated with frozen-thawed semen extended in 20% CHEY, 10% DEY, 15% DEY and 20% DEY, respectively, Table 2. High conception rate was recorded with the use frozen-thawed semen containing 15% DEY, being 65.8% followed by 20% DEY (59.3%), 20% CHEY (58.6%) and 10% DEY (58.1%), but the differences were not significant. It should be mentioned that achieved more 50% in the current study is satisfactory as compared to the previous studies using various freezing and thawing techniques.

In the Nili-Ravi buffalo bulls, [16] obtained that fertility rates were higher with semen cryopreserved in extender containing 12% LDLs compared with the control (egg yolk 20%) (72.7% vs. 50%, respectively). According to [17] suggested that a pregnancy rate higher than 50% can be regard as a good result after insemination with frozen- thawed semen.

In conclusion, our results showed that 15% duck egg yolk provided the best cryoprotective action to Egyptian buffalo bull sperm between the two avian egg yolks during the freezing-thawing process in terms of progressive motility, live spermatozoa, plasma membrane integrity and fertility rate. This conclusion is based on sperm characteristics and a full fertility trail which confirmed the beneficial effects of the inclusion of duck egg yolk in Egyptian buffalo semen cryopreservation protocols.

Table 2. Conception rate of buffalo-cows inseminated with frozen semen cryopreserved in different concentrations of duck egg yolk in extenders compare with 20% chicken egg yolk

Item	Chicken Egg Yolk 20%	Duck Egg Yolk		
		10 %	15 %	20 %
No. of inseminated females	58	62	76	54
No. of conceived females	34	36	50	32
Conception rate (%)	58.6	58.1	65.8	59.3

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EFFECT OF LOW DENSITY LIPOPROTEINS IN EXTENDER ON FREEZABILITY AND FERTILITY OF EGYPTIAN BUFFALO BULL SEMEN

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Abstract

Semen from three Egyptian buffalo bulls was collected once weekly and ejaculates with more 75% progressive motility and more 85 % normal sperm morphology prior to cryopreservation were pooled in order to have sufficient semen for a replicate and to eliminate the bulls effect. Seven extenders were used: Tris 20 % egg yolk extender with 7 ml glycerol as a control (T1), and substitution of whole egg yolk with 4, 6, 8, 10, 12 and 15 % low density lipoprotein (LDL), T2 – T6, respectively. Semen was diluted to 80 x10^6 sperm/ml, packaged into 0.25 ml straws, cooled, held at 5°C for 4 h, and then frozen in liquid nitrogen (LN) and stored at -196◦C for at least one month. Sperm progressive motility, intact acrosome and plasma membrane integrity were assesd at post dilution, equilibration, post-thawing (at 37°C for 30 sec.) and after 30 days storage in LN. This study reveled that LDL extenders were more effective in preservation of progressive motility, intact acrosome and integrity of the plasma membrane of buffalo spermatozoa than whole egg yolk extender. Sperm progressive, intact acrosome and plasma membrane integrity were much higher (P < 0.05) in the 12% LDL extender (63.3, 77.17 and 71.3% respectively) vs. 35, 40.8 and 34.7% in the control 20% EY extender at post-thawing process, respectively. Fertility rates were higher in extender containing 12% LDLs compared with the control (72.7% vs. 50%, respectively). It was concluded that LDL (12%) in extender improved the freezability and fertility of buffalo bull spermatozoa.

Key words: Buffalo Bull Semen; LDL; Freezability; Fertility

INTRODUCTION

Egg yolk (EY) is a common component of semen freezing extenders for most of the livestock species, including the buffalo (***Bubalus bubalis***) [1,2]. The major role of EY is to prevent sperm cell damage during the cooling and freezing processes. Egg yolk is generally used at a concentration of 20% (Vol./Vol.) in semen extender for bovine [3]. The use of EY in higher concentration may have deleterious effects combined with toxicity (amino acid oxidase activity) of dead spermatozoa resulting in lower post-thaw spermatozoal quality.

Andrabi et al. (2008) [4] reported that duck egg yolk compared to other avian yolks in extender improved the freezability of buffalo bull spermatozoa as judged by motility, survivability, and plasma membrane integrity, intactness of acrosome and head, mid-piece and tail abnormalities. They added that, the improvement or the decline in post-thaw quality of mammalian spermatozoa with EY is attributed to the differences in biochemical composition of the extender. Low density lipoproteins (LDL) contained in EY is largely responsible for sperm protection during cryopreservation [5,6]. The exact mechanism by which EY preserves the bull spermatozoa during freeze-thaw process is unknown [2].

Several studies have reported successful results with the addition and replacement of egg yolk by LDL in the semen freezing process of different species, like bull [7,8,9] ram [10] dogs [11] and buffalo bull semen

[12]. Therefore, the goals of this study were first to asses the cryoprotective effect of LDL as a replacement for chicken egg yolk, in extenders for Egyptian buffalo semen, on variables of semen quality after different stages of cryopreservation (i.e., after dilution, equilibration at 5° C and freezing-thawing and post-one month from freezing storage period in liquid nitrogen).

MATERIAL AND METHOD

Semen collection

Semen was obtained from three buffalo bulls at the International Livestock Management Training Center (ILMTC), Sakha Station belonging to the Animal Research Institute, Ministry of Agriculture, Egypt. Ejaculates with >75 % progressive motility and >85% normal sperm morphology prior to cryopreservation were used. One ejaculate was obtained from each bull using an artificial vagina for a period of 4 weeks.

Extender preparation

The extender for the treatment groups used in this study was composed as follow: 3.025g Tris, 1.675g citric acid, 0.75g glucose, 7 ml glycerol, 0.25 lincomycin, 0.005g streptomycin and different concentrations of LDL (4, 6, 8, 10, 12 and 15%) for 100 ml bi-distilled water. The extender for the control group differed from the treatment groups by replacing LDL with 20% egg yolk.

LDL extraction

The LDL was extracted from egg yolk according to the method described by [7] .

Semen processing

After the evaluation of motility and morphology, the fresh semen of three buffalo bulls was pooled and then divided into seven equal fraction, one fraction was diluted with the extender for the control group and others were diluted with the extender for the treatments to obtain 80 x 10^6 sperm/ml. Semen was cooled from 37 to 5ºC for 1.5h and maintained in a refrigerator at 5ºC for 4h. French straws (0.25 ml) were filled using a semen filling machine. Subsequently, these straws were placed 4 cm above the liquid nitrogen surface where the temperature was approximately -120ºC. After 10 min all straws were immersed directly into liquid nitrogen (-196º) for storage.

Semen quality assays

Assays for buffalo sperm motility, plasma membrane integrity and acrosome integrity were performed at post-dilution, equilibration, post-thaw and after 30 days of storage in liquid nitrogen. For thawing, straws were dipped into a water bath at 37ºC for 30 sec. Progressive motility was estimated according to [13], acrosome and membrane integrity were estimated according to [14, 15], respectively.

Fertility trial

A total of 141 Egyptian buffalo cows were artificially inseminated with random frozen doses from various extenders. Each female was inseminated with a single straw 8-14 h after start of estrous behavior. Using recto-vaginal technique and the universal insemination gun, the thawed semen was deposited in the uterine body just next to the anterior end of the cervix. Conception rate was confirmed by rectal palpation at least 60 days after insemination.

Statistical analysis

The data was statistically analyzed in two ways using general linear models procedure adapted by [16] for user's guide with one-way ANOVA. Duncan test within program SPSS was done to determine the differences among the means.

RESULTS AND DISSCUSION

Progressive sperm motility

Progressive motility (%) of buffalo bull spermatozoa at different stages of cryopreservation (post-dilution, post-equilibration and post-thaw) was superior ($P<0.05$) in the media containing LDL in comparison with control medium containing 20% EY (Fig.1). At post-dilution and

equilibration concentration of 12% LDL seemed to give the best results. Moreover, at post-thawing or at post 30 d storage in liquid nitrogen, sperm progressive motility was more twofold higher in the 12% LDL extender 63.3 or 61.7% vs. 35 or 26.7% in the control 20% EY extender (Fig.1).

The present results show clearly that, LDL can replace egg yolk in tris extender with better results in terms of progressive motility of buffalo spermatozoa. Furthermore, the optimum concentration of LDL has been determined to be 12%. In the bull, a concentration of 8% LDL gives the best results [7,8]. However, [12] demonstrated that the percentage of motile spermatozoa following different stages of cryopreservation in 10% LDL give the best results in Nili-Ravi buffalo bulls.

We suggested that the use of LDL could enhance the ability of buffalo spermatozoa against cold shock and improve the sperm quality during the freeze- thaw process in comparison with the medium based on egg yolk. Egg yolk is reported to contain some deleterious components which are potent to reduce semen motility[5]. The control medium is composed of 20% EY, which itself contains 50% dry matter, 6.6% of which is LDL [7,17].

The concentration of LDL in the control medium is therefore 6.6% LDL, which is very close to that use in the present 6-8% LDL medium (Fig.1), which gave the higher ($P < 0.05$) post- thaw progressive motility (45% and 50% for 6 and 8% LDL, respectively Fig.1) versus 35% in the control 20% EY. It is known that some components in egg yolk play an antagonistic role to the cryoprotective effect of LDL. This postulate may explain the higher post-thawed motility in extender containing 12% LDLs compared with egg yolk containing extender.

Acrosome and plasma membrane integrities

The extenders containing LDLs provided better protection for the acrosome than the medium containing 20% egg yolk ($P<0.05$) (Fig.2) following different stages of cryopreservation. The extender containing 12% LDL resulted in greater protection for the acrosome with an improvement of about 12.2%, 14.8%, 26.3% and 42.8% points over the control EY extender following dilution, equilibration, post-thaw and after 30 days storage in liquid nitrogen, respectively, (Fig.2).

The proportion of spermatozoa with an intact plasma membrane is superior in the medium containing 12% LDL than in the medium with egg yolk after dilution, equilibration, thawing and 30d storage in liquid nitrogen (87.7, 84.7, 71.3 and 68.5% vs. 75.5, 69.5, 34.7 and 25.8%, respectti-vely) (Fig. 3).

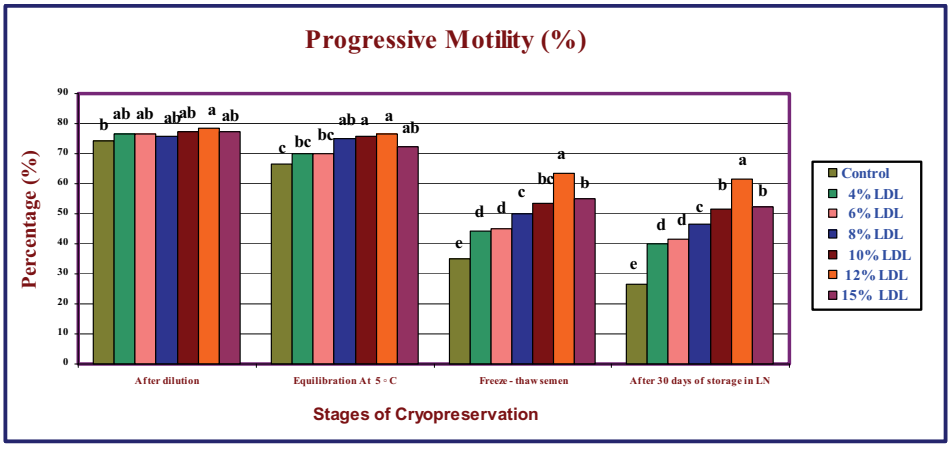

Fig. 1. Progressive motility of buffalo spermatozoa, at different stages of cryopreservation in different concentrations of LDL

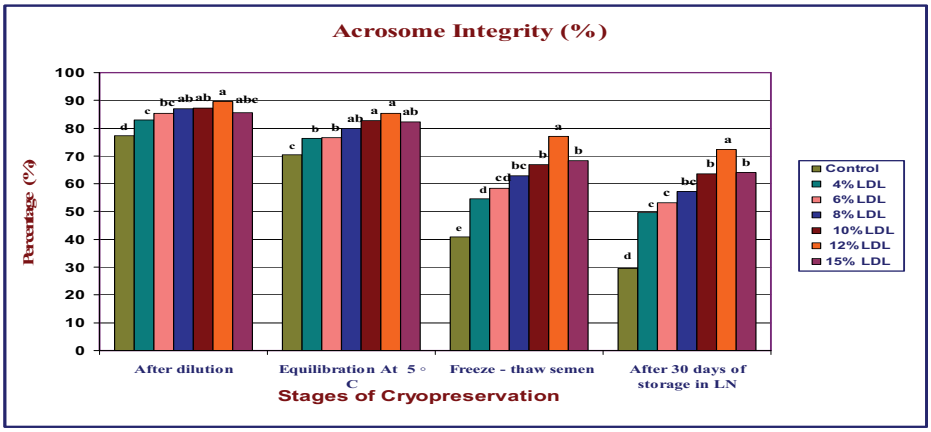

Fig. 2. Acrosome integrity of buffalo spermatozoa, at different stages of cryopreservation in different concentrations of LDL

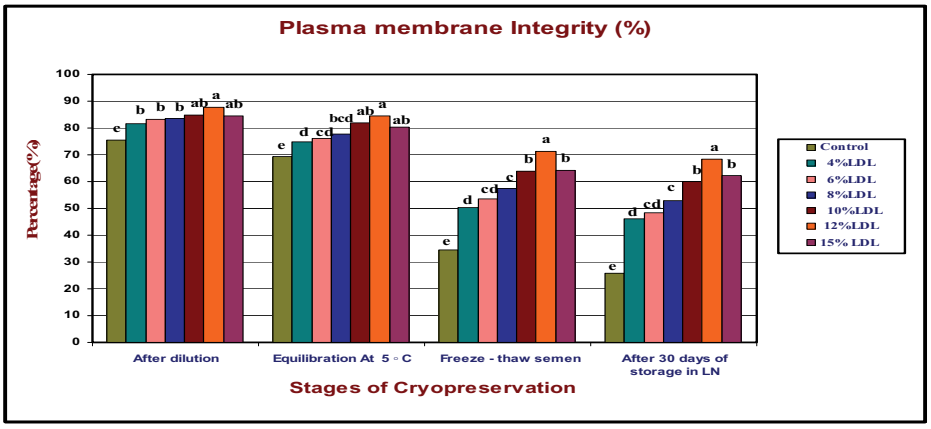

Fig. 3. Plasma membrane integrity of buffalo spermatozoa, at different stages of cryopreservation in different concentrations of LDL

The present results show that, the post-thaw structural and functional integrity of acrosome and plasma membrane of buffalo bull spermatozoa was higher in extender containing LDLs 12% compared with the control. **Amirat et al. (2004)** also reported in bulls that LDL did not induce more plasma membrane damage during the cryopreservation procedure than egg yolk. **Bencharif et al. (2008)** reported for dogs a better preservation of flagellar plasma membrane integrity for spermatozoa cryopreserved in LDL compared to egg yolk. In **1989, Courtens et al** [18] also reported that LDL were less aggressive to cell than egg yolk; there was alteration of the plasma membranes and very little acrosome disruption. They emphasized the possible adverse effect of calcium, present in high concentration in egg yolk. According to these authors, the acrosomes were modified or damaged, which could result from a rapid calcium influx into spermatozoa when the temperature is below 30ºC. Besides anthor interesting aspect that could be evaluated in impact of oxidation on membrane stability[19].

The medium containing 12% LDL provides the best protection of acrosome integrity, possibly via a direct action through the exchange or repair of acrosomal membrane phospholipids or possibly simply because

the medium is less rich in progesterone than egg yolk due to the filtering effect of the dialysis membrane [17]. The progesterone found in egg yolk plays a role in the capacitation of spermatozoa in cattle [20], horses [21] and man [22], it appears to act via an extragenomic action on human spermatozoa, via the secondary activation of calcium channels leading to an increase in intracellular Ca^{++}, which may be responsible for the capacitation of spermatozoa.

Effect of LDLs in extender on fertility rates

Fertility rates (%) in buffalo inseminated with semen cryopreserved in extenders containing different LDLs concentration (4, 6, 8, 10, 12 and 15%) were higher ($P < 0.05$) than control EY (Table 1).

The final aim of buffalo bull sperm cryopreservation is the production of fertilized eggs after artificial insemination (AI). A successful AI requires that a significant number of viable, fertile sperm are delivered at the site of fertilization in appropriate time. Fertility rates were higher with semen cryopreserved in extender containing 12% LDLs compared with the control (72.7% vs. 50%, respectively).

Table 1. Conception rate of buffalo-cows inseminated with frozen semen cryopreserved in different concentrations of LDL

Item	No. of inseminated females	No. of conceived females	Conception rate (%)
Control	20	10	50.0
4% LDL	25	14	56.0
6% LDL	25	15	60.0
8% LDL	10	6	60.0
10% LDL	25	16	64.0
12% LDL	11	8	72.7
15% LDL	25	16	64.0
Overall of LDL treatment	121	75	61.9

In the bull, [8] obtained in vitro fertilization test higher cleavage rate with semen frozen in LDL than with Optidyl$^{(R)}$, a commercial egg yolk extender, but no difference was observed on the blastoeyst rate between the two extenders. In dogs, [17] showed that the presence of LDL in the freezing extender can preserve fertility potential of spermatozoa: 6 bitches were confirmed pregnant of 6 that were inseminated.

In the Nili-Ravi buffalo bulls, [12] obtained in vivo fertilization study, higher ($P < 0.01$) fertility rates with semen cryopreserved in extender containing 10% LDLs compared with the control whole EY (20%). Also, El-Sharawy, et al (2012) [23] showed that the addition of 10, 20 and 30 mM of glutamine to the 12 % LDL extender lead to an improvement of quality of buffalo frozen semen and offer higher conception rates (72.2, 73.3 and 66.6%, respectively).

In another study high conception rate was recorded with the use frozen-thawed semen containing 15% DEY, being 65.8% followed by 20% DEY (59.3%), 20% CHEY (58.6%) and 10% DEY (58.1%), but the differences were not significant [24]. It showed be mentioned that conception rate (64 -72%, Table 1) achived in the current study is satisfactory as compared the previous studies using various freezing and thawing techniques.

CONCLUSIONS

Based on the results of this study, it could be concluded that LDL possesses remarkable cryoprotective properties for freezing-thawing buffalo spermatozoa. Higher progressive motility percentage, acrosome and plasma membrane integrity and conception rates were achived with the use of 12% LDL as compared to 20 % egg yolk semen extender.Thus it was cocluded that the optimum concentration of LDL in this buffalo semen freezing extender was 12%.

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THE INTERRELATION BETWEEN THE REPRODUCTIVE PERFORMANCE AND THE DAIRY PRODUCTIVITY LEVEL OF THE MOLDAVIAN BLACK SPOTTED CATTLE BREED POPULATION OF "SOUTH" SUBTYPE

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Abstract

The age of preparing the young cattle for reproduction and also cattle production and reproduction performances depending on the age of the first insemination, the main reproductive indices of cows and the influence of the season on the main reproductive indices in the studied population of cattle was studded. The investigations have been performed in terms of producing activity of the cattle breeding farm of the Moldova's south districts, using a group of Black Spotted cattle breed population of Moldavian "south" subtype. It wasn't established a regularity of the influence of the first insemination age on the reproductive indices and milk productivity level of the primiparous heifers of the Black Spotted cattle breed of Moldavian "south" subtype. The reduction of cow reproductive indices takes place only when milk productivity level exceeds 7000 kg per normal lactation compared with the maximum allowed requirements. The length of the service-period increased by 46,25%; The length of the calving interval increased by 3,97%; The coefficient of cow reproductive capacity decreased by 1 %. The value of the main reproductive indices of the studied group of cows manifested specific features depending on the season: The intensity of estrum manifestation prevails in the spring-summer period and constitutes 69,57% of the total females in heat during the year; The length of the service-period exceeds the maximum allowed requirements in the autumn-winter period; The best results of female fecundity have been established in the autumn and spring periods.

Key words: *Black Spotted cattle breed population of Moldavian "south" subtype**,** reproductive indices, milk productivity level, service-period, calving interval, cows' reproductive capacity.*

INTRODUCTION

The reproduction of farm animals is the basic component of the animal breeding, management and production obtaining technology. From a generally biological point of view, the reproduction ensures the continuity of animal species and breeds.

The success of farms and/or farmers it's up to an optimal fecundity. The fecundity optimization of the cow' dairy effective suppose a permanent control of a multitude factors which influence on the functionality of reproductive system. The decisive conditions for increase results of the fecundation suppose a profound knowledge of external and internal factors on the hormonal circulation.

In accordance with the multiple factors which influence realization of the reproductive function in cows, we purposed to study the interrelations between the reproductive indices and milk productivity level of the Moldavian Black Spotted cattle breed population of "south" subtype.

MATERIAL AND METHOD

The investigations have been performed in terms of producing activity of the cattle breeding farm of the some south district, using a group of Black Spotted cattle breed population of Moldavian "south" subtype.

The cow heat cycle was identified by observations of female behaviour and specific signs of excitation. The artificial insemination or introduction of semen in cows was performed by the recto-cervical method using the frozen-thawed semen in polymer straws. For the insemination, there have been used only the samples presenting at least 40% (4

points) of sperm with advancing rectilinear movements.

The primary information concerning the production and reproduction performances was obtained as a result of studying the primary zoo-technical evidence of the technician-operator's activity. In order to accomplish the established objectives we assessed the following indices:

- *Milk productivity* assessed according to:
  - ✓ The amount of milk per normal lactation;
  - ✓ Average percentage of fat content in milk per normal lactation;
  - ✓ The amount of fat per lactation.
- *Reproduction performances*, assessed using the following parameters:
  - ✓ The age of the first insemination;
  - ✓ The length of the service-period;
  - ✓ The length of the calving interval;
  - ✓ Coefficient of used reproductive capacity of cows.

The length of the calving interval was calculated analyzing technician's evidence concerning the artificial inseminations according to the following formula:

$$CI = SP + G;$$

in which: CI – the calving interval (days); SP – the length of the service-period; G – the length of the gestation period.

The service – period represents the interval (days) from parturition till the beginning of gestation.

The length of gestation represents the period from the insemination till calving. It was determined according to the results of technician's records regarding the artificial inseminations. Fecundity percentage represents the rate of successfully inseminated females from the total number of inseminated females in a certain period of time and it was established using the formula:

$$F\ \% = 100\ [\ (AG + J) : (\ AI + ÎV)];$$

in which: F % is the percentage of fecundity; (AG + J) – pregnant cows and heifers; (AI + ÎV)– inseminated cows and heifers.

The coefficient of cows' reproductive capacity was determined using the formula:

$$CCR = 365 : CI,$$

in which: CCR – the coefficient of cows reproductive capacity; 365 – the optimal period of the calving interval (days); CI – the calving interval de facto (days).

Statistical processing of the experimental results was computed by mathematical analysis of the biological phenomena [1].

RESULTS AND DISCUSSIONS

Physiologically mature are considered the young animals that have normally developed genital organs and that have reached about 65 – 70% of the adult animals body development of the same breed. The age of physiological maturity establishment is influenced by many internal genetically determined factors and also by a variety of external factors. We tried to evaluate the reproduction age (the age when young cattle reach their physiological maturity) and also the age of the first calving of the Black Spotted heifers of Moldavian "south" subtype (Tab. 1).

Table 1. The performances concerning the physiological maturity and first calving of the Black Spotted heifers of Moldavian "south" subtype

Nr. d.o.	Specification	Number of used animals	$X \pm m_{xc}$ *months*	C_v, %
1	The age of the first insemination	95	19,00 ± 0,23	5.4
2	The age of the first calving	82	28,00 ± 0,022	3.66

The analysis of data presented in table 1 proves that in the breeding and feeding conditions created within this farm, the age of heifers used for reproduction is of 19 months. When comparing this age with data from literature [6], we found out a slight difference: + 1 month. Therefore, the breeding and management conditions of heifers raised for reproduction require certain improvement measures. The age of heifers' first calving in the studied group is of 28 months. The reduction of the first calving age (less than 30 months) and of the calving interval (less than 400 days) also ensures an intensification of the rhythmicity of the total livestock number increase.

According to fig. 1 we can conclude that most of the animals (36,37%) have been successfully inseminated at the age of 18 months, 27,26% of them have been used for reproduction at the age of 19 months and,

simultaneously, 36,38% of them have been inseminated at the age of 20 – 21 months.

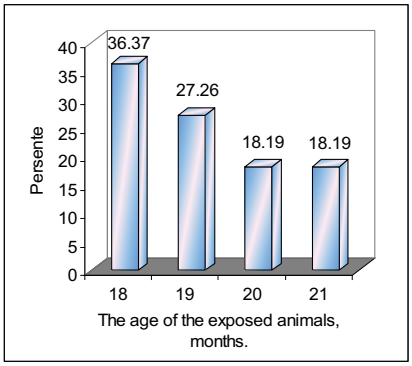

Fig. 1. Dynamics of using young cattle for reproduction

These data exceed significantly the optimal period of using young cattle for reproduction. The age when young cattle must be used for reproduction has a special importance on the economic future of cattle breeding as they will ensure the production only 3-5 months later.

Table 2. Productive and reproductive performances of the "south" subtype Moldavian Black Spotted cattle breed population of primiparous

Age of the first insemination, *months*	Milk quantity per first lactation, kg	The medium percentage of fat content, %	The amount of fat per first lactation, *kg*
18	5785,00 ± 500,26	3.71 ± 0.03	214,69 ± 1,89
19	5329,50 ± 179,59	3.73 ± 0.03	200,94 ± 7,22
20	5071,00 ± 291,08	3.70 ± 0.06	187,31 ± 9,12
21	6339,00 ± 739,07	3.67 ± 0.05	232,12 ± 24,72

The data presented in table 2 prove that milk productivity per first lactation exceeds the data anticipated by the suggested standard in order to create the breed regardless the age of heifers used for reproduction. The maximum amount of milk per normal lactation is obtained from primiparous heifers, inseminated at the age of 21 months. The lowest results of this index were established in the group of primiparous heifers inseminated at the age of 20 months.

As for the percentage of fat content, it can be mentioned a homogeneity of this index regardless the age of young cattle, used for reproduction. The difference between them exceeds the standard value.

The amount of fat per first lactation is higher in the case of heifers that were inseminated at the age of 21 months. Therefore, the cattle population raised within the publique farm shows a high productive potential exceeding the standard requirements.

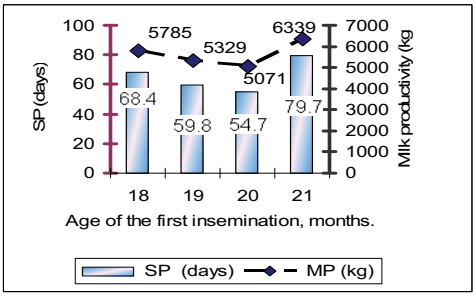

Fig. 2. Milk productivity and lenght of the service periode in corelation with the age of the heifes' first insemination

Considering the reproductive performances we can mention that the service-period varies from 54,7 days in the group of primiparous heifers inseminated for the first time at the age of 20 months up to 79,7 days in the group of those used in the breeding cyclogram at the age of 21 months. In this case there was a dependency between the amount of milk obtained per normal lactation and length of the service-period. Thus, the primiparous heifers inseminated for the first time at the age of 21 months, which recorded the highest amount of milk per lactation also recorded the longest service-period (Fig. 2). And correspondingly, the primiparous heifers inseminated at the age of 20 months recorded the lowest results of milk productivity and also the shortest service-period.

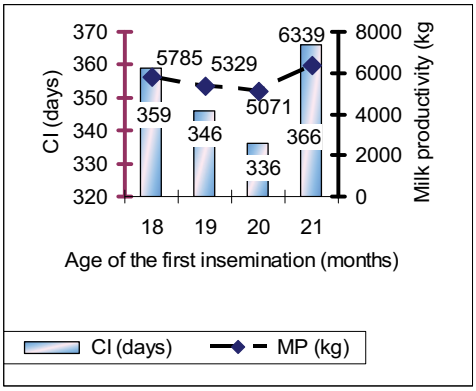

Fig. 4. The length of the service period and the length between calvings depending on the level of productivity (record lactation)

In accordance with the length of the service-period we observed the same regularity of the length of the calving interval. The lowest results were established in the group of primiparous which were inseminated at the age of 20 months – 336 days, while in the case of primiparous heifers inseminated at the age of 21 months the length of the calving interval is the highest - 366 days (fig. 3).

The coefficient of cows' reproductive capacity use varies from 1,00 up to 1.05.

The data obtained regarding the productivity performances per first lactation and also the reproductive performances confirm the data from literature concerning specific interrelations between milk productivity level and reproduction values.

On the other hand we can mention that the activity of staff responsible for the organization of the reproduction process is at an appropriate level as all the indices fall into the limits of the minimum allowed requirements.

On the basis of obtained data and information we can mention that the reproductive indices and milk productivity level are influenced, preponderantly, both by genetic factors and by fodder and management conditions and to a lesser extent by the age of first insemination.

In order to increase cattle livestock number and improve their breeding conditions, the reproduction represents a biological feature and a technico-organizational method contributing directly to this purpose [2; 5; 4].

But the achievement of certain reproductive indices depends on specific peculiarities of the breed and also on the method these ones are integrated in the breeding and management technique.

In the field of cattle breeding, the data regarding problem solving of interdependencies between milk productivity and reproductive performances have a long history and continue to be targeted by specialists because productivity growth is evolving.

Data from literature emphasize that the productivity of the Black Spotted cattle breed is higher compared with its contemporary Red Steppe breed but they lose in terms of qualities [3; 7].

Given that the Black Spotted cattle breed of Moldavian "south" subtype was created by absorption crossbreeding of the Black Spotted breed with the local Red Steppe breed, we tried to evaluate the retro influences between milk productivity level and reproductive indices of the studded population. The figure 4, presents production and reproduction performances of the studied group of animals (per record lactation).

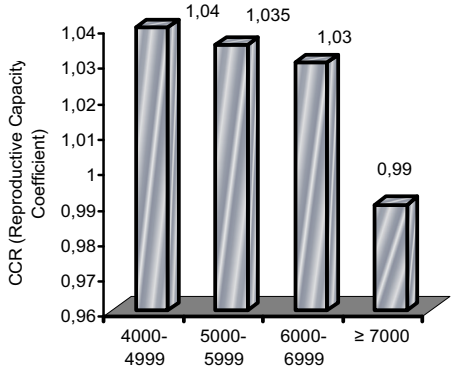

Fig. 5. The coefficient of cows' reproductive capacity depending on the milk productivity level (record lactation)

The analysis of data presented in Fig. 5 proves that milk productivity level influences to some extent cows' reproductive capacity. Thus we can mention that the lowest results were established in the group of cows

recording the productivity of more than 7000 kg of milk per lactation.

Based on the obtained results we noticed that the reproductive indices of the cow population of Black Spotted cattle breed population of Moldavian "south" subtype depend on milk productivity only after a certain level. The critical value of milk productivity indicating a trend of reduction of the main reproductive indices was the one exceeding 7000 kg of milk per lactation with the average fat content in milk of 3,65%.

The functioning of the genital apparatus is influenced by numerous internal and external factors[9; 8; 3]. The table 5 presents the practical results obtained within the animal breeding farm during the year 2011.

The data indicated in the table 3 prove that the indices of reproductive activity reduce in the autumn months. The biggest number of calvings was recorded in spring and winter and it begins to reduce in the summer period reaching its minimum values in autumn. The number of normal calvings reduced in autumn.

The rhythm of female heat cycle manifestation during the year is of great interest because it influences the uniformity of obtaining production during the year, maintenance of the sale price stability, insurance of the milk processing enterprises activity and market supply with dairy products at affordable prices

Data presented (tab. 3) reveal that the activity of cow genital apparatus is intensified in the spring-summer period. Beginning with autumn the number of in heat and fertile inseminated females reduces sharply and increases again in winter.

Percentage data regarding heat cycle manifestation in the studied population of cows prove that out of those 253 studded inseminations, 42,69% have been performed in spring, 26,88% in the summer months and the lowest percentage of inseminations was recorded in the winter season.

Table 3. The influence of the season on certain reproductive indices of cows population of Black Spotted cattle breed population of Moldavian "south" subtype

Specification	Winter	Spring	Summer	Autumn
1. Number of calvings: (*heads*)	80	90	60	18
%	33.61	37.81	25.21	7.5
including:				
- normal (%)	90.00	92.22	93.3 3	72.22
- dystocia (%)	3.75	2.22	1.67	22.22
- fetal retentions: %	6.25	5.56	5.00	5.56
2.Percentage of fecundity, %	80.95	93.52	83.82	97.15

The presented data (tab. 3) prove that cow fecundity rate varies from 80,95% up to 97,15%. The best results of female fecundity have been recorded in the autumn season. In spring there is a tendency of fecundity reduction. In the summer months, when high temperatures prevail, the fecundity rate reduced to 83,82%. The lowest results of cows and heifers fecundity have been recorded in the winter months.

Further, we evaluated the influence of the season on the length of the service-period. The obtained results are presented in figure 6.

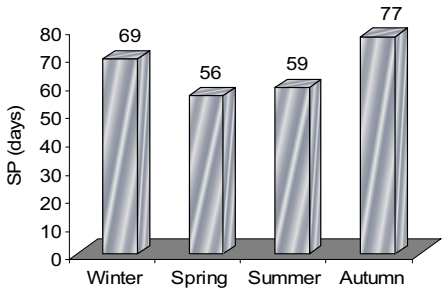

Fig. 6. The influence of the season on the length of the service-period of cows and heifers of the Moldavian "south" subtype Black Spotted cattle breed

According to presented data it can be noticed that the longest service-period was recorded in the autumn moths. During the winter this index begins to decrease. The lowest results have been recorded in the spring-summer period.

CONCLUSIONS

It wasn't established a regularity of the influence of the first insemination age on the reproductive indices and milk productivity level of the primiparous heifers of the Black Spotted cattle breed of Moldavian "south" subtype, therefore we can mention that the age of physiologically mature young cattle prepared for reproduction has a great importance on the economic future of the cattle livestock.

The reduction of cow reproductive indices raised within the APC takes place only when milk productivity level exceeds 7000 kg per normal lactation compared with the maximum allowed requirements.

- the length of the service-period increased by 46,25%;
- the length of the calving interval increased by 3,97%;
- the coefficient of cow reproductive capacity decreased by 1 %.

The value of the main reproductive indices of the studied group of cows manifested specific features depending on the season:

- the intensity of estrum manifestation prevails in the spring-summer period and constitutes 69,57% of the total females in heat during the year;
- the length of the service-period exceeds the maximum allowed requirements in the autumn-winter period;
- the best results of female fecundity have been established in the autumn and spring periods.

In order to increase the zoo-economic balance of the Black Spotted cattle breed population of Moldavian "south" subtype it is recommended to take into consideration in terms of breeding and selection measures the peculiarities characterizing the interrelations between the established reproductive performances and milk productivity level and also to further deepen the study of this topic.

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THE MANAGEMENT OF WATER STATE IN GLYCERINATED RAT HEART THE ROLE OF 1H NMR SPECTROSCOPY

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Abstract

Ln order to obtain new data concerning muscle contraction at the molecular level, the interrelation water-contractile proteins has been investigated by means of 1 H NMR Spectroscopy. Rat heart muscle from 6 and 37 months old rats has been used for proton transverse relaxation time measurements in Ri, Co and Re. at different [ATP].The distribution of negative charges in contraction and relaxation has been measured by exposing glycerinated muscle from 6 to 37 months old rats to different [Mn+2].Our data have pointed out the existence of two proton relaxation tinies:T2s and 121 accounted for two water compartments. The modification in water state are related with modifications in contractile activity. The elongation od proton transverse relaxation times is associated with a decrease in the degree of water molecules aggregation.T2s and T2l are correlated with a reduction in muscle hydration, contraction being a function of ions binding to the protein sites. These sites are implicated in determination of protein hydration state.

Key words**:** *1 H NMR, aging, glycerinated muscle, contraction, relaxation, rigor*

INTRODUCTION

Literature data [1] concerning muscular contraction phenomenon, have pointed out the appearance of long range repulsive forces within contraction state which tend to repell the myofilaments one from each other.These forces are converted into active shortening tension through passive intervention of transverse myosin crossbridges with an oblique orientation between myofilaments [2].The repulsive forces which take place during contraction are the consequence of the increase in the electric charge of myofilaments[3].

In order to obtain new data concerning muscle contraction at the molecular level,the polar groups from the contractile proteins have been investigated by means of 1 H NMR Spectroscopy, to test the water state from the close proximity of myofllaments in different experimental conditions: in contraction, relaxation and rigor from heart muscle of Wistar rat.

1H NMIR is a very useful method in biology because we can obtain very important data about mobility of some groups at the level of protein molecules,which provide informations about conformation changes which result from the chemical modifications.

The aim of our study was related with:

1.The investigation of proton transverse relaxation times of water from glycerinated muscle in Ri,Re,Co at different ATP concentrations.

2.The distribution of charges in Contraction and relaxation by exposing glycerinated heart muscle from 6 and 37 months old rats to different [Mn+2], by means of 1 H NMR spectroscopy.

MATERIAL AND METHOD

Heart muscle from 6 and 37 months old rat has been used for 1 H NMR studies; the animals have been killed by cervical dislocation and muscle samples from sartorius muscle have been processed for NMR measurements as fresh and glycerinated biological samples according with the published technique [4].Glycerinated heart muscle fragments of 2 cm long have ben washed for 15 minutes in

bidistiled water and then dried on filter paper.The next step was the placement of biological sample m pH 7.2 in order to assure the ionic equilibrium for one hour according with the published method[5]. After preincubation, the muscle fragments have ben incubated for 10 minutes in Ri ,after that have been removed and have been dried on filter paper,and then introduced in special test tubes for reading the relaxation times in an Aremi'78 1 H NMR Spectrometer in impulses of a fixed frequency of 25 MHz.

Alter the readings have been done,the tissue fragments have been introduced in relaxation solutions(0.5mM ATP) for 10 minutes.Then the Spectrograph readings have been recorded, taking care for pH of solution to be the same.

Then, the biological fragments have been introduced in Re solution 2(1mM ATP);the recordings of proton relaxing times have been done ,following another 10 minutes incubation in relaxing solution 3(2mM ATP), followed by readings at Spectrometer. Tissue fragments then have been washed ,preincubated in media without ATP for ionic equilibration and after that have been introduced in contraction media 1(0.5mM ATP), 2(lmM ATP), 3(2mM ATP), according with the protocol used for recording proton relaxation times in RI and Re state.

The composition of RI, Co and Re media has been the same as the incubation media used for optical microscopy studies[6].

Alter recording the values of proton transverse relaxation times, the muscle fragments have been weighted successively for a few days until a constant weight has been achieved, in order to estimate the dry/wet weight ratio.

The method for processing data concerning the proton transverse relaxation times has been previously described [7].

RESULTS AND DISCUSSIONS

By optical measurements done with ML4 optical microscope on sarcomere lengths in contraction state, a decrease in the active shortening capacity of sarcomeres from 1 .46u in 6 months old rats to 1 .67u in 37 months old rats for 0.5mM[ATP] has ben recorded as we For 1 mM ATP, sarcomere length recorded in young rats was 1 .67u and for 37 months old 1 .92u.Concerning 2mM ATP concentration,the mean value of sarcomere length was 1.6 u for young muscle and 1.88 for old muscle.The increase in arcomere length with ageing,is significant from statistical point of view for the three ATP concentrations.

The low milk offer has obliged Romania to import milk for assuring raw material for milk processing industry.

Table 1. Relationship between sarcomere length in heart muscle of young and old rats in Contraction state and T2s and T2l values at three [ATP].

	Heart 6 months old rat			Hear t 37 months old rat		
	T2s (ms)	T2l (ms)	Sarc.length(u)	T2s (ms)	T2l(ms)	Sarc.length(u)
Co(0.5mM)	34	230	1.74	47	280	2.01
Co.(1mM0	33	180	1.72	45	270	1.74
Co(2mM)	30	138	1.70	46	278	1.72

Table 2. Mean values of sarcomere length(u) in Heart muscle from rats of different ages in relaxation state [ATP]

	6 months	37 months
0.5mM	X=2.36+/-0.02	X=2.22+/-0.01
1mM	X= 2.42+/-0.02	X2.24+/-0.0l
2mM	X =2.228+/-0.02	X=2.26+/-0.02

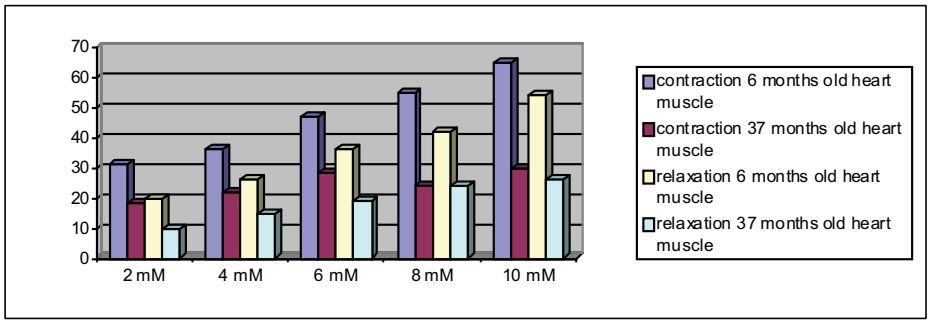

Fig. 2. There is a reduction in relaxation capacity of sarcomeres from old rat accompanied by a lengthening of T2s and T2l proton relaxation times.

Table 3. Proton relaxation times T2s in Contraction in the presence of Mn+2 from Heart muscle from 6 and 37 months old rats

	Heart of 6 month old rat		Heart of 37 months old rat	
-[Mn+2]				
	T2s	T2l	Ts2	T2l
2mM	50	100	99	150
4mM	65	150	96	160

There is an decrease in T2s proton transverse relaxation times in young rat versus old rat, as an expression of a decrease with aging in the active shortening capacity of sarcomeres.
Relationship between sarcomere length in Heart muscle of young and old rats in relaxation state and T2s and T21 values at three different [ATP].
Our studies concerning ionic charges distribution in Contraction and Relaxation using glycerinated skeletal muscle from young and old rats exposed to different concentrations of [Mn+2] have pointed out an increase in T2s in contraction in old rats.
As it can be seen, the elongation of proton transverse relaxation times is proportional with the concentration of.Mn+2 are accommodated supplementary in Contraction at the level of Concerning relaxation for all threeATP concentrations, an age dependent reduction of sarcomere length without statistical significance has been recorded. contractile proteins due to their fixation at the level of negative charges on contractile protein filaments.
In ageing muscle there is an elongation of proton transverse relaxation times T2s and T2l both for
Contraction state in the presence of an increased quantity of Mn+2.T2s and T21 are correlated with a reduction of muscle hydration in case of old muscle,contraction being a function of ions binding to the proteic sites;these sites being important in determination of hydration of proteins.

Table .4. Proton transverse relaxation times from 6 and 37 months old rats at different Mn+ concentrations

[Mn+2]	6 months old heart		37 months old heart	
	T2s	T2l	T2s	T2l
2mM	20	130	30	160
4mM	16	149	37	180

According to Elliott studies[8] the long range of charge is achieved only if subfragment 2 of myosin tail which carries aproximatively 1/3 of negative charge of molecule,has been tdted at a *45* degree towards the filament skeleton.
C.T. Dragomir [9] has studied the level of fixed charge in rabbit muscle,and he concluded that the level of fixed charges increases with the external electrolyte. For example, in the presence of 100 mM KC1 the concentration of fixed charges is aproximatively 75 mM for psoas muscle in Rigor.
1H NMR studies in presence of Mn+ related with negative charge density in heart muscle from 6 and 37 months old rats have revealed an elongation of T2s and T2l as a function of [Mn+],this being more reduced in Co than in Re which accounts for suplimentary accumulation of Mn+ in Co at the level of contractile proteins negatively charged.

CONCLUSIONS

Our data have pointed out the existence in glycerinated rat heart muscle of two proton relaxation times:T2s and T2l accounted for two water compartments. The modifications in water state are related with modifications in contractile activity.
The elongation of proton transverse relaxation times is associated with a decrease in the degree of water molecules aggregation.
T2s and T21 are correlated with a reduction in muscular hydration, contraction being a function of ions binding to the protein sites. These sites are implicated in determination of protein hydration.

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THE MANAGEMENT OF CALCIUM BALANCE IN RAT SKELETAL, CARDIAC AND VASCULAR SMOOTH MUSCLE FUNCTION THE ROLE OF CALCITONIN

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Abstract

Aim*:Our study has been concerned with investigation of the effect of Calcitonin treatment upon 45 Ca, 32P and 3H Colesterol uptake by the rat skeletal, vascular and cardiac muscle.*
Material and method*: Animals: 30 white Wistar rats (150-180 g) aged between 6-24 months old divided into two groups of 15 rats each have been taken in our study: 15 young and 15 old. Method: Both, young and old rats have received Calcitonin treatment (vials with 0.5mg/ml = 0.25 mg) 0.025mg have been injected in each animal. Controls have received injections with physiological saline solution. Rats have been killed by cervical dislocation. Muscle fragments have been collected on ice bath ,then weighted and preincubated for one hour at 37^{o}C in Hanks medium pH 7. After one hour, the muscle fragments have been incubated with 45Ca(90μl/ml). In each sample 10μl/sample has been used. Samples have been incubated for 2 hours at 37^{0}C. Nonspecificaly bound radiomarkers have been extracted with 1N Hcl for 24 hours and then a determination of specific bound radioactivity as well as the extracted one using a beta scintillator in liquid phase. 32P has been used with a specific activity of 7 mCi/ml,0.5μCi/sample. 3H Cholesterol has been used also for our experimental studies, with a total activity of 1mCi.0.1mCi/mlhas been used for working solution using 8μl from dilution. The radioactivity has been evaluated with a Beta Betrhold Scintilation Counter.*
Results: *Calcitonin experimentaly administrated has a clear influence upon 45Ca uptake in muscle tissue. Calcitonin treatment has an influence upon 3H Cholesterol uptake in skeletal muscle from treated rats versus controls.*
Conclusions: *Our data have an important clinical value in monitoring calcium level in patients in order to avoid cardiac arithmias and vascular perturbations.*

Key words**:** *calcitonin,skeletal muscle,cardiac muscle, vascular muscle,calcium level,3H cholesterol,45 Calcium*

INTRODUCTION

Hormones are produced by endocrine and neuroendocrine cells and mediate mainly systemic effects Tashjan A.(1970). Cytokines are produced by numerous cell types and mediate local effects. The production of calcitonin (CT) peptides follows either the classical hormonal expression which is believed important for calcium metabolism or cytokinelike expression which is induced by inflammatory stimuli. To describe this plasticity, the term "hormokine" was proposed. The concept is based on the discovery of the ubiquitous expression of CT peptides (i.e., ProCT, CT gene-related peptide (CGRP) and adrenomedullin (ADM)) during sepsis.

Calcitonin (CT) was discovered 40 years ago, when it was assumed to be a hormone with a yet-to-be-determined role in human physiology (Becker KL, et al. 2004). Since then, CT has been found to be only one entity among related circulating peptides which have pivotal roles in the metabolic and inflammatory host response to microbial infections (Becker KL, 2001).

These peptides share marked structural homologies and include procalcitonin (ProCT), calcitonin gene-related peptide (CGRP) I and II, adrenomedullin (ADM), and Amylin. (**Cooper GJ** 1994)

Calcitonin influence in key points in calcium metabolism (**Hay DL, Christopoulos 2005**).

Its administration is important in mentaining Ca levels in normal range(**Jaeger P,Jones et al. 1986**).

The aim of this study was to investigate the mode of action of this molecular agent at the cell membrane level of some unspecialized cells in calcium balance such as muscle tissue.

Our investigations have been done on skeletal, cardiac and aortic muscle tissue from control and treated rat with calcitonin (acute treatment-24 hours).

MATERIAL AND METHOD

Our study has been done on 30 Wistar rats (150-180 g) aged between 6-24 months old: 15 young and 15 old. Both, young and old rats have received Calcitonin treatment (vials with 0.5 mg/ml = 0.25 mg) 0.025 mg have been injected in each animal. Controls have received injections with physiological saline solution. Rats have been killed by cervical dislocation. Muscle fragments have been collected on ice bath, then weighted and preincubated for one hour at 37°C in Hanks medium pH 7. After one hour, the muscle fragments have been incubated with 45Ca (90µl/ml). In each sample 10 µl/sample has been used. Samples have been incubated for 2 hours at 37^0C.
Nonspecificaly bound radiomarkers have been extracted with 1N Hcl for 24 hours and then a determination of specific bound radioactivity as well as the exctracted one using a beta scintillator in liquid phase. 32P has been used with a specific activity of 7 mCi/ml, 0.5 µCi/sample. 3H Cholesterol has been used also for our experimental studies, with a total activity of 1mCi.0.1mCi/mlhas been used for working solution using 8 µl from dilution.
The radioactivity has been evaluated with a Beta Betrhold Scintilation Counter.

RESULTS AND DISCUSSIONS

Fig 1 presents the hystogram of 32P uptake in skeletal, cardiac and smooth muscle; a significant increase in 32P uptake in old rats has been recorded versus yong rats.
Fig. 2 Presents the uptake of 45Ca in slkeletal, cardiac and smooth muscle form control and calcitonin treated rats. A decrease in 45Ca uptake in skeletal and cardiac muscle has been recorded in calcitonin treated rats versus controls. No significant difference has been observed for aortic tissue.

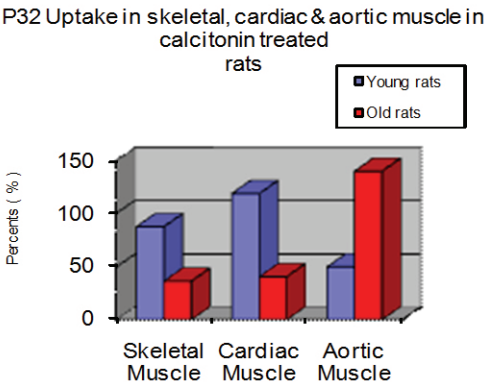

Fig. 1 - ^{32}P uptake in young and old rat skeletal, cardiac and aorta

The different uptake of 45Ca in the three types of muscle can explain somepharmacological aspects which appear in patients treated with calcitonin. Cardiac aritmias which appear after calcitonin injection as well as some vascular disturbances may be the couse in the answer of cell receptors and their influence upon membrane Ca exchange .Receptor binding of calcitonin can be in a direct proportion with the daily dose clinicaly utilised.

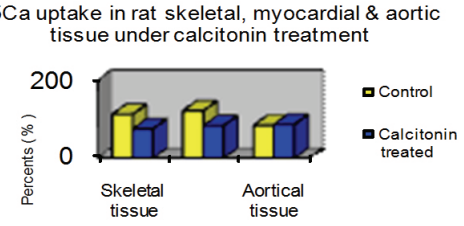

Fig. 2 - ^{45}Ca uptake in rat skeletal, cardiac and aorta from calcitonin treated rats.

Fig. 3 Presents the hystogram of 3H Cholesterol uptake in rat skeletal, myocardial and aortic tissue from cotrol and calcitonin treated rats.
An increase in 3H Cholesterol uptake in skeletal muscle from treated rats has been recorded, in comparison with cardiac and aortic muscle, where a decrease in 3H Cholesterol has been recorded.

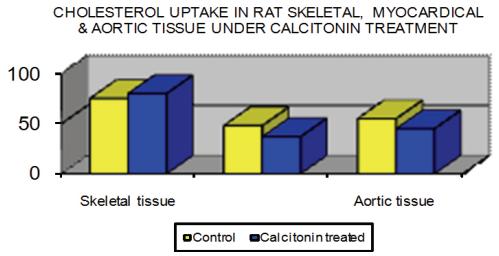

Fig. 3. 3H Cholesterol uptake in skeletal, cardiac and aorta from contol and treated rats with Calcitonin

CONCLUSIONS

Calcitonin experimentaly administrated has a clear influence upon 45Ca uptake in muscle tissue.

Calcitonin treatment has an influence upon 3H Cholesterol uptake in skeletal muscle from treated rats versus controls.

Our data have an important clinical value in monitoring calcium level in patients in order to avoid cardiac arithmias and vascular perturbations.

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ESSAY ON ESTIMATION OF UNDEMONSTRATIVE SPARE OUTPUTS DISCLOSED BY REPRODUCTION BIOTECHNOLOGIES IN SHEEP BREEDING

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Abstract

Among genetic species of farm animals sheep has the highest artificial biodiversity. There are plenty of breeds due to the many kinds of biological production of human interest and to the large areas from plains to mountains and fro Equator to Poles engaged in sheep breeding. Sheep are producing wool (thin, meddle or thick), lambs, mouton (lean or fat), milk, pelts, furs or leather, each of them acting as single selection criterion or as component of selection indexes. Each kind of resulted product has its own commercial value and its way of appreciation it. In many cases the commercial value of sheep breeding is related to the ewes' fertility. On the other hand ewes have seasonal sexual activity including a longer or shorter period of anoestrus when produce nothing related to their fertility. Biotechnological means as artificial insemination, estrus induction, arouse of ovulation rate, embryo transfer, MOET, embryo cloning, in vitro fertilization, transgenice engineering are able to influence the economic efficiency of sheep breeding. The present essay is configuring modalities of estimating the increase of economic value of sheep products when research programs of developing reproductive biotechnologies in ewes implemented in sheep farming.

Key words: *artificial insemination, in vitro fertilization, moet, reproduction, sheep,*

INTRODUCTION

Generally speaking animal farming aims to offer for the market goods in order to get profit. The profit level depends on the relation existing for each product between the price per unit of product and the cost of the same unit. A good price of product is obtained when the product is much required and small quantities of it are offered by producers.

The output of farms is given by the obtained price per product unit and by the quantity of produced goods doesn't matter if they were sold or not yet. Costs are classified in variable costs which modifies together with the produced quantity of goods, fixed costs, which are inherent for production and maintain their level even the production is stopped, and added fixed costs as taxes, publicity or other expenses of the kind [4].

The difference between the total output and the variable costs is giving the farm gross margin. The farm gross margin minus the fixed costs gives the farm profit or loss, or gross profit. The gross profit minus the added fixed costs gives the net profit of the farm.

In order to measure value each country uses its currency. There is an each day currency quotation changing the quotas of peculiar currencies. Prices are frequently changing with the offer and require of the market. That modifies the level of costs as well.

For these reasons the authors of this essay will not use the Romanian currency in the economic analysis of reproduction biotechnology effect in sheep breeding. They will imagine analyses for each peculiar case.

MATERIAL AND METHOD

Considering the complexity of the present theme the essay will discuss the diversity of goods produced with the sheep and their biodiversity [8], will underline the maine reproduction particularities of this genetic species, will show the virtues of the applied biotechnics in reproduction with ewes [10] and will suggest opportunities to use them in order to disclose undemonstrative outputs of sheep flocks, based on their former experiments [1].
In the last part of the essay the effect of the most interesting opportunities will be economically estimate using adequate measure units but having in mind the need for a sure gross margin of the farm economy, what means low variable costs, and a good gross profit, what means using the natural environment as much as it is possible.

RESULTS AND DISSCUSSION

Biodiversity in sheep

Among the domesticated animal genetic species, sheep is the one giving the most diversified kind of goods [6].
Sheep are producing wool, at list three kinds of wool related to the fiber diameter: thin wool (< 24μ), meddle wool (28μ – 33μ) and thick wool (> 37μ). There is also white wool and colored wool. Related to the wool production there are breeds covered with the denomination of Merino Sheep. The most famous breed is the Australian Merino. A preserved old breed is the Rambouilet Merino, in France. There are also Merino breeds in Spain (as native breeds), in some European countries including Romania, in Russia, in other countries having dry climate. Unfortunately wool is not enough appreciated, now days, except in Australia. That means there is no desire to increase wool production in other countries.
Sheep are producing meat. There are at least three kinds of sheep meat. The Mouton, what is a lean meat obtained mostly from young animals grown up to 30 -35 kg of body weight. This kind is required in Western European counties and other countries undertaken English influence. Countries having dry climate and low vegetables' production, like Arabian countries or Mongolia prefer a fat mutton containing vitamins in their suet. There countries, like Romania, for instance, that consume sucking lambs.
In case of mutton production there is interest for more meaty carcasses from some local ewes, for faster growing lambs and for more sucking lambs to be slaughtered.
For good mutton English breed like Southdown, Suffolk, Romney Marsh, Lincoln, Leicester, Border Leicester and so on are appreciated. For fat carcasses the Romanian local breeds Tsurcana and Tsigaia are very good.
Milk production is desired especially in the undeveloped countries where the extensive farming is in use. Thus good mobility of ewes is appreciated and has to be preserved. There is much need of labor in production of milk. Nevertheless in intensive farming two specialized sheep breeds for milk production are in vogue. The Friesian breed, in Netherland, is created for a humid climate and Awassy, in Israel, is created for a dry climate.
Pelts from very young lambs are much appreciated. There are two kinds of wanted pelts. Karakul pelts on ringed fibers and "mouton d'aure' "pelts prepared from young lambs skin of different middle fiber wool sheep. For this purpose large new born lambs are desired.
Skins of adult sheep are also wanted for covers or fur coats. A special quality is offered by the skin of Romanov sheep breed whose coat has shorter thick fibers and little longer thin ones. Such kind of skin is much appreciated in fur cups' manufacture. This kind of fur isn't possible to have for other breeds or crosses.

Some reproduction particularities in ewes

Sexual activity in ewes is under photoperiodic influence. Increasing of dark part of day has cumulative effect in stimulating and liberation of gonadotrophic hormones from the pituitary gland [2] [6]. That is due to the natural selection acting in formatting of *Ovis* genetic species that synchronized the mother ewes' needs of food to the growth intensity of herbs. This trait was saved in domesticated sheep since the outdoor farming system has been practiced along the times. Par consequence the

inter lambing interval is formatted from the mating season of some estrus cycles, the pregnancy of 5 months standard length, the suckling period when young lambs are fed on milk, a milking period if the ewes are milked and a seasonal anestrous before the next mating season (Fig. 1).

The anestrus is due to the photoperiodism. In addition it is sustained by the presence of lambs so long they are sucking [9].

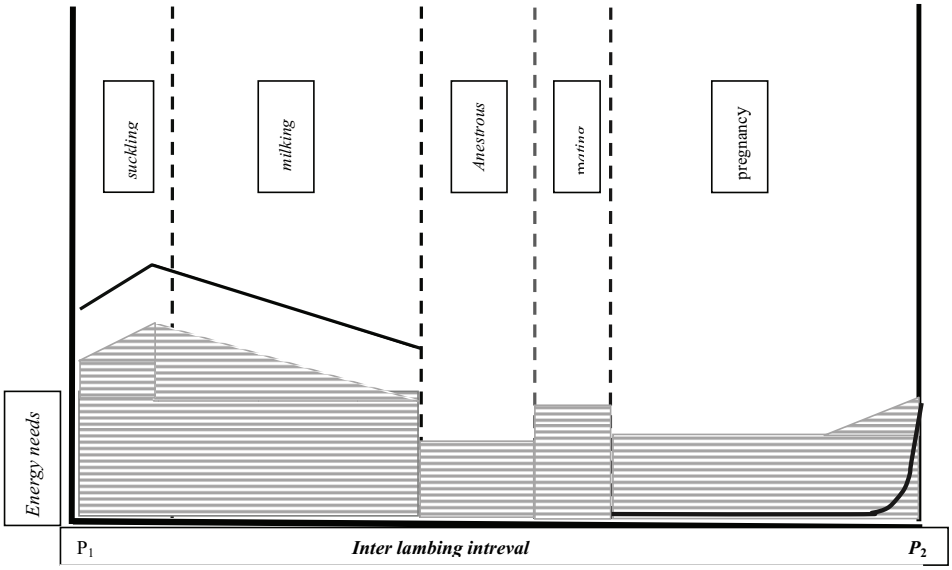

Fig. 1. Components of the inter lambing interval in ewes (after M. Paraschivescu)

Two parameters are genetically determined the 148 – 150 days as length of the pregnancy and 17 days as length of the estrus cycle. Under farming condition the mating season in some breeds is longer than it is in local breeds...Uzualy one folicule is dehiscent in a natural estrus cycle but there are some breeds with frequent gemelarity. In extensive farming the first mating is taking place in the second year of life. In the intensive farming the first lambing is moved in the first year of life.

Essential reproduction biotechnologies in ewes

Many experiments concerning reproduction biotechnologies in farm animals started with ewes [4]. Currently there is rich knowledge about artificial insemination in sheep. Long term preservation of semen is ensured by freezing. Last progress concerning ova fertilization was done by intrauterine insemination of semen especially when deep frozen semen is used [3].

Estrus synchronization both in mating season and out of mating season is solved braking the estrus cycle in diestrus in case of the sexual season by progesterone in the sexual season or by a complete substitute hormonal treatment out of season [5]. Thus blind artificial insemination can be applied for entire flock at the same time.

Super ovulation is controlled as well using FSH of ovine or porcine origin in three days before proestrus or with one dozes of 350 IU of PMSGn immediately before proestrus [7]. Proestrus is obtained when controlled diestrus is deblocate or at the end of a complete substitute hormonal treatment when the action is provided out of the mating season.

Multiple ovulation in sense of collecting embryos by repeating super ovulation is provided only once at the next estrus cycle after

embryo washing. That could be explained by the little number of ovarian cycles during the sexual season or may be because before embryos are collected surgically [3].
Long term conservation of embryo by freezing is a solved question. The lent (English method) freezing, the rapid freezing and the vitrification freezing are applied with satisfactory results. The most used crioprotector is glycerin [11].
The success rate of embryo transfer goes up to 60%. Ruther successful attempts have been made to have twins from receptors females [11].
Better results are wanted concerning the fertilization rate of super ovulated ova in order to increase the number of transferable embryos before freezing them. Better synchronization of the gynecological state of receptor ewes with the age of the transferred embryos is also desired.
Important results have been obtained with in vitro fertilization (IVF) [11]. On this basis transgenic organisms carrying foreign DNA have been obtained. This is a very important fact for pharmaceutics industry.
Cloning of sheep organism was successfully provided. We have to remember the famous Dolly case. Now is said some hundreds of clones of Dolly's type have been obtained. In this case clones have been obtained from adult somatic cells, but clones can be obtained from embryonic cells, too. Hopes for the future are connected to improving de endoscope technique of collecting embryos in order to save the life of genetically valuable donors.
There are also hopes to increase the fertilization rate of super ovulated ova by improving the inseminated spermatozoa capacitating. Many hopes are related to the frequency of twins or triplets obtained from the receptors ewes, associated to small number of transfer embryos' lost. Of great importance for the pharmaceutical. Al of these hopes aims to decrease the cost of applied MOET in selection or in commercial flocks.

Disclosing undemonstrative spare outputs by reproduction biotechnologies in sheep

The target of disclosing undemonstrative spare outputs by reproduction biotechnologies in sheep is very complex because of the great number of possible biotechnologies that could be used and the high diversity of possible targets to be attained.
To pass over difficulties we will make an attempt to classify the main fields reproduction biotechnologies to be applied and to appreciate their specificity. The main fields are:
a) to determine *genetic progress* of the breeding stock of pedigree animals selected to conceive in closed reproduction the new generations of pedigree animals of one sheep breed;
b) to improve the quality or to increase the quantity of products from a sheep flock with commercial purpose;
c) to multiply transgenic organisms by cloning them from adult somatic cells;
d) to preserve "*ex situ*" breeds in critical state of extinction.
Better *genetic progress* can be obtained with higher selection intensity or with higher precision of selection. Higher selection intensity is possible when individual fertility is increased. Precision of selection is the best when progeny' performances are considered versus self performances' test, or sibs' and ancestors' test of performances are used. Artificial insemination is able to highly increase the fertility of rams [6] . Thus AI permits to select with higher precision the best son of a ram when the selection criterion is appreciated on the sons and before they have to be at puberty. This is the case of mutton breeds, of thin or middle wool breeds or even of Karakul pelt breed. For traits that cannot be appreciated on rams, the case of milk production where the minim numbers of 30 pairs daughter – contemporary are required the AI become necessary. But using or not using the artificial insemination is a question of costs. Since AI is not expensive, it might be used to know the genetic abilities of rams in all flocks of excellence. Very interesting discoveries could be done concerning the genotype of rams in Karakul flocks producing pelts of different colors. Thus pelts of wanted colors could be obtained at will not by hazard.
Using ET or other associated to ET technologies in order to increase selection intensity for genetic progress seems to be too expensive for such purpose.

To produce more for the market or to produce more valuable products from the same flock is the goal of any shepherd. AI can help a shepherd possessing of Tsurcana breed producing low growing lambs and fat carcasses when adults to have better lambs to be sold at about 6 month of age for mutton. That is very simple all the flock when first in heat is artificially inseminated and after artificial insemination rams of the flock breed will be left free in the flock for natural mating. It is estimated that approximately 50% of the ewes will be pregnant from AI and the other 50% of ewes will became pregnant with rams of the local breed. Further the first born lambs, of both sexes, will be grown for mutton and the last lambs will be sold for slaughter as sucking lams if males and will be kept in the flock to replace the old females [4].

The same procedure could be applied in case of ET. Then the wall flock is synchronized as receptors of embryos of mutton breeds. Half of them will deliver mutton lambs and the other will deliver males to be slaughter and females to be kept for completing the flock. This time the commercial value of mutton lambs will be higher [11].

Other schemes with ET are applicable as well. Let's say a shepherd has an Awassy flock and he is interested to sell more milk [8]. If he transfer to the al ewes in the flock 1 karakul embryo and half of them became pregnant the other half of the flock will became pregnant with Awassy rams left free in the flock after ET. Out of these naturally mated ewes will be born females equivalent to about 25% of the number of ewes in the flock. That is enough to maintain the size of the flock if the necessity culling of ewes allows a mean production life of 4 years per ewe. That means that about half of the ewes will bore one

Karakul lamb slaughtered for its pellet at three or less days of age. Thus these ewes will have a longer milking period based on the beginning of lactation when the daily milk production is more. In this case the shepherded win the value of the pelts and the value of the plus o milk from the first about 45 days of lactation. It is for sure that the sum of the goods motioned before depasses the costs of the embriotransfer.

The same scheme applied in a Karabasha flock could allow to tray to transfer two embryos to each receptor ewe. There is a chance to have twins of usual size since Karabasha ewe is known to give heavy lambs.

Or what is if in Karabasha sheep two Karabasha embryos are transferred 7 days after ewes were matted. Then it is possible to have a double prolificacy of the flock resulting from single double and triple parturitions. That also will value more than the costs of the embryo-transfer.

If the life of donors is saved when the embryo are washed, that will reduce the embryo transfer costs, then more opportunities are opened in applying reproduction biotechnologies in sheep. In this case production of embryos has to be concentrated out of mating season of ewes.

In vitro fertilization is the biotechnology permitting to access the ova or the zygote before ova fertilization. That allows sequences of foreign genetic species of DNA to be transferred in sheep embryos. The DNA sequence transferred command syntheses of a protein of interest. Usually the transgenesis success is recognized by the presence of transferred genes in the milk of genetically modified organism obtained. Cloning of superior animals from adult cells give the possibility to reproduce transgenic organisms without sexual reproduction, a very important fact for pharmaceutical industry.

Finally the *"ex situ"* preservation of populations in critical states of extinction while they have less than 100 individuals' contingency, is based of deep freezing of semen or embryos. Of course embryos must be preferred since they dispose of complete genetically information.

CONCLUSIONS

Estimating the undemonstrative spare outputs of sheep breeding disclosed by reproduction biotechnologies in sheep breeding pretends much imagination because of the multitude of known procedures and because of diversity of products obtained from sheep flocks.

The main sources of added outputs are a higher fertility of ewes from induced by super

ovulation gemmelarity, eventually associated with higher commercial value of new born lambs, or by producing and saving embryos during the long anestrous between two pregnancies.

Of great importance is creating by transgenesis GMOs for pharmaceutical purposes and to reproduce them by cloning from somatic adult cells.

Some difficulties in developing reproduction biotechnologies with ewes result from the ruther small body size of animals. It is possible to pass over these difficulties with good endoscopic equipment.

ACKNOWLEDGEMENTS

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THE MANAGEMENT OF 45 CA UPTAKE IN SKELETAL, CARDIAC AND SMOOTH MUSCLE TISSUE IN RATS OF DIFFERENT AGES. THE IMPACT OF ACUTE AND LONG TERM TREATMENT WITH D3 VITAMIN

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Abstract

The aim of the present study was to investigate the effect of acute and long term treatment with D3 vitamin upon 40 Wistar rats aged between 6-24 months old. Significant modification in ^{45}Ca uptake in striated muscle from young rats with acute and long term treatment with D3 - vitamin has been noticed. Acute and long term treatment with D3 - vitamin in old rats determined an increase in ^{45}Ca at the level of heart and aorta. The results have an important clinical significance in administration of D3 - vitamin in aging people because of the Ca accumulation danger on cardiac muscle and aorta leading to acceleration of functional disorders of the cardiovascular system affected by atherosclerosis process.

Key words**:** *vitamin D3, 45Ca uptake,skeletal muscle, cardiac muscle, smooth vascular muscle,aging*

INTRODUCTION

It has been well-established that vitamin D plays an essential role in the regulation of calcium and phosphate homeostasis and in bone development and maintenance (DeLuca, 2004). Classically, vitamin D is known to exert its actions on target organs, such as the intestine, the kidney, the parathyroid glands, and bone. Over the last two decades, however, there has been increasing evidence that vitamin D plays an important role in many other tissues including skeletal muscle. Early clinical descriptions of a reversible myopathy associated with vitamin D deficiency and/or chronic renal failure recognized a potential association between vitamin D and muscle (Boland, 1986). The identification of the vitamin D receptor (VDR) on muscle cells (Zanello et al., 1997; Bischoff et al., 2001) provided further support for a direct effect of vitamin D on muscle tissue.

The aim of study was to investigate the effect of acute and long term treatment with D3 vitamin upon Ca uptake in rat heart,skeletal and smooth vascular muscle.

MATERIAL AND METHOD

Animals groups: Our study has been conducted on 60 female Wistar rats divided into two groups: 30 young (6 months old) and 30 old (24 months old).

From these, 20 controls rats have been used (10 young & 10 old), 20 rats with acute treatment with D3 - vitamin, 20 with cronical treatment with D3 - vitamin. D3 vitamin (600.000 U) has been diluted 1/10, 0.1ml has been used for i.m. injection/animal according with the experimental model used in our laboratory.

For radioisotope studies of ^{45}Ca uptake experiments we have used fragments of skeletal, cardiac & smooth muscle from young & old rats wit acute and long term treatment with D3 - vitamin. The fragments have been incubated at 37^0 C for an hour in $IC_{65.}$ The tissue fragments have been incubated with ^{45}Ca with an overall activity of 1.79 mCi / ml. The working solution was of 90 uCi / ml using 10 ul / sample according to the described protocol (F. Revnic R.J.G.G 3,8,27 1987).

Radioactivity has been estimated using a Beta Berthold liqiud scintillator counter.

RESULTS AND DISCUSSIONS

Long term treated rats with D3 vitamin have shown an increase in ^{45}Ca uptake in skeletal muscle which may represent two different processes:

1 - Ca binding to the extracelular compartment of the transport of calcium in cell;

2 - the sequestration of Ca in subcellular compartment.

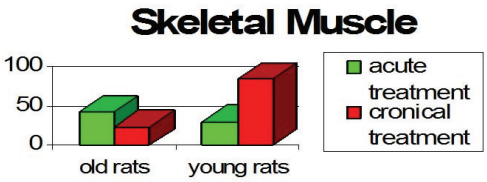

Fig. 1. ^{45}Ca uptake by skeletal muscle from young and old rats with acute and long term treatment with D3 - vitamin.

Fig. 2 presents ^{45}Ca uptake by the cardiac muscle histogram from young and old rats with acute and long term treatment with D 3 vitamin. A reduction in ^{45}Ca uptake in young rats with acute and long term treatment versus old rats has been observed.

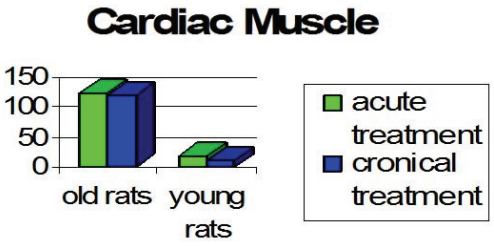

Fig. 2. ^{45}Ca uptake by cardiac muscle from young & old rats with acute and long term treatment with D3 vitamin.

The results have a clinical significance for D3 vitamin administration in elderly patients because of the Ca accumulation danger in cardiac cell, which leads to functional perturbations of the heart by deviation of mitochondrial activity of ATP synthesis towards the removal of Ca excess from mithocondria.

Fig. 3 presents the mean values of ^{45}Ca uptake from young and old rats with acute and long term treatment with D3 vitamin.

In young rats a reduction in Ca has been observed versus old rats accounting for the existence of a defencing mechanism at the aorta level towards accumulation of ^{45}Ca while, in old rats an increase has been observed.

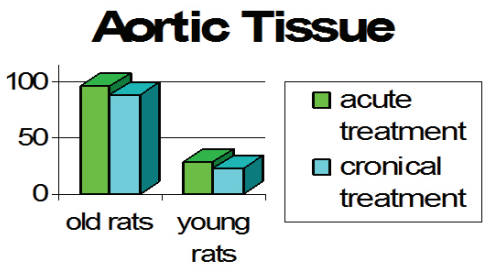

Fig. 3: ^{45}Ca uptake by rat aorta with acute and long term treatment with D3 vitamin.

CONCLUSIONS

Acute and long term treatment with D3 vitamin in young and old rats have revealed a different behaviour of skeletal muscle comparatively with cardiac muscle and aorta concerning ^{45}Ca uptake.

Heart and aorta from acute and long term treated old rats with D3 vitamin show an increase in ^{45}Ca uptake comparatively with treated young group. This means a stimulation of receptors affinity for ^{45}Ca under the effect of D3 vitamin treatment. In elderly patients where, because of atherosclerosis, the cardiovascular is affected, D3 vitamin administration must be done with very much precaution.

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ANALYZE OF REPRODUCTION ACTIVITY IN DAIRY COWS IN VRANCEA REGION

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Abstract

The basis of cattle development, livestock increasing, quality improvement, milk yield and economic efficiency increasing is the reproduction activity process. The researches in the present paper were carried out in Vrancea region, in the south-east of Romania, on a 1000 Holstein Frisian cows livestock, in different lactations, raised in different location. There were analyzed the main reproduction activity indicators resulted following the artificial insemination biotechnology activity: the age at first calving, number of inseminations per one pregnancy, the fecundity index, the length of service-period and calving interval. The primary data were statistically processed, being calculated the main statistic population parameters: average, its error, standard deviation, coefficient of variability. There were recorded the data following every pregnancy, from the first one to the third and there was established the moment of the best results. The obtained results represented key-points in technologic flow optimization of the artificial insemination biotechnology in the studied region, having in view the sustainable agriculture development principles.

Key words**:** *artificial insemination, reproduction indices*

INTRODUCTION

To achieve the proposed objectives, there were notice the reproduction activity data in the whole country and also in the studied region, from the Romanian Agency for Animal Reproduction recordings and the regional recordings too.

MATERIAL AND METHOD

There were analyzed:

- The evolution of the reproductive livestock in Romania and Ruginesti Vrancea County during 2008 – 2011
- Total artificial inseminations out of total matings in Romania and Ruginesti Vrancea County during 2008 – 2011
- Artificial inseminations percentage out of total matings per year in Ruginesti Vrancea County
- Reproduction indices in cattle in Ruginesti Vrancea County during 2008 - 2011

Following the result interpretation during 2008-2011, we have made a comparison to emphasize the reproduction evolution in during this interval. There were compared:

- cattle livestock
- reproductive cattle livestock
- number of matings
- number of artificial inseminations
- number of descendants obtained by heifers and cows

number of frozen semen doses.

RESULTS AND DISCUSSIONS

In Fig. 1 it is presented the evolution of the cattle livestock during 2007 - 2011. The cattle livestock decreased every year from 2,2 million heads in 2007 to the lowest recorded value in 2011, almost 1,8 million heads. The deepest decreasing was recorded in 2011, almost 10 % beside 2010.

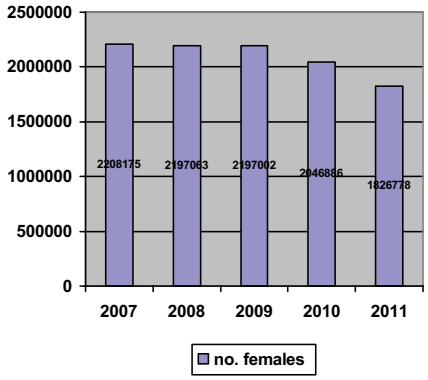

Fig. 1. Total Romanian cattle female during 2007-2011

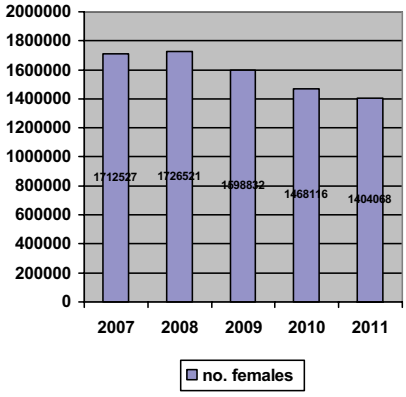

Fig. 2. Reproductive Romanian cattle female during 2007-2011

The highest value of the Romanian reproductive cattle livestock was recorded in 2008, as 1726521 females and the lowest value in 2011, as 1404068 females. This difference is correlated to the decreasing of the total cattle livestock at the country level.

The insemination percentage at the country level evolved from 49,4% in 2007 to a maximum of 56,9%, in 2008, followed by a decreasing to 2010, of 49,9%, then becoming over 55% in 2011.

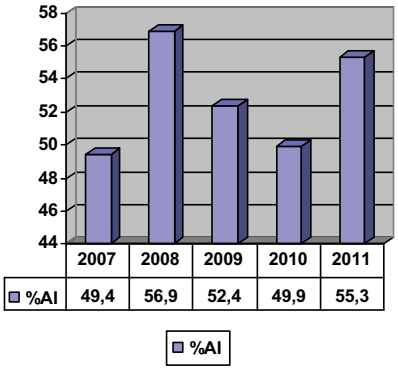

Fig 3. Cattle artificial insemination in Romania during 2007-2011

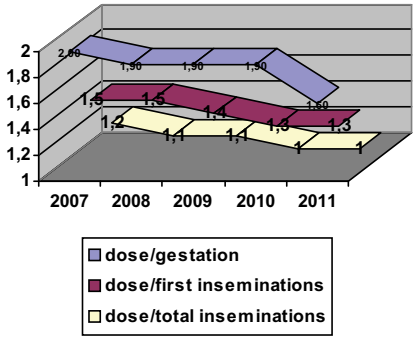

Fig. 4. Number of frozen semen doses used 2007-2011

In Fig. 4 it is presented the average consumption at the country level of the semen doses necessary to obtain one pregnancy, for the first insemination and for total inseminations.
Due to the lower price of the semen doses it may notice a decreasing from year to another year of these indicators.

Table 1. Evolution of the reproductive livestock during 2008-2011

Year	2008	2009	2010	2011
Reproductive livestock	358	301	350	375

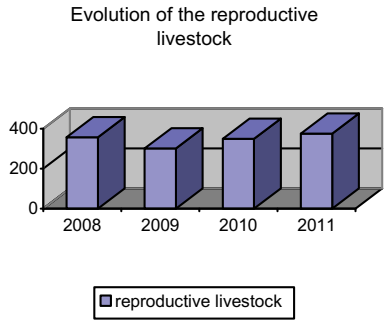

Fig. 5 - Evolution of the reproductive livestock during 2008-2011

In Table 1 and Fig. 5 it may notice the evolution of the reproductive livestock in the studied region. It may notice an increasing of the livestock with a peak in 2011, contrarily the country level trend.

Table 2. Artificial inseminations and matings in the studied region

Year	2008	2009	2010	2011
AI+matings	156	214	310	367
AI 1	131	200	305	360
AI 2	25	14	14	7

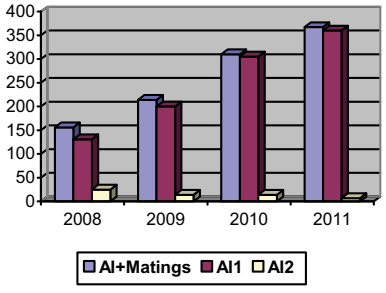

Chart 6 - Artificial inseminations and matings in the studied region

In Fig. 6 and Table 2 it is quantified the activity of artificial inseminations and matings at Ruginesti Vrancea region. It may notice that how along a few years the number of artificial inseminations increased from 131 in 2008 to 360 in 2011 that representing an increasing of over 250%. The same time, it may notice the decreasing of the number of females which needed the second insemination.

Table 3. Artificial insemination percentage during 2008-2009

YEAR	2008	2009	2010	2011
ARTIFICIAL INSEMINATION PERCENTAGE	40%	71%	91%	91%

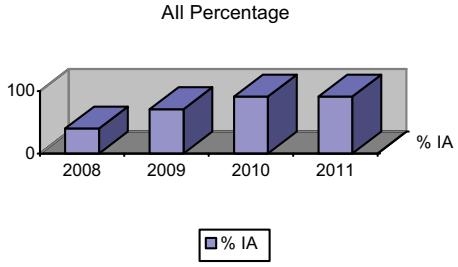

Chart 7 – Artificial insemination percentage during 2008-2009

In Fig. 7 and Table 3 it is presented the evolution of the artificial insemination percentage at the studied region level. This has increased having a maximum of 91% in 2010 and also in 2011. This value shows a special activity achieved by the insemination technicians in this region.

CONCLUSIONS

It may conclude that in the analyzed region, the values of the recorded reproduction indicators are superior in the last year of recording, 2011, followed by the special management regarding the reproduction activity.

Table 4 shortly presents the reproduction indices at the studied region level.

Table 4. Reproduction indices in cattle in the analyzed during 2008 – 2011

Year	2008	2009	2010	2011
Conception rate	83,97%	93,45 %	95,61%	98,08
Pregnancy percentage	100%	98,59 %	99,6 %	100%
Service period	112 days	72,5 days	70 days	59 days
Average number of inseminations per one pregnancy	1,16	1,08	1,04	1,02
Calving interval	397 days	357,5 days	355 days	344 days
Medium age at first calving	23 months	23 months	23 months	23,4 months
Fertility	91,93%	101,02%	102%	107,3%

ACKNOWLEDGEMENTS

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TECHNOLOGIES OF ANIMAL HUSBANDRY

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BEE COLONIES COMFORT IN DIFFERENT TYPES OF HIVES

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Abstract

In order to test various hive systems in growing and exploitation of bee colonies, and in order to estimate comparative advantages and disadvantages, assessment of biological bee comfort, as well as economic efficiency of their exploitation in small and medium beekeeping farms, was carried out an experiment on comparative study of maintaining bee colonies in different types of hives: horizontal and vertical, both with Dadant frames. Two similar batches of bee colonies were created. The first batch of 20 colonies was put in horizontal hives and second batch with 25 colonies– in vertical hives. The main sources of honey in the area were: Acacia, Linden tree and spontaneous flora. In beekeeping season of 2011 have been studied main biological and morpho productive characters of bee colonies, such as: colony strengthens, resistance to overwinter and diseases, queens prolificacy, brood viability, total quantity of honey collected in nest after harvest. Appreciation of morpho productive characters was done according to our methodology developed by the new zootechnic regulation according to bee colonies valuation, growing and certification of genitor beekeeping materials, approved by decision of Government of Republic of Moldova no. 306 of 28.04.2011. It was found that the types of hives, where were housed the experimental bees, all other equal conditions of maintenance and exploitation, have not had any impact on the biological process of bees overwinter. This is confirmed by the fact that the average strengthens of bee colonies in both experimental groups, being equal at the beginning of experiment (1,78 kg in the autumn of 2010, in entry of overwinter) remained same in spring of 2011 (1,49 kg out of the winter). Therefore, overwinter resistance of bee colonies in both groups was also identical, averaging 83.1%. At the same time, the hive types, tested in experiment, had a significant influence on reproduction process and development of bee colonies in high beekeeping season. Thus, the queens prolificacy from 2nd batch with bee colonies located in vertical hives during the season, was higher compared to the Ist batch, accommodated in vertical hives, with 60 eggs/24 hours, or with 3.5% (B > 0.95). A better prolificacy activity of queens in vertical hives can be explained, in our view, by the fact that they have a better comfort of laying, compared with those from horizontal hives. We found that in horizontal hives, queens prefer for laying the area near bee entrance and it is explained by the fact that this place is better aired and ensure the brood with sufficient oxygen. In vertical hives queens laying is more uniform and it is spread on more honeycombs. This is due to a better and uniform ventilation in entire hive, which gives enough oxygen to brood. All this, has led to an active laying of queens from vertical hives, also to a bigger amount of capped brood and as a result, essential increase of bee colony strengthens. At the beginning of first harvest (locust tree), bee colonies placed in vertical hives reclaimed at a bigger rate than those placed in horizontal hives. Regarding bee colonies strengthens, those from 2nd batch exceeded significant, at this stage, those from 1st batch, with 0.33 kg or 9,4% (B > 0,99). Bee colonies from vertical hives entered overwinter significantly more powerful than bees from horizontal hives, which creates premises for a stronger development and better productivity in the next beekeeping season. Due to a quicker development, bee colonies kept in vertical hives accumulated, during active season, a bigger quantity of honey in the nest. Thus, the total quantity of honey accumulated in nest, bee families maintained in vertical hives have significantly exceeded those from horizontal hives with 7.5 kg, or 19,1% (B > 0.999). Economic effect obtained at exploitation of vertical hives only from honey production is 375 MD lei, or 23.8 euro per bee colony. Based on obtained results were made following conclusions. 1. Vertical hives compared to horizontal hives, offer to bee colony more comfortable biological conditions. 2. Maintenance of bee colonies in vertical hives ensures an increase of queens laying -3.5% and of average annual strengthens of the bee colony – with 6.0%. 3. Use of vertical hives contributes to increasing of honey production with 19.1%. 4. Bee colonies exploitation in vertical hives ensures economic efficiency at least 23.8 euro per bee colony.

Key words**:** *Apis mellifera Carpatica*, effect economic, hives, horizontal, testing, vertical

INTRODUCTION

Beekeeping from Republic of Moldova is practiced, most by amateur beekeepers, and a smaller number of professional beekeepers. On the whole, in our country, there are about 120 thousand *Apis mellifera Carpatica* bee colonies and over 4,5 thousand beekeepers. Technology

of bee colonies growing and exploitation is, predominantly, extensive and sedentary. Only a small part of professional beekeepers practice beekeeping at pasture. Bee colonies transportation to pasture shall be carried out, as a rule, with trucks, trailers and less with special pavilions. Most of loading-unloading of bee hives with colonies is carried out manually and less with special means. In this context, the type of hives and their weight has a fairly large technological importance.

In many beekeeping households, so far, there is no unified technology of bee colonies growing and exploitation. In beekeeping practice are used different types of hives, both horizontal and vertical, with different types of frames. The advantages and disadvantages of these types of hives and frames are generally known from literature of specialty [1, 3, 5, 6, 7].

According to Silaev's report, from 2007 (5), Russia, home of bees, has an important place in production of beekeeping products. Evolution of bees' houses has passed a long way, from tree hollow, up to contemporary beehive with removable frames. In Russia, more used are vertical hives with 12 frames and two stores, the hives with two bodies with 10 frames and one or two stores, and horizontal hives with 20 and more frames. At the choice of hive type is necessary that its construction would correspond to some requirements, such as: good overwinter of bees, quick increase of bees power in spring, facility and efficiency of anti swarming procedures, high productivity of work to tend bee colonies and honey extraction, comfortable transportation to pasture, easy construction and its low cost.

According to D. Istratie's research, from 2010 [1], Romania had been highlighted, comparing, the economic efficiency of bee colonies maintenance in two systems of horizontal hives LAYENS and DADANT, with the purpose to find solutions which can limit the negative influence of climatic factors on damage to bee biology and beekeeping production.

Special researches on testing various hive systems in bee colonies growing and exploitation on territory of Republic of Moldova have not been done yet.

Based on this, we proposed to test different systems of hives in bee colonies growing and exploitation, in order to estimate advantages and disadvantages, biologic comfort of the bees, as well as economic efficiency of their exploitation, in small and middle beekeeping households.

MATERIAL AND METHOD

Researches have been done on bee colonies of *Apis mellifera Carpatica* race, in 2011. For the experiment, in fall of 2010 were formed two similar batches of bee colonies. Bees from first batch, in number of 20 colonies, were introduced for overwinter into horizontal hives with 20 Dadant frames. Bee colonies from second batch, with a bee population of 25 colonies, were introduced into vertical hives with body of 10 Dadant frames and stores with ½ Dadant frames. The thickness of the wood walls at both types of hives was 22 mm. Vertical hives had detachable bottoms of metal mesh. The hives were placed at stationary apiary of Zoology Institute from Science Academy of Moldova, which is located in a forest glade of forest sector nb. 21 of Canton no. 4, fold forest Ghidighici, Straseni. As honey sources in this area, serve Acacia, Linden tree and spontaneous flora. Bee hives with colonies from both batches were placed under same conditions, at a distance of 2 m one from another, with bee entrance faced to south. In winter of 2010-2011, the minimum air temperature, in the area, was 0 C.

Beekeeping season of 2011 have been studied the main bee colonies biological and morphoproductive characters such as: bee colonies power, resistance to overwinter, queens prolificacy, brood's viability, resistance to diseases, the total quantity of honey collected in nest after harvest. Morpho productive characters assessment was done according to methodology developed by us in the new zoo technical regulation regarding bee colonies valuation, growing and certification of beekeeping genitor material, approved by decision of Government of Republic of Moldova no. 306 of 28.04.2011 [2].

The power of bee colonies has been approved by bee amount existing in the nest at that moment. Assessment was done three times a year: spring examination (02 April), end of

spring (31 May) and autumn examination (29 September). After these three appreciations was determined average power of each bee colony. Bee quantity (kg) was determined by multiplying the number of intervals between frames, occupied uniformly by bees, with coefficient of 0,25 for Dadant standard frame.

Bee colonies resistance to overwinter was assessed after the amount of surviving bees over winter, using information from autumn examination of 2010 and from spring of 2011. The bees' resistance to overwinter was determined by the correlation of bee quantity after winter with bee quantity before winter, expressed as a percentage.

Queens prolificacy (eggs/24 hours) was determined at the end of the spring examination (May 31) by dividing capped brood cell number from the nest to 12 (cycle of capped brood development, days), thus resulting the egg number laid in 24 hours. The cells number with capped brood from the nest was determined by measuring with Netz frame of squares number (5 x 5 cm) filled with capped brood and multiplying it by 100, revealing the total number of cells with capped brood.

Assessment of bee colonies brood viability was done twice - on 15th and 30th of June 2011, by marking with matches, in the corners, a part of honeycomb densely egg laid, with 400 cells (10 x 10 cm). After 4 days, were counted cells with larvae and total number of cells on marked surface, which represents the brood viability, expressed as a percentage. The final brood viability was determined by calculating the average of the two assessments.

Resistance to diseases was determined through following the hygienic behaviour of bee colonies, using standard test, whereby brood from a compact surface was euthanized in order to establish the speed and accuracy with which the bees identify and remove the dead brood. The evaluation was done twice (on 19th May and 02nd June, 2011) on each bee colony. The brood was euthanized at capped stage (Stern) by pricking with a fine needle through cell caps from a part of honeycomb in the nest, on a square surface of 5 x 5 cm (100 cells) marked in corners with matches. After 24 hours, since the introduction into the nest of the comb with euthanized brood, was determined the number of cells in which the brood has been removed. The correlation of cells number with removed brood and with initially euthanized brood from the marked surface of the comb, made up the resistance to diseases, expressed as a percentage. Average of the values between the two evaluations made the final resistance to diseases.

Total quantity of honey collected in the nest was determined at each bee colony, by adding the quantity of honey-wares, extracted during harvest season, with quantity of honey collected in the nest, and left after autumn examination, as food for bees for overwinter period. The quantity of honey-wares was determined for each bee colony apart, at each extracting, by weighing the combs with honey before and after extraction (accurate to 0,1 kg), weight difference being the amount of extracted honey-wares. The quantity of honey left in nest as bee food was determined at autumn examination, by weighing combs with honey and their reduction (from the total weight thereof) of summary weight of standard frames with combs without (for frame-type Dadant-0.6 kg).

Experimentally obtained information was processed according to variation biometric statistics, after the methods of Plohinschii N. A. 1969 [4].

RESULTS AND DISCUSSIONS

The results of researches, presented in the table, show that the types of hives, where were kept the bees during our experiment, all other equal conditions of maintenance and exploitation, have not had any impact on the biological process of overwinter of the bees. This is confirmed by the fact that the average power of bee colonies from both experimental batches, was equal at the beginning of the experiment (the fall of 2010, before overwinter) remained same in the spring of 2011 (after overwinter). Therefore, the resistance to overwinter of bee colonies from both batches was also identical, averaging 83,1%.

At the same time, the types of hives tested in our experiment had a significant influence on reproduction process and bee colonies development during beekeeping season.

Table 1. Testing results of different types of hives for maintenance and exploitation of *Apis mellifera Carpatica* bee colonies

Name of morpho productive characters	Horizontal hives n = 20 $M_1 \pm m_1$	Vertical hives n = 25 $M_2 \pm m_2$	$M_2–M_1$	td
Bee colonies power in autumn, 2010, kg	1,78 ± 0,07	1,78 ± 0,05	0,00	0,00
Bee colonies power after overwinter, 2011, kg	1,49 ± 0,07	1,49 ± 0,06	0,00	0,00
Overwinter resistance 2010-2011, %	83,1 ± 1,8	83,1 ± 1,5	0,0	0,00
Queens prolificacy, eggs/24 hours	1702 ± 25	1762 ± 15	+60*	2,07*
Bee colonies power at first harvest, kg	3,50 ± 0,09	3,83 ± 0,04	+0,33**	3,36**
Bee colonies power in autumn, 2011, kg	2,01 ± 0,02	2,08 ± 0,02	+0,07*	2,50*
Bee colonies average annual power, kg	2,33 ± 0,05	2,47 ± 0,03	+0,14*	2,41*
Brood viability, %	89,0 ± 0,5	89,6 ± 0,4	+0,6	0,94
Resistance to diseases, %	85,3 ± 1,2	87,3 ± 0,9	+2,0	1,33
Total quantity of honey, kg	39,2 ± 1,0	46,7 ± 0,9	+7,5***	5,33***

Remark: * - B > 0,95; ** - B > 0,99; *** - B > 0,999

Thus, the queens prolificacy from bee colonies placed in vertical hives, during beekeeping season, was higher, compared to bee colonies placed in horizontal hives, with 60 eggs/24 hours, or 3,5% (B > 0,95). A better prolificacy activity of the queen from vertical hives is explained, in our view, by the fact that they have a better comfort for laying, compared with those from horizontal hives. We found that in horizontal hives, queens prefer to lay on the place near bee entrance, what is explained by the fact that this place is better ventilated and ensures the brood with sufficient oxygen. In vertical hives Queens are laying more uniform and it is spread on many combs. This is due to better and uniform ventilation in entire hive, which favours necessary provision of the brood with oxygen. All this has led to the queens active laying in vertical hives, and a bigger number of capped brood and as a result, essential increase of bee colonies power.

At the beginning of first harvest (locust), bee colonies placed in vertical hives recovered much quicker than the bees from horizontal hives. After their power, bee colonies from second batch, were much stronger, at this stage, as those from first batch, with 0,33 kg or 9,4% (B>0,99).

Dynamics of bee colonies development can be demonstrated most clearly by illustrating of their power diagram at different periods of time. It was found that the power of bee colonies placed in horizontal hives increased, in active season, from 1,49 ± 0,07 kg - after overwinter, up to 3,5 ± 0,09 kg – at first harvest, reaching up to 2,01 ± 0,02 kg – in fall, at beginning of overwinter. The bee colonies power kept in vertical hives increased, in this period, from 1,49 ± 0,06 kg - out of the overwinter, up to 3,83 ± 0,04 kg - at first harvest, reaching up to 2,08 ± 0,02 kg – in fall, at beginning of overwinter. Therefore, bee colonies kept in vertical hives entered into overwinter, significantly more powerful, than bees from horizontal hives, which creates premises for a stronger development and higher productivity in the next beekeeping season.

In bee colonies placed in vertical hives can be noticed a weak increasing of their resistance to diseases, compared with bees from horizontal hives. However, the difference after this biological characteristic, between the two batches of bee colonies is not significant.

Due to more accelerated development rhythm, bee colonies kept in vertical hives have collected during the active season, a bigger quantity of honey in the nest.

Thus, after the total quantity of honey collected in the nest, bee colonies placed in vertical hives have significantly exceeded the bee colonies from horizontal hives with 7.5 kg, or 19.1% (B>0.999). This difference is certain according to the highest level of resolution after Student [4]. The economic effect obtained at the exploitation of vertical hives, makes in honey production 375 MD lei, or 23.8 per bee colony. Taking into account the fact that, simultaneously with honey production, from bee colonies are acquired other bee products (beeswax, propolis, etc), quite important too, so economic efficiency of vertical hives, instead of horizontal ones, can grow up to 25-30 Euros per bee colony.

CONCLUSIONS

1. Vertical hives offer to bee colonies more comfortable biological conditions, compared to horizontal hives.
2. Maintenance of bee colonies in vertical hives guarantees an increase of queens prolificacy - with 3.5% and colonies average annual power - with 6.0%, compared with horizontal hives.
3. Use of vertical hives for maintaining bee colonies contributes to increase honey production with 19.1% compared to horizontal hives.
4. Bee colonies exploitation in vertical hives ensures an economic efficiency at least of 23.8 per bee colony, compared with horizontal hives.

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STATE AND PRIORITIES OF LIVESTOCK IN PRIVATE HOUSEHOLDS

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Abstract
We analyzed the situation of livestock in private households in the Republic Moldova. These households have the largest herd of livestock in the country and produce more than two-thirds of overall livestock products. Animal productivity and economic efficiency are at low level. We propose measures to modernize and intensify the livestock sector which will ensure increased productivity of animals, quality and competitiveness of products of animal origin.

Key words**:** *livestock, private households, products, Republic Moldova*

INTRODUCTION

Products of animal origin have an important role in human nutrition. These are social products, important for ensuring food security, including nutrition of children, the elderly and socially vulnerable people. Currently, in the Republic of Moldova the production of animal products is based on animal husbandry in the private sector. For these reasons, we carried out a statistical analysis of the situation of the population in the livestock sector, based on a sample of 2332 households that had agricultural land of an area of less than 10 ha. From the analysis of the results of these investigations we determined the need for measures to increase the production of products of animal origin.

MATERIAL AND METHOD

In the research we used information on households regarding sown areas, their structure, livestock, agricultural production volume, revenues from the sale of agricultural production. Research results also contain data on some households that individually process land, produce and sell food and raw materials for processing (case study).
Proposals to modernize and increase production of animal products were developed using the results of research conducted by the Academy of Science, the Agricultural University, and scientific research institutions.
The research was based on applying statistical and mathematical methods to determine the population of households and peasants to be included in the study (sample) data processing and inference of obtained data for the totality of households; obtaining data from households through investigations by interviews, with especially employed and interviewers; documentation (records) conducted systematically in the researched subject households.

RESULTS AND DISCUSSIONS

We carried out statistical studies of agricultural activities of personal, auxiliary households of citizens, occupied with raising livestock and poultry breeding and horticulture on lots of the house and gardens, which are situated both in the city and near the locality in question. The information can be found in the tables no.1-5.
The ultimate goal of the research is to obtain complex and authentic information on the activity in the category named by agricultural producers, including the sector of land, livestock and poultry, volume of production obtained, size and structure of costs and expenditure on the agricultural production structure, the use of agricultural production and income from agricultural activity.

Table 1. Livestock in all categories of producer, as of January 1, on thousand heads

	Year				On an average	Year				On an average
	2004	2005	2006	2007	2004 -2007	2008	2009	2010	2011	2008 - 2011
Cattle	373	331	311	299	328	232	218	222	216	222
of which, cows	256	231	217	207	228	169	160	161	154	161
Pigs	446	298	461	532	459	299	284	377	478	360
Sheep and goats	938	942	938	947	941	853	866	915	905	885

Table 2. The number of livestock per 100 households to rural, from 1 January

	Year				On an average 2004-2007	Year				On an average 2008-2011
	2004	2005	2006	2007		2008	2009	2010	2011	
Cattle	40	36	34	33	36	26	24	25	25	25
of which, cows	28	26	25	24	26	20	19	19	18	19
Pigs	44	40	47	53	46	27	25	33	40	31
Sheep and goats	99	102	103	104	102	93	96	102	102	98

Table 3. Production of main animal products in households, *total, thousand tones*

Production	Year				On an average 2003 -2006	Year				On an average 2007 – 2010
	2003	2004	2005	2006		2007	2008	2009	2010	
Meat *(in live weight)*	108	109	109	117	111	129	83	93	111	104
Milk	564	604	634	604	602	585	527	559	576	562
Eggs, *thousand pcs.*	406	443	478	479	452	465	356	392	442	414

Table 4. Livestock productivity in agricultural enterprises, kilograms

Production	Year				On an average 2003 -2006	Year				On an average 2007 – 2010
	2003	2004	2005	2006		2007	2008	2009	2010	
Average annual milk yield calculated per one cow	2493	2561	3018	2913	2746	2710	2743	3098	2993	2886
Average daily overweight, g: - cattle	262	275	321	323	295	297	325	378	348	337
Average daily overweight, g: - pigs	136	166	187	200	172	218	268	311	315	278

Table 5. Level of profitableness (unprofitableness) of production sold by agricultural enterprises, percentage

Production	Year				On an average 2003 -2006	Year				On an average 2007 – 2010
	2003	2004	2005	2006		2007	2008	2009	2010	
Animal production, on an average	-8,6	7,4	25,0	20,1	11,0	-3,0	18,4	21,4	20,7	14,4
Cattle and poultry for slaughter (in live weight), on an average	-35,1	-19,2	1,8	4,0	-12,0	-14,8	15,3	17,4	19,9	9,5
Including: cattle	-47,9	-43,8	-23,8	-26,8	-35,6	-41,2	-20,9	-19,6	-39,9	-30,4
pigs	-39,0	-30,0	-7,7	-4,5	-20,3	-12,8	25,2	25,4	34,4	18,2
sheep and goats	-26,6	-20,8	-32,2	-22,0	-25,4	-44,1	-44,1	-36,8	-34,5	-39,9
poultry	-9,0	12,8	20,3	15,7	10,0	0,2	17,1	16,2	16,1	12,4
Milk	-3,3	0,4	9,9	10,4	4,4	0,5	-1,4	8,5	13,7	5,4
Eggs	17,3	31,8	50,8	50,7	37,7	10,6	36,9	34,3	27,9	27,4

Small-sized agricultural producers - this category of producers includes personal auxiliary households, peasant farms (farms) with land lots up to 10 ha, registered in the established way, and people who have received the trading sectors of land equivalent, but have not registered its household in the established manner.

Auxiliary personal households of citizens – the form of agricultural production through the work carried out by the members of their households (lots to House and gardens) in order to satisfy the needs for food and other needs.

Farms – the shape of entrepreneurship activity carried out on the basis of land use and heritage privately owned or used by the farmer in dealing with the production, processing and sale of agricultural production.

Of all the agricultural lands of the Republic of Moldova, farms represent 30 %, lands with a medium surface of land area less than 10 ha 25.9%, and about 14% are in possession of auxiliary households (lots in addition to homes and gardens).

The majority of private sector households (65.3%) does not have a specific specialization and produce several types of agricultural products. Specialized in the manufacture of cereal farms are only 22.7%, 7.8% in the production of grapes. There are no specialized households in the production of animal products. In the structure of sown areas, fodder plants represent less than 5%.

Of the total agricultural production, private households produce 26-34% of cereals, 21-28 % soy, 40-45% of grape, 28-38 of fruit, 24-25 % of sun flower production.

Average harvest in tons per 1 ha is 2, 5 - 3, 2 t grain, 1, 6 - 2, 2 t soy, 4, 0 - 4, 4 t grapes. In the private sector are raised 94% of cattle, including 97% of cows, 75% of pigs, and 98% of sheep, goats and equine from all livestock in the country. The number of livestock per 100 households to rural population constitutes 25 cattle, inclusive 19 cows, pigs 31 and 98 sheep and goats.

On average for 2007-2009 in all categories of farms were produced 132 thousand tons in live weight of meat of species of animals and birds; 579 thousand tons of milk and 656 thousand eggs in the respective households; 104 thousand tons of meat, 562 thousand tons of milk and 414 thousand eggs. The average annual milk yield per cow in agricultural enterprises was 2886 kg; the addition of average daily weight gain in cattle was 337 g, for pigs 278 g.

The level of profitability of production sold by agricultural enterprises on average for 2007-2010 was at 18.2% for pork meat, 12.4% for poultry meat, 27.4% for eggs and 5.4% for milk. Production of beef, ovine and sheep meat was with loss of 30.4-39.9%.

From the analysis presented we can conclude that in private households producing products of animal origin requires the development and implementation of measures that would ensure the modernization and enhancement of this activity in order to increase the income of the rural population and to produce competitive products on domestic and foreign markets.

A broad analysis of the sector for the production and processing of milk in Moldova reveals the following:

• Cow herds are in permanent decline;

• 97% of cow herds are kept in rural households by the population (peasant homes);

• The health and ecological situation in rural areas of the country has deteriorated;

• Milk produced in private households is a seasonal produce and of lower quality, making it difficult to ensure the capacity of processing raw material during autumn and winter, as well as manufacturing by processing enterprises of competitive and quality production;

• Capacities of processing companies are used in an average 22.6%.

The situation can be further improved by the gradual passage of flocks of cattle from the homes of citizens to outside the village, by revitalizing and modernizing existing facilities and by construction of new modern installations.

Of the most important measures we mention the following.

The elaboration and implementation of a strategy for the development of the livestock sector in the long and in the middle term of all the categories of households according to the strategy of the production and processing of milk.

The strategy requires ensuring conservation and diversification of the genetic fund of cattle, sheep and goats. To grant special support to invest in the livestock sector development for the creation of race farms with the use of global genetic resources in animal husbandry and increase the share of farm-bred animals up to 25-30% of the reproduction population of cattle, sheep and goats.

Increase of the volume of financing for scientific research in order to develop and implement the new breeds, lines and populations of livestock production and improved quality of adaptation to local conditions of animal welfare.

The encouragement of livestock producers to implement the achievements of science and technology for the creation of new facilities and to upgrade existing ones in order to enhance the effectiveness of the production of products of animal origin.

CONCLUSIONS

In the middle and long-term the goal is not to increase the number of animals, but substantial gains by improving yields, improvement of breeds and rearing technologies and exploitation of farm animals. Classical systems of food production are less able to cover all the needs of food of animal origin, being replaced by intensive methods and technologies of production, based on the conquests of science and the achievements of modern agricultural equipment.

It is necessary that in the Republic Moldova the practice of growth and exploitation of animals acquired in economically developed countries should be more and more of an industrial character, to implement means of mechanized work and even automated technologies for breeding, feeding and maintenance at the same time, using biological and increasingly more valuable material.

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THE EFFECT OF ALGAL SUSPENSION "CLORELLA VULGARIS" USING IN ARTIFICIAL RAISING OF QUEENS

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Abstract
Obtaining of bees products depends on the conditions of maintenance of bees' families, organization of breeding work, selection, the honey and the quality of queens obtained from specialized apiary. The goal of the research was to determine the influence of using the algal suspension "Clorella Vulgaris" in acceptance larvae inoculating for queens rearing, length, diameter and mass of queen - cells, and mass of mated or none mated queens. There had been noted that using of algal suspension "Clorella Vulgaris" with sugar in the diet of queens nurse family, contributes the queen - cells development, influencing the mass, length and diameter of queen - cells. During the active season for lack of natural harvesting (nectar, pollen), is beneficial to use algal suspension "Clorella Vulgaris" to supplement biological mixture of sugar 1:1, the amount of one liter per nurses family nutrition, from the moment of larvae inoculation to capped queen - cells (5 days).

Key words**:** *algal suspension, bees, beehives, food, honey, queen - cell, queens sugar, syrup*

INTRODUCTION

The main task of beekeeping is to ensure food of high nutritional and biological value. Obtaining of bees products depends on the conditions of maintenance of bee families, organization of breeding work, selection, the honey and the quality of bee queens obtained from specialized apiary.

Bees collect from flowers of the plants nectar and pollen, which process in food - honey and bee bread. Bees' food contains all needed vital nutrients - protein, lipids, carbohydrates, minerals, vitamins [5].

For bee family life processes is required a considerable amount of food - honey and bee bread. Strong family during the year consumes 90 kg honey during winter rest - about 10 kg, while in the active vital periods- spring, summer and autumn - about 80 kg (to maintain life of adult individuals, larvae feeding, wax secretion, energy consumption during flight, nectar processing in honey).

At queens rearing are formed the following groups of families: mother, father, started, nurses (growing), families incubators, helpful, care and production families [1, 2].

In cases when the reserve amount of family food is insufficient, the bees must be fed. For growth of juveniles is using sugar syrup in a concentration of 50% (1 kg sugar 1 liter water) [6].

After 6 hours after orphanzing there is introduced in the nanny family frames with artificial queen - cells with inoculated larvae and it is administrated sugar syrup to stimulate the adoption of a large number of larvae [3].

Nescubo p.m., Crahotin N.F., Rogova V: A. [7] – fed the bees in the experimental group with hlorela paste mixed with sugar syrup. Daily each bee family received 300-350 g of nutrient mixture. Analogue the same amount of sugar syrup, not containing added, there was to the families in the control group: one part water and one part sugar. Over 12-15 days in experimental families, was noted an increase of juveniles increased compared to control families, fed with sugar syrup without biological supplement.

According to the authors, clorella can be used for increasing family nutrition, the production

of bee venom and royal jelly, selection improving and bees work. The study of the influence of algal suspension at artificially raise of queens has also scientific and practical interest.

Using clorella suspension as additional food for bees significantly expand up the opportunities of bee family increases the queens' prolificacy and bees activity, increase their immunity and disease resistance [4].

Based on the presented research goal is to determine the influence of algal suspension "Clorella Vulgaris" at acceptance larvae transfuse for queen growing , length, diameter and mass of queen - cells mass of pared and not pared and queens.

MATERIAL AND METHOD

To fulfill the decided objectives, the object of investigations had served the bees families of the Carpathian breed from apiary "Albinarie", Ialoveni, Republic of Moldova.

To study the influence of algal suspension "Clorella Vulgaris" at acceptance of inoculated larvae for queens rearing, the length, width and weight of lidded queen - cells also the mass of pared and not pared queens were formed 3 groups of growing bees families.

The first group growing families received one liter of sugar syrup (1:1);

Group II - were additionally supplied and worked with reserves of their honey;

Group III - received a liter of algal suspension "Clorella Vulgaris" with sugar 1:1.

Bess families of group I and III were respectively fed using one liter of solution, when the larvae were inoculated than daily till capped queens – cells (during 5 days).

Data were processed by statistical variation method, after Mercurieva E. [8] N. Plohinschii [9] and using computer programs Microsoft Excel.

RESULTS AND DISCUSSIONS

Research has shown that the first series of experiments, at 16 June growing families, had each 9-11 combs in the nest and power was 8-10 areas between populated of honeycombs with bees. Growing bee families had received each 1.0 liter of syrup and there were each inoculated by 30 larvae (Table 1).

Of the total number of accepted inoculated larvae had accepted bees queen rearing 20-25 larvae, or from 66.7 to 83.3%**.**

Raised queen - cells in families that were fed with algal suspension with sugar had the mass of 1.12 mg or by 0.42 mg higher which is 160.0%, longer by 0.33 cm ($B \geq 0.999$) that is 114.2% and diameter 1.31 cm or higher by 0.14 cm ($B \geq 0.99$) which is 112.0% compared with control group I. The extremities of queen - cells in group III were between 2.3 and 3.0 cm and diameter - 1.2 to 1.5 cm (table 2).

In determining the weight of not pared queens there was not found significant differences between groups, averaging between 182.56 and 200.67 mg (Table 3). Coefficient of variation of this index was 5.88 and 10.56%.

The mass of mated queens was on average 247.5 mg in group III (with minimum 236, maximum 253) in group I - 249.5 mg (range 247-252) and in group II - 229.0 mg (209 and 244.0) (Table 4).

Coefficient of variation of body weight in experimental group was 2.0% and in group I control - 1.42% and in second control - 7.87%.

In the second series of experiments starting from July 16, growing families had 11 to 12 combs in the nest and the power of 10 areas of honeycombs populated by bees. Growing families were fed with 1.01 l of syrup each in groups I and III during 5 days and also were inoculated every 30-35 larvae per family.

Experiments conducted on 16.06.2011 - 16.07.2011 were repeated.

The result shows that nurses' family had accepted to raise queens growing in group I, 19 in group II - 22 and in group III - 18 inoculated larvae (Table 5), constituting 54.3% respectively in batches, 64, 7% and 60,0%.

At evaluation of capped queen - cells on July 26, was found that their mass formed in group I - 1.0 g in group II - 1.05 g and in the experimental group with application of algal suspensions Clorella Vulgaris - 1.14 g (Table 6).

Table 1. Influence of algal suspension on inoculated larvae acceptance (16. 06. 2011)

Group	Nr. of honey combs in the nest, pieces	Power of growing family, between frames space	Quantity of administrated syrup, l	Number of inoculated larvae	Nr. of accepted larvae	
					number	%
I – Syrop1:1	11	10	1,0	30	25	83,3
II – Honey	9	8	-	30	20	66,7
III – Algal suspension + sugar1:1	10	9	1,0	30	20	66,7

Table 2. Influence of algal suspension on length, diameter and mass of queen - cells (26. 06. 2011)

Group	Nr. of queen - cells	Index	X ± Sx	V, %	Limits	Index in % compared to the control
I – Sugar syrup 1:1	25	Mass, g Length , cm diameter, cm	0,7 ± 0,50 2,32 ± 0,020** 1,17 ± 0,015	35,71 4,31 6,41	0,5 – 1,0 2,2 – 2,6 1,1 – 1,4	100 100 100
II – Honey	20	Mass, g Length , cm diameter, cm	0,60 ± 0,046 2,21 ± 0,032 1,18 ± 0,011	34,20 6,43 4,13	0,5 – 1,0 2,0 – 2,5 1,1 – 1,3	85,7 95,2 100,9
III – Algal suspension + sugar 1:1	20	Mass, g Length , cm diameter, cm	1,12 ± 0,17** 2,65 ± 0,005*** 1,31 ± 0,040**	21,46 0,27 4,32	0,5 – 1,5 2,3 – 3,0 1,2 – 1,5	160,0 114,2 112,0

The significance between the differences: ** B≥0,99; *** B≥0,999

Table 3. Body mass of not pared queens, mg (29. 06. 2011)

Group	Nr. of queens	X ± Sx	V, %	Limits
I – Honey syrup 1:1	6	200,67 ± 6,302	7,69	174 - 217
II – Honey	9	182,56 ± 6,425	10,56	165 - 212
III – Algal suspension + honey 1:1	27	182,83 ± 0,37	5,88	158 - 208

Table 4. Body mass of pared queens, mg (16. 07. 2011)

Group	Nr. of queens	X ± Sx	V, %	Limits
I – Honey syrup 1:1	2	249,5 ± 2,50	1,42	247 – 252
II – Honey	3	229,0 ± 10,408	7,87	209 – 244
III – Algal suspension + honey 1:1	5	247,5 ± 3,50	2,00	236 – 253

Table 5. Influence of algal suspension at inoculated larvae acceptance (16. 07. 2011)

Group	Nr. combs in nest	Power of growing family, between frames space	Quantity of administrated syrup, l	Number of inoculated larvae	Nr. accepted larvae	
					number	%
I – honey syrup +water 1:1	12	10	1,0	35	19	54,3
II – Honey	11	10	-	34	22	64,7
III – Algal suspension + honey 1:1	11	10	1,0	30	18	60,0

Table 6. Influence of algal suspension on the length, width and mass of queen - cells (26. 07. 2011)

Group	Nr. of queen - cells	Index	X ± Sx	V, %	Limits
I – Syrup (honey +water) 1:1	18	Mass, g Length, cm Diameter, 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
II – Honey	8	Mass, g Length, cm Diameter, 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
III – Algal suspension+honey1:1	17	Mass, g Length, cm Diameter, cm	1,14 ± 0,016* 2,84 ± 0,026*** 1,31 ± 0,016**	5,62 3,73 5,04	1,03 – 1,24 2,6 – 3,0 1,2 – 1,4

The significance between the differences: ** B≥0,99; *** B≥0,999

Table 7. Body weight of not mated queens' mg (29. 07. 2011)

Group	Nr. of queens	X ± Sx	V, %	Limits
I – Syrup (honey +water) 1:1	16	175,69 ± 2,12	4,82	157 - 186
II –Honey	8	180,37 ± 4,91	7,70	159 - 199
III– Algal suspension+honey1:1	17	178,87 ± 2,01	4,64	166 - 204

Table 8. Body weight of mated queen's mg (05.08.2011)

Group	Nr. of queens	X ± Sx	V, %	Limits
I – Syrup (honey +water) 1:1	4	223,67 ± 19,18	17,15	181 – 270
II – Honey	-	-	-	-
III – Algal suspension+honey1:1	5	232,4 ± 5,45	5,25	219 – 252

Best queen – cells were grown in group III, which families have been fed during the larval stage of algal suspension, their mass being 0.09 g higher than in control group II ($B \geq 0.95$). Also queen – cells of group III were by 0.17 cm longer ($B \geq 0.999$) and diameter of - 0.09 cm ($B \geq 0.99$).

The coefficient of variation of mass of queen - cells was 5.62% in the experimental group, 8.99% in group I and 10.82% in group II. Respective coefficient of variation of queen - cells length was 3.73, 5.93 and 2.64%, and the diameter of 5.04, 5.08 and 5.77%.

Body mass of not mated queens on July 29 had the average in group I - 175.69 mg, in group II - 180.37 mg and the experimental - 178.87 mg (Table 7). The coefficient of variation respectively 4.82, 7.70, 4.64%.

Body mass of mated queens was established in August 5 223.67 mg (group I) and 232.4 mg (experimental group III) difference 3.9% (inauthentic), (Table 8).

Coefficient of variation of body mass of mated queens in group I was 17.15%, and of the queens of group III only 5.25%.

CONCLUSIONS

1. Use of algal suspension "Clorella Vulgaris" with sugar in the feeding of queens' family nurse increased the better developing of queen - cells, influencing the mass, length and diameter of the queen - cells.

2. During the active season for lack of natural harvesting (nectar, pollen), is beneficial to use algal suspension "Clorella Vulgaris" as a biological supplement 1:1 mixture of sugar, the amount of one liter per family for nurses family nutrition, from the moment of larvae inoculation to queen – cells capping (5 days).

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ORGANOLEPTIC, CHEMICAL AND MICROBIOLOGICAL QUALITY OF TABLE EGGS OBTAINED IN DIFFERENT EXPLOITATION SYSTEMS FOR LAYING HENS IN ROMANIA

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Abstract

Table eggs represent one of the most valuable sources of proteins for the human diet. Therefore, their quality is a very important aspect for food safety and for public health. During a period of 6 months, samples of table eggs were collected as it follows: 20 samples of each category of exploitation systems for laying hens (organic, free-range, aviary and cage production). Duplicates of the following examinations were performed: Roche index (10 samples/week); total content of proteins, lipids, water and ash (two samples/week); CFU/eggshell as well as presence of Salmonella in the contents using three different selective agar media (8 samples/week). From the total number of 480 samples of each category, results showed the following: for the Roche index, organic eggs were evaluated at 10-11, for free-range and aviary ones, values reached 13 and for cage production systems, 8-9. The content of proteins (%) was higher for free-range eggs (12.48). The content in lipids showed high values for cage production eggs (11 %), but with close values for the other categories, reaching the minimum to 10.7 % in aviary eggs. As for the water content, the highest value was observed for free-range eggs, and for ash the lowest value was observed for cage production eggs, while the highest for organic eggs. Microbiological quality of table eggs revealed a significant bacterial load for the eggshell, of up to 5.06 log CFU/eggshell for free-range eggs, and 5.04 log CFU/eggshell for organic ones. Industrial systems revealed a lower bacterial load, of up to 4.83 log CFU/eggshell for aviary-obtained eggs and 4.67 log CFU/eggshell for cage production eggs. The number of samples in which Salmonella presence was detected was at it follows: 33 % of the organic table eggs samples, 28 % for the free-range table eggs, 25 % for the aviary-obtained table eggs and 14 % for the cage production ones. From the consumer point of view, eggs obtained in free-range and aviary systems could be the most appreciated ones. Chemically, results are very close from a category to the other, while the microbiological quality may differ, as a wide range of factors contribute to a certain bacterial load on the eggshell as well as for the presence of Salmonella.

Key words: laying hens, eggs, quality, Salmonella, exploitation systems.

INTRODUCTION

Table eggs represent a very important source of proteins, through their structure being built to provide vital nutrients to the embryo, during its development [13][23].

In the European Union, breeding and exploitation systems designed for egg production are now designed focusing on the welfare of laying hens, without a decrease in egg production and profit as well. Their classification nowadays is resumed to “cage” or “non-cage ones” [17].

Husbandry techniques can differently influence the quality characteristics that are important when evaluating table eggs, among them: yolk color, chemical composition and microbiological quality [22][23].

In different parts of the world, yolk color is considered one of the most important factors when evaluating egg quality [3], consumers preferring coloration to be between 10 and 14 on the Roche yolk color fan scale [9].

The chemical composition of table eggs is another important quality factor, being already acknowledged that table eggs are the

richest source of proteins among all food products. Yolk represents 36 % of the whole egg weight and it consists mainly on lipoproteins [13]. The albumen contains also a high amount of proteins, small quantities of mineral elements and hydrosoluble vitamins [23].

Considering microbiological quality of table eggs available on the market, selection at the farm level already ensures that dirty of cracked eggs are discarded. Still, egg washing is banned in the E.U., but the safety is ensured by a tough and rigorous selection [6]. The eggshell may become contaminated with different aerobic bacterial species, due to direct or indirect action of different factors: age [18][4][15], air quality [12], percentage of dirty or cracked eggs [15][8].

Salmonella spp., especially serotype Enteritidis is considered a high risk foodborne pathogen. It causes human salmonellosis through ingestion of different food products, among these table eggs being considered as the most important source [7]. *S.* Enteritidis is able to contaminate table eggs through vertical as well as horizontal transmission, both ways being intensely investigated lately [11][10][16].

The present paper aims to reveal the results of a study considering table eggs quality, reflected on Roche yolk color value, chemical composition showed by the values of the main nutrient classes evaluated as well as microbiological quality, revealed through the results obtained when evaluating eggshell microbial load as well as *Salmonella* spp.'s presence in the samples.

MATERIAL AND METHOD

In order to evaluate the egg quality, during a period of 6 months, 20 samples of table eggs were collected from random stores. They were classified considering the exploitation system they would originate from, according to the package information provided to the customer: organic, free-range, aviary and cage production. Duplicates of the following examinations were performed: Roche index (10 samples/week); total content of proteins, lipids, water and ash (two samples/week); CFU/eggshell as well as presence of Salmonella in the contents using three different selective agar media (8 samples/week).

Eggs were stored at 4-6^{o}C until examination.

Roche yolk color fan was used when evaluating the yolk color and natural light instead of artificial one was used, according to the standard method [22][23].

Chemical composition was evaluated using four samples/week, each category of exploitation system being represented by a duplicate of two samples, afterwards being obtained a mean of the two results.

The total protein content (%) was obtained using Kjeldahl method, the content of total lipids (%) was obtained using Soxhlet method, the water content (%) was obtained using the drying oven method and the ash content (%) was obtained through calcination [20][21].

The total number of germs on the eggshell was obtained using decimal dilutions number and Plate Count Agar [1]. For the identification of *Salmonella* in the egg contents' samples, three different selective culture media were used, for each sample: MacConkey agar, Salmonella-Shigella (SS) agar and Xylose-Lysine-Deoxycholate (XLD) agar [1]. The sampling and examinations took place from October 2010 until March 2011.

Statistical analysis was performed using SAS 9.2 software [19].

RESULTS AND DISCUSSIONS

Roche yolk color fan values

The results obtained for this analysis are as it follows: 8 and 9 for cage originating eggs, 10 and 11 for organic eggs, 12 and 13 for both free-range as well as for aviary originating eggs (table 1). Table 2 shows the overall frequencies for each value of Roche yolk color fan examination.

Chemical composition reflected in general nutrients classes

The results for the chemical composition are included in table 3. The highest as well as the lowest values for the percentages of the different nutrient classes, by category, were included in table 4. The lowest levels

observed for each class of nutrients were as it follows proteins – 11.29 % for cage eggs, lipids – 10.02 % for aviary eggs, water – 66.06 % for organic eggs and ash – 0.761 % for aviary eggs.

Table 1 – Frequencies (number of samples) for each category of examined table eggs considering Roche yolk color fan value

Roche Value	Organic eggs frequency	Free-range eggs frequency	Aviary eggs frequency	Cage eggs frequency
8	-	-	-	53
9	-	-	-	67
10	35	-	-	-
11	85	-	-	-
12	-	75	14	-
13	-	45	106	-

Table 2. One-Way Frequencies for overall Roche yolk color fan values.

Roche Value	Frequency	Percent	Cumulative frequency	Cumulative percent
8	53	11,04	53	11,04
9	67	13,96	120	25,00
10	35	7,29	155	32,29
11	85	17,71	240	50,00
12	89	18,54	329	68,54
13	151	31,46	480	100,00

On the other side, the highest levels were 12.97 % total protein content for free-range eggs, 11.74 % total lipids content for aviary eggs, 73.38 % water content for free-range eggs and 0.994 % ash content for both organic and cage eggs.

Table 3 – Global nutrients' levels for each category of examined table eggs (on 24 values obtained using duplicate samples for each category) (means ± standard deviation) (0 = organic eggs; 1 = free-range eggs; 2 = aviary eggs and 3 = cage eggs)

Proteins content (%)[1]	Lipids content (%)	Water content (%)[2]	Ash content (%)[3]
11.64 ± 0.19	10.90 ± 0.20	68.93 ± 1.52	0.918 ± 0.042
12.47 ± 0.28	10.84 ± 0.29	71.53 ± 0.95	0.909 ± 0.040
11.96 ± 0.27	10.71 ± 0.58	69.97 ± 1.37	0.856 ± 0.057
11.84 ± 0.32	11.00 ± 0.23	68.69 ± 1.16	0.847 ± 0.055

[1] $p < 0.0001$ by category;
[3] $p < 0.0001$ by category and by month of study; $p = 0.0096$ for category x month;
[4] $p < 0.0001$ by category; $p = 0.0005$ by month of study.

The correlations between the different general classes of nutrients of table eggs were included in table 5. There has been observed a highly significant correlation between the content of proteins and the water one ($p < 0.0001$).

Table 4 – Global nutrients' minimum and maximum levels for each category of examined table eggs (on 24 values obtained using duplicate samples for each category) (0 = organic eggs; 1 = free-range eggs; 2 = aviary eggs and 3 = cage eggs)

Proteins content (%)		Lipids content (%)		Water content (%)		Ash content (%)	
Min.	Max.	Min.	Max.	Min.	Max.	Min.	Max.
11.31	11.95	10.61	11.29	66.06	71.36	0.851	0.994
12.03	12.97	10.52	11.48	70.04	73.38	0.824	0.968
11.52	12.47	10.02	11.74	67.65	71.89	0.761	0.946
11.29	12.48	10.71	11.46	66.72	70.78	0.772	0.994

Table 5. Pearson correlation coefficients for the general nutrients' classes examined for each category of table eggs (N = 96; Prob > |r| under H0: Rho = 0)

	Proteins	Lipids	Water	Ash
Proteins	1.00000	-0.02066 0.8416	0.46914 < 0.0001	0.12963 0.2081
Lipids	-0.02066 0.8416	1.00000	-0.09315 0.3667	0.16673 0.1045
Water	0.46914 < 0.0001	-0.09315 0.3667	1.00000	0.17251 0.0928
Ash	0.12963 0.2081	0.16673 0.1045	0.17251 0.0928	1.00000

Microbiological evaluation of the eggshell

The values obtained for the microbiological evaluation of the eggshell are included in table 6. All results were highly significant, with the highest mean observed for organic and free-range eggs, while the lowest was allotted to cage eggs category. During 2010-2011, eggs available on the market were still obtained in systems with conventional cages, at that time, this practice was still legally applied. Concerning this category, Protais et al. [18] obtained values for log CFU/eggshell between 4.1 and 4.23, lower than those obtained in this study. However, the results obtained for cage eggs during this study are in agreement with De Reu et al. [4].

They obtained for the same category of eggs, a value of 4.8 logCFU/eggshell. In addition, Huneau-Saläun et al. [12] showed a value of 4.4 logCFU/eggshell, considering conventional cage eggs, and Cepero et al. [2] obtained a value of 4.52 logCFU/eggshell.

Aviary eggs showed a 4.82 logCFU/eggshell value. This is very high compared to the values of Vučemilo et al. [24]. They obtained values of 3.73, 3.91 and 3.98 logCFU/eggshell, when studying different ages of the egg producing-laying hens. However, the mean value is in agreement

with the one obtained by De Reu et al. [6] which is closer 4.95 logCFU/eggshell. Protais et al. [18] obtained values of 4.9, 5.22, 5.41 and 5.44 logCFU/eggshell, these being higher than the values obtained in this study.

Table 6. Microbiological evaluation of the eggshell (log CFU/eggshell)

Category	logCFU/eggshell[1]
Organic	***5.01 ± 0.03**[2]***
Min.	4.91
Max.	5.07
Free-range	***5.01 ± 0.04**[3]***
Min.	4.91
Max.	5.09
Aviary	***4.82 ± 0.08**[4]***
Min.	4.64
Max.	4.99
Cage	***4.64 ± 0.14**[5]***
Min.	4.15
Max.	4.87

* $p < 0.0001$
[1] mean ± standard deviation
Variance: [2] 0.00123; [3] 0.00166; [4] 0.00684; [5] 0.02243;

Organic eggs and free-range ones revealed the highest values concerning the microbial load of the eggshell. De Reu et al. [5] also obtained values of more than 5.00 logCFU/eggshell: 5.30 and 5.86 logCFU/eggshell. However, Huneau-Saläun et al. [12] obtained 4.79 logCFU/eggshell.

Salmonella spp. presence in the contents of table eggs

From the total number of 384 samples examined for the presence of *Salmonella* spp., the results showed no samples containing this pathogen in the yolk, but 110 samples with *Salmonella* spp. present in the albumen. All positive samples of albumen were observed on the three types of selective media used in this study, with an overall percentage of 28.65 %.
For each category taken into account for the examination, the number of samples with positive results as well as the percentage allotted is included in table 7.

Table 7. Number of samples with positive and negative results for *Salmonella* spp. presence in the albumen for each category of table eggs

Category of table eggs	Number of negative samples (no.)	Percentage of negative samples (%)	Number of positive samples (no.)	Percentage of positive samples (%)
Organic	64	66.67	32	33.33
Free-range	68	70.83	28	29.17
Aviary	65	67.71	31	32.29
Cage	77	80.21	19	19.79

The highest numbers of samples with positive results as well as the highest percentage were observed for organic eggs, and with a very small difference followed by aviary eggs. On the other side, the smallest value was observed for cage eggs, only 19 samples being tested positive for the presence of *Salmonella* spp. in the albumen.
Horizontal transmission of *Salmonella* spp. is strongly influenced by the quality of the eggshell. De Reu et al. [5] investigated the ability of *S.* Enteritidis' ability to penetrate the eggshell, following the inoculation. After 3 weeks of storage, it was found that *S.* Enteritidis was recovered from the content of 32 % of the examined eggs, and the bacterial contamination of the contents was higher on the eggshell of the penetrated eggs, in comparison to non-penetrated ones. Another authors showed the same in another study, performing a comparison between different ages of the egg-producing layers. This resulted in a correlation of *S.* Enteritidis' levels in the egg contents with the hen age, being proved that as hen ages, the shell quality decreases, microorganisms of different species being able to contaminate the eggs much easier [14].

CONCLUSIONS

Free-range and aviary eggs have higher values of Roche index (12 and 13, respectively), showing that these eggs would easily be more appreciated by the consumer, on this trait's basis.
The differences considering the general nutrient classes among the different categories of table eggs were not highly significant, but the content of proteins was positively correlated with the water one ($p < 0.001$).
The eggshell contamination with different microorganisms was evaluated through the total number of germs. This analysis revealed a high contamination for organic and free-range eggs, probably due to contact with exterior environment, the soil as well as the natural factors contributing to an easier contamination of the eggshell.

The highest number of *Salmonella* spp. free-table eggs was observed for cage eggs, while the highest number of positive samples was observed for organic eggs. Aviary eggs were also very close considering this value. These two exploitation systems can enhance the chances of *Salmonella* spp. contamination of table eggs.

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POLICY OF KNOWLEDGE MANAGEMENT IN UNIVERSITIES. FROM THEORY TO PRACTICE

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Abstract

Higher education plays an essential role in society, creating new knowledge, transferring it to students and fostering innovation. Mass access to higher education and the spectacular expansion of research are two concepts that become increasingly competitive and developed heavily in the recent years. If initially, the former enhanced the resources for the latter in the modern era of scientific discovery, the postmodernism brought equilibrium between the two concepts, in the production of knowledge time. Nowadays, there´s a strong consensus around the idea that there´s a new paradigm which forces people and organizations to reposition themselves. This new paradigm has evolved around concepts such as learning organizations and knowledge-based organizations, designations that emphasize teamwork, decentralization, organizational learning and knowledge. The proposed survey in this paper is the outcome of an European project whose main purpose was to help school leaders to improve ICT usage in teaching and learning, by better managing the information and knowledge available in their organizations. This expertize is now used at university level.

Key words: knowledge management, university, learning organizations

INTRODUCTION

Higher education institutions have "significant opportunities to apply knowledge management practices to support every part of their mission," [1]. Last century, a russian economist called Knodratieff helped us understand economy and its history by identifying long economic cycles (also called Kondratiev long waves or supercycles). The first economic cycle occurred between 1787 and 1842, based on the steam machine, iron, loom and textile industry. Between 1843 and 1897, railways, building materials and foundry were the economic basis for the second economic cycle. The third one started in 1989 and lasted until 1950, having as major technologies steel, electricity, mechanics, automobile industry, oil, gas production and mineral chemistry. The fourth one began after the 2nd World War, having as basic technologies nuclear energy, satellites, commercial aviation, transistor, semiconductors that contributed to important evolutions in fields like Microelectronic and chips, telecommunications, robotics, advanced chemistry, biotechnology, and so on.

Although process management is still seen as important, human capital development is now the buzz word. The main ingredient for success is knowledge. We live in a knowledge society. In fact, knowledge is increasingly recognized as the most important organizational resource. And that´s why its management is too important to be left to chance.

The last 20 years have seen a growing interest in the topic of knowledge management as a discipline. From 1997, a surge of books, magazines and websites have come onto the scene and today most large organizations have some form of knowledge management initiative, by creating knowledge teams, appointed CKOs (Chief Knowledge Officers). Knowledge is firmly on the strategic agenda.

Organizations are realizing that their real advantage lies in what they know, the knowledge of their people. In fact, organizational success depends more and more on the appropriate use and deployment of distinctive capabilities.
The work force is increasingly mobile (nobody expects to work for the same organization for the entire career), which creates problems of knowledge continuity for the organizations and places continuous learning demands on the knowledge worker.
Connectivity is not only ubiquitous but has also changed expectations; workers are expected to be on at all times and to be able to respond in minutes, not in weeks.

The 2001 survey by Knowledge Management and IDC found that of those organizations that adopt KM, the top reasons are to:
- Retain expertise of personnel (51.9%)
- Increase customer satisfaction (43.1%)
- Improve profits, grow revenues (37.5%)
- Support e-business initiatives (24.7%)
- Shorten product development cycles (23.0%)
- Provide project workspace (11.7%)

In what concerns universities, the main ground for knowledge workers, complementary reasons justify the growing importance of knowledge management. Funding for education is tighter (mainly in the economic and financial crisis that Europe has been facing since 2008), accountability is a buzz word, after OECD think tank´s suggestions and recommendations, external pressures for measurable improvements are increasing as well as demands for improved information about student outcomes. Simultaneously, the perils of information overload force all levels within a university organization to understand how they can more effectively collect, disseminate and share knowledge and transform it into effective decision making and action.
For professors, true knowledge workers who are not paid for their muscles but for their brains and the way they use it, knowledge is simultaneously an input, medium and output for their work.
And as we are about to prove, although KM may be expensive, stupidity is more.

MATERIAL AND METHOD

The survey was based on the outcome of an European project [3] aiming to help:
- Identifying major strengths and possibilities in EU VET schools;
- promoting school leaders competencies in knowledge management systems on ICT usage
- adapting a self-evaluation tool for ICT use in schools, to evaluate the strengths and weaknesses of ICT use and plan new types of approach;
- helping school leaders to understand the process and background for facilitating the design process and implementing new learning and performance environments for ICT usage that involve all learning agents (students, teachers, trainers, enterprises, etc);
- creating a monitoring system for school leaders to follow up the integration of ICT;
- implementing and rooting ICT development in the organization structural framework;
- ensuring that training needs analysis, delivery and evaluation are oriented towards the organization strategic goals;
- promoting a more user-oriented approach in teacher training;
- promoting more non-formal and informal ways of educational agents to learn and develop their ICT competences;
- implementing more effective and efficient communication systems in schools;
- enhancing the creation and sharing of ICT knowledge;
- promoting a quality frame of mind in educational agents;
- disseminating this knowledge throughout school leaders in Europe;
- guaranteeing equal opportunities for men and women.

In the same way, the main purpose of this research is to help university leaders to improve ICT usage in teaching and learning, by better managing the information and knowledge available in their organizations.
ICT information and knowledge is being used to improve teaching and learning. The survey is divided in 3 main sections (Benefits, Strategies/tools and University´s performance on ICT Knowledge Management) and a rather simple scoring system.

Scope	local (L),regional (R) national (N),european (E) international (I)
University Legal Status	public (PB) private (PR)
Area	A - AGRICULTURE, FORESTRY AND FISHING B - MINING AND QUARRYING C – MANUFACTURING D - ELECTRICITY, GAS, STEAM AND AIR CONDITIONING SUPPLY E - WATER SUPPLY; SEWERAGE, WASTE MANAGEMENT AND REMEDIATION ACTIVITIES F – CONSTRUCTION G - WHOLESALE AND RETAIL TRADE; REPAIR OF MOTOR VEHICLES AND MOTORCYCLES H - TRANSPORTATION AND STORAGE I - ACCOMMODATION AND FOOD SERVICE ACTIVITIES J - INFORMATION AND COMMUNICATION K - FINANCIAL AND INSURANCE ACTIVITIES L - REAL ESTATE ACTIVITIES M - PROFESSIONAL, SCIENTIFIC AND TECHNICAL ACTIVITIES N - ADMINISTRATIVE AND SUPPORT SERVICE ACTIVITIES O - PUBLIC ADMINISTRATION AND DEFENCE; COMPULSORY SOCIAL SECURITY P – EDUCATION Q - HUMAN HEALTH AND SOCIAL WORK ACTIVITIES R - ARTS, ENTERTAINMENT AND RECREATION S - OTHER
Size (staff)	a 20 21 a 50 51 a 250 251 a 500 501 a 2000 More than 2000
Country	

1. Main benefits of a knowledge management system for ICT usage in teaching and learning?

	Not important				Very important
Reduce costs					
Increase relevant information access					
Other...					

2. List of activities/strategies that help university members create, gather, organize, disseminate, use and exploit knowledge on ICT usage for teaching and learning

	Not important				ervery important	We don´t use
Document Management (paper and electronic)						
Email						
Phone						
Meetings						
Other.....						

3. ICT tools are being used in your university to manage knowledge on ICT usage in teaching and learning?

	Not important				Very Important	We don´t use
Content Management Systems (CMS)						
Blogs						
Document Management system						
Foruns						
Virtual Communities						
Wikis						
Other.....						

4. Knowledge Management system on ICT usage. Assesment on university performance on ICT Knowledge Management

Our university has a system for acquiring, organizing, sharing and applying knowledge on ICT usage for teaching and learning.

Strongly disagree ☐☐☐☐☐ Strongly agree

We know who are the best experts on ICT usage.

Strongly disagree ☐☐☐☐☐ Strongly agree

There´s hardly any duplication of effort in our university when it comes to ICT usage and learning.

Strongly disagree ☐☐☐☐☐ Strongly agree

In the daily work, professors have easy access to the right information at the right time, in the right place.

Strongly disagree ☐☐☐☐☐ Strongly agree

Formal networks exist to facilitate dissemination of knowledge on ICT usage for teaching and learning.

Strongly disagree ☐☐☐☐☐ Strongly agree

Communication systems and ICT tools are used to promote learning and team work.

Strongly disagree ☐☐☐☐☐ Strongly agree

Our university has got a structured knowledge repository on ICT usage for teaching and learning that is easily accessed and understood.

Strongly disagree ☐☐☐☐☐ Strongly agree

Our University Board recognizes the potential of knowledge assets on ICT usage for educational purposes and develops strategies to manage them.

Strongly disagree ☐☐☐☐☐ Strongly agree

Our University Board promotes collaborative learning on ICT usage.

Strongly disagree ☐☐☐☐☐ Strongly agree

Our university has a clear vision and strategy that articulates knowledge management on ICT usage with the university mission and main objectives.

Strongly disagree ☐☐☐☐☐ Strongly agree

All members recognize the importance of Knowledge Management on ICT usage for teaching and learning as an important asset.

Strongly disagree ☐☐☐☐☐ Strongly agree

In our university, there are formal roles and responsibilities for managing knowledge on ICT usage for teaching and learning.

Strongly disagree ☐☐☐☐☐ Strongly agree

Tacit knowledge (what professors know how to do with ICT but cannot express) is valued and transferred throughout our university.

Strongly disagree ☐ ☐ ☐ ☐ ☐ Strongly agree

In our university, there are librarians or information management staff that coordinate knowledge repositories on ICT usage and act as focal points for provision of information to support decision making.

Strongly disagree ☐ ☐ ☐ ☐ ☐ Strongly agree

Resources are committed for ongoing training and competencies development on ICT usage by professors.

Strongly disagree ☐ ☐ ☐ ☐ ☐ Strongly agree

Professors are evaluated and rewarded for sharing and reusing knowledge and information on ICT usage.

Strongly disagree ☐ ☐ ☐ ☐ ☐ Strongly agree

Our University Board rewards all university members for *thinking outside the box.*

Strongly disagree ☐ ☐ ☐ ☐ ☐ Strongly agree

In what concerns knowledge management on ICT usage, leaders model behaviors and actions through actions and not just words.

Strongly disagree ☐ ☐ ☐ ☐ ☐ Strongly agree

Our university encourages and facilitates knowledge sharing on ICT usage.

Strongly disagree ☐ ☐ ☐ ☐ ☐ Strongly agree

In our university, there is a climate of openness and trust and people are not afraid to lose power or influence by sharing their knowledge on ICT usage.

Strongly disagree ☐ ☐ ☐ ☐ ☐ Strongly agree

Improving learning results is acknowledged as a major goal of knowledge management on ICT usage.

Strongly disagree ☐ ☐ ☐ ☐ ☐ Strongly agree

Our university is driven by constant flexibility and desire to innovate.

Strongly disagree ☐ ☐ ☐ ☐ ☐ Strongly agree

In our university failure is seen as an opportunity to learn and reasonable mistakes on ICT usage are seen as investments.

Strongly disagree ☐ ☐ ☐ ☐ ☐ Strongly agree

Our university has created ways to link knowledge management on ICT usage to learning results.

Strongly disagree ☐ ☐ ☐ ☐ ☐ Strongly agree

Our professors are aware of the need to proactively manage knowledge on ICT usage for teaching and learning.

Strongly disagree ☐ ☐ ☐ ☐ ☐ Strongly agree

Knowledge management on ICT usage for teaching and learning is part of our university culture.

Strongly disagree ☐ ☐ ☐ ☐ ☐ Strongly agree

In our university collaboration is the norm and people are continuously learning how to learn together in order to improve knowledge on ICT usage for teaching and learning.

Strongly disagree ☐ ☐ ☐ ☐ ☐ Strongly agree

In our university, technology is a key enabler in ensuring that the right information is available to the right person at the right time, in the right place for the right reason.

Strongly disagree ☐ ☐ ☐ ☐ ☐ Strongly agree

In our university technology helps to enhance relationships and collaboration between all educational agents.

Strongly disagree ☐ ☐ ☐ ☐ ☐ Strongly agree

Technology is a key enabler in the creation of an institutional memory (eg. digital repositories) accessible to educational agents according to their needs.

Strongly disagree ☐ ☐ ☐ ☐ ☐ Strongly agree

When a team or a teacher completes a task involving ICT and its usage, distil and document what was learned

Strongly disagree ☐ ☐ ☐ ☐ ☐ Strongly agree

Technology brings the professors close to their students

Strongly disagree ☐ ☐ ☐ ☐ ☐ Strongly agree

In what concerns ICT usage in teaching and learning, university members are able to capture and transfer our best practices.

Strongly disagree ☐ ☐ ☐ ☐ ☐ Strongly agree

Our ICT staff is well prepared to maintain and technically support our information systems.

Strongly disagree ☐ ☐ ☐ ☐ ☐ Strongly agree

In what concerns knowledge on ICT usage for teaching and learning, our technology helps to connect people to contents.

Strongly disagree ☐ ☐ ☐ ☐ ☐ Strongly agree

Our university has good data and information infrastructures.

Strongly disagree ☐ ☐ ☐ ☐ ☐ Strongly agree

Our university has developed a specific set of indicators to manage knowledge on ICT usage in a systematic way.

Strongly disagree ☐ ☐ ☐ ☐ ☐ Strongly agree

The goals for improving our knowledge on ICT usage for teaching and learning are clear and understood by everyone.

Strongly disagree ☐ ☐ ☐ ☐ ☐ Strongly agree

Knowledge gaps on how to use ICT in our university are systematically identified and well-defined processes are used to close them.

Strongly disagree ☐ ☐ ☐ ☐ ☐ Strongly agree

CONCLUSIONS

Knowledge Management (KM) principles recognize that it is important for organizations to "know what they know." The true core competence of any organization is the ability to create new knowledge, learn continuously, identify and solve changing problems. We could summarize the main drivers behind the increased interest in KM in four major trends: Organizations are more multisite, multilingual and multicultural in nature; Organizations are doing more and doing it faster (increased pace and workload) also needing to work smarter as knowledge workers. And knowledge workers are increasingly being asked to think having little time to digest huge amounts of incoming data and information.

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THE EFFECT OF STORAGE TIME IN DIFFERENT TEMPERATURE ON NATIVE CHICKEN EGG HAUGH UNIT AND YOLK INDEX

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Abstract

Three hundred sixty native chicken eggs was test on the effect of storage time in different temperature on haugh unit and yolk index. The aim of this research is to get the effect of storage time in different temperatures : 30^0 – 32^oC (RH 54%), 28^0 – 29^0 C (RH 58%) and 15^0 – 18^0 C (RH 80%); on native chicken eggs haugh unit and yolk index. This experiment used Completely Randomized Design (CRD) on three different temperatures (30-32^0C, 28-29^0C and 15-18^0C) and each treatment was repeated six times. Results indicated that the lowest temperature (15-18^0C) has the highest haugh unit (89.96) and yolk index (0.45); and when the temperature raised (28-29^0C and 30-32^0C) the haugh unit and yolk index was lower (72.80 and 0.29; 73.15 and 0.30 respectively).

Key words**:** *native chicken, haugh unit, yolk index and storage time.*

INTRODUCTION

In Indonesia, there are many types of local chickens with different characteristics from one species to another species. Native chicken is a local non-specific chicken, which kept by many rural communities. The ability of native chickens to produce eggs during a certain period, are varies depending on the maintenance system (Diwyanto and Prijono, 2007)[2]. It is known that the egg quality besides the genetics traits, will be influenced by the storage time of the eggs. One of the official standards quality in a sense that specific numerical values have been assigned and recognized. The measurement of egg white or albumen quality in Haugh Unit. Haugh unit values ranging from 100 down to a practical minimum of 20 (Nesheim et.al., 1979)[4]. Native chickens in Indonesia, has a Haugh unit average of 82.43 (Sri Sulandari et al, 2007)[5]. The height of the albumen is one of the principal characteristic used to judge the interior egg quality. Height of 8 to 10 mm are considered as indicators of superior interior quality (Bell and Weaver, 2002)[1]. The quantity of thick albumen in the freshly laid egg is affected by genetics, duration of continuous production and environmental factors.

The yolk index is a measure of the standing-up quality of the yolk; obtained by dividing the height of the yolk by its average diameter. Average values for fresh eggs usually fall between 0.42 and 0.40 (Bell and Weaver, 2002)[1]. According to Gavril and Usturoi (2011)[3], the yolk index of Romania hens, which egg was stored until 35 days in different temperatures, are between 0.23 to 0.36. According to Bell and Weaver, (2002)[1], as the yolk becomes flattened, the yolk index is low. When the value is 0.25 or more lower, the yolk is so weak.

MATERIALS AND METHOD

The quality properties (the Haugh Unit and Yolk Index) of the native chickens, were analyzed on 360 eggs that have been storage for 42 days in different temperature (30-32^0C, 28-29^0C and 15-18^0C) and each treatment was repeated six times. The experiment used Completely Randomized Design (CRD).

RESULTS AND DISCUSSION

1. The Haugh Unit

In Table 1, there are the haugh unit of the eggs that have been stored for 42 days, at 30^0 – 32^oC (RH 54%), 28^0 – 29^0 C (RH 58%) and 15^0 – 18^0 C (RH 80%).

Table 1. The Haugh Unit of the eggs that stored in different temperatures

	REPLICATIONS	T-1 (30^0 – 32^0C; RH 54%)	T-2(28^0 – 29^0 C; RH 58%)	T-3 (15^0 – 18^0 C; RH 80%)
	I	87.40	83.47	93.30
	II	79.45	85.65	93.85
	III	68.90	75.48	92.15
	IV	70.48	67.25	90.90
	V	60.78	60.78	81.88
	VI	69.80	66.25	87.72
	TOTAL	436.81	438.88	539.80
	AVERAGES	72.80	73.15	89.96

The averages of Haugh Unit scale are between 72.80 till 89.96. The lowest is from the eggs that have been stored in 30^0 – 32^0 C (RH 54%) and the highest is the eggs that have been stored at 15^0 – 18^0 C (RH 80%). The lowest temperatures gave the highest Haugh Unit; because of the increase of the temperature, it will evaporate the albumen and then the Haugh Unit scale will be more lower. According to Bell and Weaver (2002)[1], one of the occasioned that the Haugh Unit scale decreased by the environment factor (the temperature and RH).

2. The Yolk Index

In Table 2, there are the Yolk Index of the eggs that have been stored for 42 days, at 30^0–32^oC (RH 54%), 28^0–29^0 C (RH 58%) and 15^0–18^0 C (RH 80%).

Table 2. The Yolk Index of the eggs that stored in different temperatures

	REPLICATIONS	T-1 (30^0 – 32^0C; RH 54%)	T-2 (28^0 – 29^0 C; RH 58%)	T-3 (15^0 – 18^0 C; RH 80%)
	I	0.42	0.43	0.52
	II	0.37	0.35	0.48
	III	0.30	0.29	0.44
	IV	0.24	0.26	0.45
	V	0.21	0.23	0.43
	VI	0.20	0.22	0.42
	TOTAL	1.74	1.78	2.74
	AVERAGES	0.29	0.30	0.45

Results indicated that the range of the native chicken yolk index that have been storage for 42 days in Bandung, West Java Indonesia is 0.20 up until 0.48 with the average 0.34. When the temperature rise, the yolk index more lower (0.45 for 15^0 – 18^0 C and 0.29 for 30^0 – 32^0 C). Comparing with Gavril and Usturoi (2011)[3] result, that the range of the native chicken yolk index that have been storage for 35 days is 0.23 up until 0.36 with the average 0.30. The fact about the different of yolk index very point out by environment factors. The reality of temperature in Bandung, about 15 – 32^0 C. Beside that, the length of storage also has effect on yolk index, where the eggs was storage for 42 days; different with Gavril and Usturoi (2011)[3] results where the eggs was storage only just for 35 days.

CONCLUSIONS

The effects of storage time in different temperature on native chicken eggs haugh unit and yolk index, showed that :

1. Native chicken eggs haugh unit will be lower when the temperature rise, at temperature 15^0 – 18^0C the haugh unit 89.96 but at temperature 30^0 - 32^0C the haugh unit average are 72.80.
2. Native chicken eggs yolk index will also lower as the temperature rise. At temperature 15^0 – 18^0C the yolk index 0.45 but at temperature 30^0 - 32^0C the yolk index average are 0.29.

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CORRELATIONS BETWEEN PHENOTYPIC ASSOCIATIONS Hb/K AND QUANTITATIVE PRODUCTION TRAITS IN THE BOTOSANI KARAKUL SHEEP

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Abstract

The existence of a linkage between the determinant loci of haemoglobin and blood potassium in the ovine species suggested to us the approach of correlations between various combinations of haemoglobin and potassium phenotypes and the quantitative production parameters (meat, wool, milk) in the Botosani Karakul breed. The electrophoretic and flame photometric tests pointed out four phenotypic combinations (of the six possible) between the haemoglobin and potassium types: HbAB/LK, HbAB/HK, HbBB/LK and HbBB/HK. The four phenotypic combinations Hb/K are characterized by different levels of their production metabolism according to the age and sex of animals. The experimental data show that the subpopulations of animals with phenotypic combinations HbAB/HK and HbBB/HK are more productive than the subpopulations of animals with phenotypic combinations HbAB/LK and HbBB/LK. These correlational aspects recommend the use of phenotypic combinations Hb/K as biochemical genetic markers to improve the production traits of the Botosani Karakul breed.

Key words**:** *haemoglobin phenotype, potassium phenotype, production traits, sheep*

INTRODUCTION

Potassium is an important chemical element for the erythrocyte physiology having considerable implications on the biophysical and biochemical mechanisms of ionic transport from the cell membrane level (4, 6).
There have been reported a number of studies showing that between the determinant loci of haemoglobin (Hb) and potassium (K) there is a linkage, phenomenon that influence the intermediate metabolism, which, in its turn, reflects on the production metabolism of farm animals (2, 4,5, 6, 7).
In a previous paper, we found some associations of different combinations of haemoglobin and potassium phenotypes with the qualitative features of lamb pelts (shape and size of hair curls, quality, lustre and colour of hair fibres) in sheep belonging to the Botosani Karakul breed (2).
The present study tries to find such correlations between the phenotypic combinations Hb/K and the quantitative production traits (meat, wool, milk) of the same sheep breed.

MATERIAL AND METHOD

The correlational analysis of phenotypic combinations Hb/K and production traits was carried out on three sheep populations belonging to the Botosani Karakul breed, differentiated among them by age and sex: 156 lambs (0-3 months), 162 adult ewes and 129 adult rams.
The haemoglobin and potassium phenotypes of animals were identified from samples of venous blood obtained by jugular venipuncture.
The sheep haemotypifying at the Hb locus was performed by the starch gel electrophoresis method (1).
The discontinuity of kalemic system at the locus K was revealed by the flame photometric method (3).
The quantitative production parameters, specific to age and sex, were estimated by

weighing and measuring methods, as follows:
In lambs 0-3 months: body weight at birth, lamb pelt surface at birth, body weight at weaning, body weight gain at 90 days and daily average gain;
In adult ewes: body weight at mating, wool production, milk production, milking period and daily average milk production;
In adult rams: body weight at mating and wool production.
In terms of statistics, there were calculated the arithmetical average ($\bar{x}$), standard deviation (s) and variability coefficient (v%); the Student test (t) was used to compare the production differences among the subpopulations constituted depending on the phenotypic combinations Hb/K of the individuals.

RESULTS AND DISCUSSIONS

As a result of different migration speed of haemoglobin, the patterns of haemoglobin electrophoregrammes show that in the haemoglobin system of the Botosani Karakul breed are expressed only two haemoglobin phenotypes: HbAB (intermediate type) and HbBB (slow type); the fast type HbAA is missing (2).
Depending on the levels and discontinuity of potassium ion distributions, the flame photometry revealed in the Botosani Karakul sheep existence of two potassium phenotypes: LK (low potassium type) and HK (high potassium type) (2).
Theoretically, in ovine species, there are six possible phenotypic combinations among the genetic variants of haemoglobin and potassium: HbAA/LK, HbAA/HK, HbAB/LK, HbAB/HK, HbBB/LK and HbBB/HK. In the Botosani Karakul breed, because of the absence of haemotype HbAA, only four such combinations are expressed (HbAB/LK, HbAB/HK, HbBB/LK and HbBB/HK) (2).
The four phenotypic combinations Hb/K are particularized by different levels of their production metabolism according to the age and sex of animals.
a) Correlations between phenotypic combinations Hb/K and quantitative production traits in lambs (Table 1,Table 2).
The weighting most lambs at birth were those which had in their genetic structure the phenotype HbAB and phenotype HK. Also, the lambs on type HBB/LK recorded a considerable body weight at birth. The slighter newborn lambs are those with the phenotypic combination HbBB/HK. The body weight of lambs of type HbAB/HK is close to the average populational level.
As a result, both the lambs HbAB/HK and the HbBB/LK differ significantly compared to lambs HbBB/LK in terms of body weight at birth. Also, the body weight difference between the lambs HbAB/LK and HbAB/HK is appreciable, the value of “t” being situated in the adjacency of the first critical threshold of significance (5%). Body weight differences among the groups of lambs with the other phenotypic associations Hb/K are small and unsignificant.
According to the Brody method, there is a relative linearity between the surface of lamb pelts and body weight at birth. The only significant difference among the body surfaces of the different lamb groups is noted only between the lambs HbAB/HK and those HbBB/HK, and the difference between the lambs HbBB/LK and those HbBB/HK just approaching the first critical threshold of significance.
Correlative aspects of phenotypic associations Hb/K with production parameters that define the growth rate of lambs (body weight at weaning, body weight gain at 90 days and daily average gain) have revealed that this rhythm is influenced by body weight at birth and their hereditary endowment concerning the haemoglobin and potassium types. Thus, the highest growth rate is of the lambs HbAB/HK, and the lowest growth rate occurs in the lambs HbBB/LK. In the case of the growth rate too, the statistical aspects concerning the production differences among lamb groups with different phenotypic combinations Hb/K are identical to those found among the same groups of lambs at their birth.

Table 1. Production traits in lambs belonging to the Botosani Karakul breed

Production trait	Statistical parameter	Phenotypic combination			
		HbAB/LK n=18	HbAB/HK n=25	HbBB/LK n=25	HbBB/HK n=88
Body weight at birth (kg)	$\bar{x}$ ±s $\bar{x}$	4.20±0.14	4.54±0.17	4.05±0.13	4.36±0.07
	s	0.70	0.70	0.65	0.70
	v%	16.54	15.49	16.07	16.11
Lamb pelt surface at birth (cm^2)	$\bar{x}$ ±s $\bar{x}$	2616±61	2759±68	2552±61	2677±32
	s	302.93	288.87	306.75	302.77
	v%	11.58	10.47	12.02	11.31
Body weight at weaning (kg)	$\bar{x}$ ±s $\bar{x}$	14.86±0.82	16.59±0.61	14.80±0.48	15.94±0.28
	s	4.09	2.58	2.39	2.61
	v%	27.51	15.55	16.15	16.37
Body weight gain at 90 days(kg)	$\bar{x}$ ±s $\bar{x}$	10.66±0.74	12.05±0.44	10.75±0.35	11.58±0.20
	s	3.68	1.88	1.74	1.90
	v%	34.48	15.57	16.18	16.40
Daily average gain (g/day)	$\bar{x}$ ±s $\bar{x}$	118±8	134±5	119±4	129±2
	s	39.79	20.98	19.33	21.34
	v%	33.72	15.66	16.24	16.54

Table 2. Testing the production differences between haemoglobin/potassium phenotypic combinations in the Botosani Karakul lambs

Production trait	**Phenotypic combination**	**"t"**	**Liberty degrees**
Body weight at birth	HbAB/LK – HbAB/HK	1.71^{+}	41
	HbAB/LK – HbBB/LK	0.71	41
	HbAB/LK – HbBB/HK	0.89	104
	HbAB/HK – HbBB/LK	2.57*	48
	HbAB/HK – HbBB/HK	1.13	111
	HbBB/LK – HbBB/HK	2.07*	111
Lamb pelt surface at birth	HbAB/LK – HbAB/HK	1.56	41
	HbAB/LK – HbBB/LK	0.68	41
	HbAB/LK – HbBB/HK	0.78	104
	HbAB/HK – HbBB/LK	2.46*	48
	HbAB/HK – HbBB/HK	1.24	111
	HbBB/LK – HbBB/HK	1.80^{+}	111
Body weight at weaning	HbAB/LK – HbAB/HK	1.45	41
	HbAB/LK – HbBB/LK	0.06	41
	HbAB/LK – HbBB/HK	1.08	104
	HbAB/HK – HbBB/LK	2.55	48
	HbAB/HK – HbBB/HK	1.11	111
	HbBB/LK – HbBB/HK	2.06	111
Body weight gain at 90 days	HbAB/LK – HbAB/HK	1.47	41
	HbAB/LK – HbBB/LK	0.10	41
	HbAB/LK – HbBB/HK	1.03	104
	HbAB/HK – HbBB/LK	2.54*	48
	HbAB/HK – HbBB/HK	1.10	111
	HbBB/LK – HbBB/HK	2.08*	111
Daily average gain	HbAB/LK – HbAB/HK	1.56	41
	HbAB/LK – HbBB/LK	0.10	41
	HbAB/LK – HbBB/HK	1.14	104
	HbAB/HK – HbBB/LK	2.63*	48
	HbAB/HK – HbBB/HK	1.05	111
	HbBB/LK – HbBB/HK	2.23*	111

b) Correlations between phenotypic combinations Hb/K and quantitative production traits in adult ewes (Table 3,Table 4). The adult females which have achieved the biggest body weight at mating were those who presented the phenotypic combination HbBB/LK. Also, the body weights of adult ewes of types HbAB/HK and HbBB/HK are close to those of females HbBB/LK. Subpopulation of ewes with phenotypic combination HbAB/LK recorded a significantly lower body weight than the other three groups of ewes, the difference to the females HbBB/HK being even distinctly significant.

Females with intermediate haemoglobin in association with high serum potassium (HbAB/HK) produced the largest quantity of wool, and the smallest quantity of wool was obtained from females that also have intermediate haemoglobin but associated with low potassium level (HBAb/LK). The females with haemoglobin of type B in association with both types of blood potassium have also achieved important quantities of wool, especially those with phenotypic combination HbBB/HK. Therefore, in terms of wool production, the females HbAB/LK are clearly distinguishable to the other three subpopulations of females, significantly compared to ewes HbBB/LK and distinctly significantly compared to females which have high potassium levels associated with both haemoglobin types (HbAB/HK and HbBB/HK). Wool production differences among the female groups with the other phenotypic combinations give unsignificant values of the Student test.

The highest milk production was obtained from females HbAB/HK, and the lowest milk production is given by females HbAB/LK. Also, from females with slow haemoglobin associated with both potassium types, important milk quantities are obtained. In the case of milk production too, ewes with phenotypic combination HbAB/LK are significantly different compared to ewes with high potassium level associated with both haemoglobin types (HbAB/LK and HbBB/HK), and the difference between females with low potassium level associated with both haemoglobin types (HbAB/LK-HbBB/LK) is close to the 5% significance threshold. The production differences among the other phenotypic combinations Hb/K are unsignificant.

The total milk production is determined mainly by the lactogen potential of animal and less by the milking period. However, low milk production of females HbAB/LK is correlated with the shortest milking duration. The longest period of lactation does not occur in females HbAB/HK (with the highest milk production), but in females HbBB/LK. Females HbBB/LK give more milk than ewes HbBB/HK because their lactation period is more prolonged. Females with the highest milk production (HbAB/HK) have a shorter lactation period than that of ewes with slow haemoglobin associated with both potassium types (HbBB/LK and HbBB/HK).

Consequently, the daily average milk production for each ewe subpopulation Hb/K is dependent on the total milk production and milking period.

Table 3. Production traits in adult ewes belonging to the Botosani Karakul breed

Production trait	Statistical parameter	Phenotypic combination			
		HbAB/LK n=14	HbAB/HK n=22	HbBB/LK n=16	HbBB/HK n=110
Body weight at mating (Kg)	$\bar{x} \pm s_{\bar{x}}$	43.73±0.88	48.63±2.03	49.14±1.72	47.40±0.58
	s	4.14	7.60	6.87	6.06
	v%	9.47	15.62	13.98	12.78
Wool production (Kg)	$\bar{x} \pm s_{\bar{x}}$	1.90±0.14	2.60±0.17	2.40±0.16	2.49±0.07
	s	0.64	0.63	0.66	0.69
	v%	33.90	24.23	27.50	27.71
Milk production (l)	$\bar{x} \pm s_{\bar{x}}$	52.94±4.92	77.04±7.90	68.54±5.62	67.17±2.53
	s	23.09	29.54	22.50	26.55
	v%	43.62	38.34	32.83	39.52
Milking period (days)	$\bar{x} \pm s_{\bar{x}}$	156±7	166±7	177±5	171±3
	s	32.44	23.76	18.75	29.01
	v%	20.77	14.31	10.59	16.96
Daily average milk production (ml/day)	$\bar{x} \pm s_{\bar{x}}$	339±27	464±51	387±31	393±19
	s	124.99	192.19	125.46	204.28
	v%	36.87	41.42	32.42	51.98

Table 4. Testing the production differences between haemoglobin/potassium phenotypic combinations in the Botosani Karakul ewes

Production trait	Phenotypic combination	"t"	Liberty degrees
Body weight at mating	HbAB/LK – HbAB/HK	2.50*	34
	HbAB/LK – HbBB/LK	2.65*	28
	HbAB/LK – HbBB/HK	2.94**	122
	HbAB/HK – HbBB/LK	0.22	36
	HbAB/HK – HbBB/HK	1.29	130
	HbBB/LK – HbBB/HK	0.96	124
Wool production	HbAB/LK – HbAB/HK	3.23**	34
	HbAB/LK – HbBB/LK	2.11*	28
	HbAB/LK – HbBB/HK	3.22**	122
	HbAB/HK – HbBB/LK	0.94	36
	HbAB/HK – HbBB/HK	0.74	130
	HbBB/LK – HbBB/HK	0.51	124
Milk production	HbAB/LK – HbAB/HK	2.73*	34
	HbAB/LK – HbBB/LK	1.87^{+}	28
	HbAB/LK – HbBB/HK	2.13*	122
	HbAB/HK – HbBB/LK	1.01	36
	HbAB/HK – HbBB/HK	1.44$^{?}$	130
	HbBB/LK – HbBB/HK	0.22	124
Milking period	HbAB/LK – HbAB/HK	1.00	34
	HbAB/LK – HbBB/LK	2.13*	28
	HbAB/LK – HbBB/HK	1.65^{+}	122
	HbAB/HK – HbBB/LK	1.59	36
	HbAB/HK – HbBB/HK	0.87	130
	HbBB/LK – HbBB/HK	1.10	124
Daily average milk production	HbAB/LK – HbAB/HK	2.36*	34
	HbAB/LK – HbBB/LK	1.05	28
	HbAB/LK – HbBB/HK	1.40	122
	HbAB/HK – HbBB/LK	1.49	36
	HbAB/HK – HbBB/HK	1.56^{+}	130
	HbBB/LK – HbBB/HK	0.16	124

c) Correlations between phenotypic combinations Hb/K and quantitative production traits in adult rams (Table 5, Table 6)

The most weighting adult rams at mating are those with phenotypic combination HbAB/HK. An important body weight is recorded in rams with high potassium level but associated with slow haemoglobin (HbBB/KH), too. The groups of adult males with low potassium level associated with both haemoglobin types recorded the same body weight, both groups being the slightest of population. Therefore, in statistical terms, the groups of rams with big body weights (HbAB/HK and HbBB/HK) are significantly differentiated compared to the groups of rams with small body weights (HbAB/LK and HbBB/LK).

In the case of wool production too, the most productive rams were those which have had in their biochemical genetic dowry the intermediate haemoglobin type AB associated with potassium type HK, but also those which had high potassium levels but associated with slow haemoglobin (HbBB/HK). The smallest productions of wool were obtained from rams with low potassium levels associated either with heterozygous haemoglobin type (HbAB/LK) or, especially, with homozygous haemoglobin type (HbBB/LK). But, statistically, the most important difference occurs between the two groups of rams that have the highest wool productions. Also, between the males HbBB/HK (with low production) and males HbAB/HK (with high production) the production difference is considerable, being situated in the adjacency of the 5% threshold.

Table 5. Production traits in adult rams belonging to the Botosani Karakul breed

Production trait	Statistical parameter	Phenotypic combination			
		HbAB/LK n=13	HbAB/HK n=17	HbBB/LK n=10	HbBB/HK n=89
Body weight at mating (Kg)	$\bar{x} \pm s_{\bar{x}}$	71.06±2.14	79.59±2.37	71.01±3.11	76.79±1.26
	s	8.81	8.55	9.82	11.88
	v%	12.40	10.74	13.83	15.47
Wool production (Kg)	$\bar{x} \pm s_{\bar{x}}$	4.14±0.21	4.64±0.36	3.93±0.31	4.54±0.12
	s	0.89	1.28	0.97	1.14
	v%	21.40	27.59	24.68	25.11

Table 6. Testing the production differences between haemoglobin/potassium phenotypic combinations in the Botosani Karakul rams

Production trait	**Phenotypic combination**	**"t"**	**Liberty degrees**
Body weight at mating	HbAB/LK – HbAB/HK	2.67*	28
	HbAB/LK – HbBB/LK	0.01	21
	HbAB/LK – HbBB/HK	2.09*	100
	HbAB/HK – HbBB/LK	2.30*	25
	HbAB/HK – HbBB/HK	1.15	104
	HbBB/LK – HbBB/HK	1.73^{+}	97
Wool production	HbAB/LK – HbAB/HK	1.27	28
	HbAB/LK – HbBB/LK	0.54	21
	HbAB/LK – HbBB/HK	1.47	100
	HbAB/HK – HbBB/LK	1.63	25
	HbAB/HK – HbBB/HK	2.71**	104
	HbBB/LK – HbBB/HK	1.85^{+}	97

*

The correlation analysis of combinations of haemoglobin and potassium phenotypes (Hb/K) with quantitative production traits (meat, wool, milk) in the Botosani Karakul breed revealed, generally, irrespective of age and sex, the productive superiority of sheep which contain in their biochemical genetic dowry the phenotypic combination HbAB/HK. Also, important productions are obtained from animals which possess slow haemoglobin associated with high potassium level (HbBB/HK). On the other hand, from sheep with low blood potassium associated either with intermediate haemoglobin (HbAB) or with slow haemoglobin (HbBB) there were obtained (as the case) lower productions. Statistically speaking, the values of Student test show that, generally, between the animal groups with high productions and the animal groups with low productions, there are differences that have various significance degrees.

On the occasion of this paper, in the Botosani Karakul sheep, we found some peculiarities regarding the specific of correlations or associations of phenotypic combinations Hb/K with the quantitative production traits (meat, wool, milk), on the one hand, or with the qualitative traits (shape and size of hair curls, quality, lustre and colour of hair fibres of lamb pelts), on the other hand. If in case of quantitative productions, the phenotypic combinations HbAB/HK and HbBB/HK are correlated with the highest production levels, then, relating to the qualitative productions, the phenotypic combination HbBB/HK is the best associated with the most valuable morphological and histochemical features of lamb pelts and the phenotypic combination HbBB/LK with the largest range of colours of hair fibres (2).

Therefore, in the selection of Botosani Karakul sheep, some biochemical markers, as the combinations of haemoglobin and potassium phenotypes, can be used to improve their productions depending on the purpose: the combinations HbAB/HK and HbBB/HK for the quantitative productions (meat, wool, milk), the combination HbBB/HK for the morphological and physicochemical features of lamb pelts and

the combination HbBB/LK for colour varieties and their shades.

CONCLUSIONS

In the Botosani Karakul sheep, four phenotypic combinations between the haemoglobin electrophoretic patterns and the blood potassium levels (HbAB/LK, HbAB/HK, HbBB/LK and HbBB/HK) are found; the combinations HbAA/LK and HbAA/HK are missing.

Some correlations were established between the four combinations of the haemoglobin and potassium phenotypes existing in this breed, on the one hand, and the quantitative production traits (meat, wool, milk), on the other hand.

Irrespective of age and sex, the quantitative production capacity of the Botosani Karakul sheep is higher in subpopulations of animals with phenotypic combinations HbAB/HK and HbBB/HK and lower in subpopulations of animals with phenotypic combinations HbAB/LK and HbBB/LK.

Many of these production differences among the animal groups with different phenotypic combinations Hb/K present statistical assurance having various significance degrees.

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WATER BUFFALO FOR OUR NEXT GENERATION IN EGYPT AND IN THE WORLD

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Abstract

The total number of buffaloes in Egypt reached about 5.317million in 2011, of which 42 percent were dairy cows, 6 percent buffalo bulls, 32 percent heifers less than two years old and 20 percent male calves less than two years old. While the annual growth rate for the buffalo population approached 3 percent over the last two decades, it still only accounts for 1 percent of the cattle population. The aggregate share of buffalo milk, from all types of production systems is about 81 percent of total milk production in Egypt. The cost of milk production from buffaloes is also less than the cost of reconstituted imported powdered milk at the international market price. Most common name: Baladi Other local name: Beheri, Menufi, Egyptian. Livestock population is in great part located in the Nile delta and Nile valle, Total population size 5371000. The distinction between different types of Egyptian buffaloes is only environmental. It is the most important and popular livestock for milk production in Egypt. Buffalo productivity in Egypt is about 210-280 days/lactation, an average of seven lactations and a milk yielding of 1 600 kg. The age at the first calving is 34-41 months. The average animal production per year represented 30.8% of national agricultural production, the average annual red meat production reached 495,000 ton during the same period, contributing by 45% to overall meat produced. Artificial insemination is used in one percent of the medium to large herds. There are six AI stations owned by the Government and one by the University, possessing a total of 70 bulls. Artificial insemination is still performed at research level; usually only one semen dose is offered at each oestrus, conception at the first oestrus being 30 percent. Milking is done by hand, twice a day, mainly by women. Average slaughter weight is 500 kg, at the age of 18-24 months. Carcass yield is 51 percent. Overall growth rate is 700 g/day. Dairy performance: Lactation duration 210-280 days Milk yield 1 200-2 100 kg Milk fat 6.5-7.0 percent. Buffalo exceed cattle in their ability to convert poor quality and forage to meat or milk. The greatest buffalo losses are often among calves. Newborn buffalo calves, like cattle calves, can die in large numbers due to viruses, bacteria, and poor nutrition. Artificial insemination, embryo transfer, cloning. Biotechnology is important to improve feed utilization and disease control for the next generation.

Keywords: *Artificial insemination; Beheri; Calves; Egyptian buffaloes; Menufi.1.*

INTRODUCTION

Egypt is in the north-eastern corner of Africa between latitudes 21^{O} and 31^{O} North and longitudes 25^{O} and 35^{O} East (see Figure 1a) with a total area of 1 001 450 km^2; the country stretches 1 105 km from north to south and up to 1 129 km from east to west. It is bordered in the north by the Mediterranean Sea, in the east by the Gaza Strip, The Red Sea, in the south by Sudan and in the west by Libya.

Egypt is predominantly desert and arid and semi-arid rangelands (see Figure 1b) and can be divided into 4 major physical regions The Nile Valley and Delta, Western Desert, Eastern Desert and Sinai Peninsula.

Egypt is divided into twenty-six governorates (see Figure 1c) , which include four city governorates (Alexandria, Cairo, Port Said and Suez), nine in Lower Egypt (in the Nile Delta region), eight in Upper Egypt along the Nile River from Cairo to Aswan, and the five frontier governorates covering Sinai and the deserts that lie west and east of the nile[12]

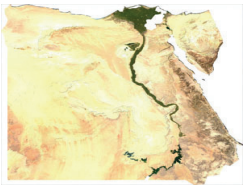

Fig. 1. Egypt showing the vast desert area and the Nile Valley and Delta

Egypt is known as one of the oldest agricultural civilizations; the River Nile allowed a

sedentary agricultural society to develop thousands of years ago. It has a predominantly rural population (the percentage of rural inhabitants is estimated at about 58%) and according to World Factbook the July 2011 population was estimated at 82 079 636 with a growth rate of 1.96%. The capital city is Cairo with an estimated population of 10.902 million, while Alexandria has 4.387 million persons (2009 estimates). Figure 2 shows the population distribution and density in Egypt[12]..

Fig. 2. Map of Egypt's Administrative Divisions/Governorates

Livestock form an important component of the agricultural sector, representing about 24.5% of the agricultural gross domestic product with value of around EGP [Egyptian pounds] 33.6 billion [US$6.1 billion] in 2007 In 2005 local production covered about 92.5, 82.2, 100, 81.9, 100, 100 and 100% respectively for milk, red meat, white meat, fish, eggs, wool and leather. Each of cattle, buffalo, sheep, camel, and goat populations contributes about 51.6, 33.2, 6.5, 5.9 and 2.7% of local red meat production, respectively, which reached 629 000 tonnes in 2005. There is no surplus of animal production for export except some limited numbers of sheep and goats. The sector is depending mainly on the private sector, with the majority of animal Breeders being smallholder farmers and the share of the government sector is less than 2% of the total animal numbers. The ruminant sector is well-integrated with cropland since Egypt has limited natural pastures. Animal production is highly dependent on cattle and buffaloes as milk-producing animals, as well as male animals and un-reproductive females are fattened for meat. The cattle population totalled 4.6 million head, while the buffalo population reached 4.3 million head in 2006.

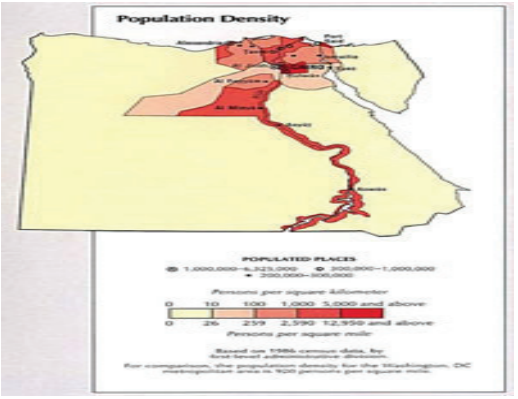

Fig. 3. Map of Egypt showing Population Density

Regarding small ruminants, the sheep population reached 5.4 million head, while the goat population exceeded 3.9 million head in 2006. The camel population was about 120 thousand head, while horses and asses exceeded 3.2 million head in 2005The cattle population is concentrated in both Middle Delta and Middle Egypt regions with percentages 22.4 % and 26.2%, respectively. While 32.2% of the buffalo population is in the Middle Delta region and 22.4% is in the Middle Egypt region. Nevertheless, 31% of the sheep population is concentrated in Upper Egypt, compared to 22.38% in Western Delta region. The goat population is concentrated in both Upper Egypt and Middle Egypt regions with percentages of 36 % and 23.5%, respectively[12]..Indigenous cattle represent about 60% of the all cattle, while mixed-breed cattle represent about 37% and imported cattle about 3%. It These averages represented about 0.34%, 2.98%, and 0.96% to the total annual average of cows and buffaloes and goats in the world, respectively, in the same period [13].

Is worth mentioning that 65% of the cattle population in the Western Delta region is mixed-breed, while in Middle Egypt the percentage of mixed-breed is 18.5% only.

Meat and milk productivity of both cattle and buffalo experienced significant increases during the period 1980-2007. Average cow milk production increased from around 675

kg/head/season in 1980 to around 1.3 tonnes/head/season in 2007, due to increased number of indigenous cows mixed with foreign cows. As to buffaloes, milk production increased from around 1.15 tonnes/head/season in 1980 to around 1.4 tonnes/head/season in 2007, as a result of increased mechanization of farm operations. With regard to meat production, average weight of the cow carcass increased from around 132 kg/head in 1980 to around 200 kg in 2007, due to establishing fattening farms as well as improving animal feeding practices. The average weight of the buffalo carcass increased from around 129 kg/head in 1980 to around 176 kg in 2007, as a result of expanding the first and second stages of the young male animals fattening project.

Agricultural production is dominated by peasant agriculture in which livestock are often kept for multiple functions. summarized the multiple functions of animal husbandry into the following categories: Food production function, insurance, capital accumulation and income generating functions and internal integration function. Among output uses are subsistence consumption, direct supply of farm inputs, cash through sale of live animals or their outputs,

savings and investment through increasing size and quality of herds and social functions such as holding wealth

In Egypt the average annual number of cows and buffaloes and goats reached about 4.38, 5.31, 5.42 million head, respectively, for the period (2011).

What is a Buffalo?

Buffalo are members of the bovine group of animals. They are cloven footed ruminants With 4teats There are two main species of buffalo.[15].

1- The African wild Buffalo (Syncerus)

2- Asian Buffalo (Bubalus) which for the most are domesticated(Bubalus bubalis).

Water (domestic) buffaloes is ~ 150x 10 6 in 5 countries world wide:1/9 the Nr. of cattle in the world. It is an economically important livestock species in many Asian and Mediterranean countries.3- American buffalo (Bison)[1].

Buffalo population and strategies in Egypt

The total number of buffaloes in Egypt reached about 5.317million in 2011, of which 42 percent were dairy cows, 6 percent buffalo bulls, 32 percent heifers less than two years old and 20 percent male calves less than two years old. While the annual growth rate for the buffalo population approached 3 percent over the last two decades, it still only accounts for 1 percent of the cattle population. The aggregate share of buffalo milk, from all types of production systems is about 81 percent of total milk production in Egypt([19][20].

Baladi general information

Most common name: Baladi Other local name: Beheri, Menufi, Egyptian Taxonomic classification Breed Current domestication status: domestic Country: Egypt Main location of variety within country: Livestock population is in great part located in the Nile delta and Nil valley.

Main use:

1-food: milk

2-food: meat.

Risk status: not at risk POPULATION Year of data collection: 2011 Total population size 5371000 Percentage of females being bred pure: 100 Number of males in AI service: 0

Population trend: increasing Additional information: The distinction between different types of Egyptian buffaloes is only environmental. It is the most important and popular livestock for milk production in Egypt[4].There are different research institutes at the Ministry of Agriculture and in Kafr el sheikh Univ. at the University Giza (Cairo) involved in developing projects concerning buffaloes and buffalo products. The breed is the River Egyptian. The buffaloes are spread along the river Nile, in the Delta Region and at the Fayum Oasis. Buffalo productivity in Egypt is about 210-280 days/lactation, an average of seven lactations and a milk yielding of 1600kg. The age at the first calving is 34-41 months[8].

Breeding and selection of dairy buffaloes in egypt the cattle: Information System/Egypt (CISE) of the Cairo University records about 290 small (one to five animals), 27 medium (six to 20) and six large herds. Due to a lack of financing, fat and protein content cannot be recorded. The Ministry of Agriculture and Land Reclamation (MALR) through the Animal Production Research Institute (APRI) records four State herds, belonging to APRI, the sizes

of which are respectively 50, 70, 75 and 80 and 500 breedable females. The Breeders' Service Unit of APRI provides free complete milk analysis and Somatic Cell Count for the enrolled herds[8].

CISE is the only institution in Egypt performing data analysis centrally, producing monthly herd summaries and individual milk yield information. Calculation of the genetic merit of recorded buffaloes and breeding bulls is in progress.The average milk production of milk recorded buffaloes (year 2002) is 2 030 kg (312 days) and the fat content is 8.2 percent. There are six breeding stations with a total of 60 bulls with an average age of five years. These stations belong either to APRI or MALR. All smallholders (one to five animals) take their buffaloes to the breeding stations, as well as 20 percent of the medium size (6 to 20) owners. The red meat producers face several problems that hamper both vertical and horizontal expansion of production[5].

The field study reveals some significant problems that can be listed in terms of relative importance as follows:

a) The high prices of concentrates.

b) The lack of fodder which is associated with weak control over its manufacturers.

c) The high prices of calves to be fattened.

d) Inadequate veterinary car and the high prices of veterinary medicaments.

e) The low supply of summer fodder.

f) The need to protect the local industry against the imported red meat.

g) The financing problems facing the red meat producers

Recommendations:

1. The rise in processed fodder prices (from LE 38/ton in the 1980s to 400-450/ton at present) entails the review of cost items with the fodder plants in order to produce it at lower prices, which will help producers reduce meat production costs, raise production capacities and eventually upgrade the farms' economic efficiency. 2. Find solutions for the inadequacy of animal feed such as: improve fodder qualities, encourage research to develop new low-priced types of fodder and strengthen the control over fodder industry to ensure standard products. 3. Support the programs that are concerned with the genetic improvement of local cow breeds in order to develop breeds of higher transformation rates, which will eventually contribute to the promotion of red meat production

4. Under the bovine production pattern, the study shows that the recommended operating capacities for maximized profit are 10, 16, 5 and 341 head/cycle for small-scale, medium-scale, graduates and cooperatives/projects farms respectively

5. Under the buffalo production pattern, the recommended operating capacities for maximized profit are 3, 49, 25 and 1562 head/cycle for small-scale, medium-scale, graduates and cooperatives/projects farms respectively 6. The results indicates that the cooperatives/ projects farms achieve the lowest cost of bovine and buffalo meat (LE 2.25 and 3.86/kg. gross respectively), followed by medium-scale farms (LE 3.2 and 4.6/kg gross respectively)

Therefore, both systems must be supported as an effective means for the development of red meat local production. In bigger herds, breeding bulls are mainly raised from their own male calves although 20 percent of them buy adult buffaloes (two to three years) from different owners. In all cases, breeding bulls are chosen on the basis of pedigree and performance results of the dams, when provided by CISE.

Artificial insemination is used in one percent of the medium to large herds. There are six AI stations owned by the Government and one by the University, possessing a total of 70 bulls.

Artificial insemination is still performed at research level; usually only one semen dose is offered at each oestrus, conception at the first oestrus being 30 percent. Domestic buffalo are generally regarded as having low reproductive efficiency. This largely because of the conditions under which the majority of them are raised, being small holder farming systems with harsh environments, poor nutrition and minimal managerial inputs. However, they can have good fertility when managed and fed properly. Modern methods in molecular genetics are helping to unravel the evolutionary and genetic status of the river and swap types of buffalo, but from a practical viewpoint, the disparity in the number of chromosomes in the

2 types needs to be considered in cross-breeding programs in order to avoid decline in fertility. Buffalo, cows and bulls are capable of breeding through out the year but often show seasonal fluctuations in fertility because of climatic and nutritional factors that modulate ovarian and testicular functions. The physiology and endocrinology of reproduction in buffalo are basically similar to those in cattle, but some important differences exist that must be considered in attempts to improve reproductive efficiency through the use of modern reproductive technologies [7][12].

Buffalo breeds and management systems: Buffaloes were introduced into Egypt from India, Iran and Iraq approximately during the temperament middle of the 7th century.

Principle usage: milk, meat, fertilizer- no longer used for plowing, pulling, riding, etc. The distinction between the different types of Egyptian buffaloes is only environmental. It is the most important and popular livestock for milk production in Egypt. Population size: 5,317 000

Description: Blackish grey in colour, horn form varies from lyre to sword-shaped. The head is long and narrow, the jaws are long and strong. Ears are long and dropping. The neck is rather long, thin and straight. The forelegs are rather short and heavy boned. Ribs are wide, deep and well sprung. The rump is sloping and the tail setting is low. Height at withers of adult male is 178 cm, body weight is 600 kg. Height at withers of adult female is 144 cm, body weight is 500 kg. Distribution: All over the country, mainly in peri-urban areas and the Nile delta. Husbandry: The farmer keeps manure in a solid state inside the animal enclosure. The solid manure is taken twice a year and spread in the fields before planting. The animals are slaughtered only in slaughterhouses, following the Islamic practice of cutting the jugular vein. Milking is done by hand, twice a day, mainly by women. Average slaughter weight is 500 kg, at the age of 18-24 months. Carcass yield is 51 percent. Overall growth rate is 700 g/day. Dairy performance: Lactation duration 210-280 day Milk yield 1 200-2 100 k Milk fat 6.5-7.0 percent Products: The following cheeses are produced with the addition of cow milk: Domiati, Karish, Mish, Rahss.

SOURCES OF EGYPTIAN WATER BUFFALOESS: Villagers in medieval Egypt adopted the water buffalo, and become the most important domestic animal in modern Egypt. Buffalo population increased gradually and has reached over 5 million head. Buffaloes now supply Egyptian with more meat, milk, cooking oil, and cheese.

Dimensions: height at withers: 125-150cm- Weight: 350-600 kg- Color: dark grey- Horns: extend backwards(sickle shape).

Egyptian buffaloes are considered one of the most important farm animals that kept for dual purposes (milk/meat production). There can be vicious when handling (temperament can depend a lot on environment and level of confinement). Milk production: 4-10 kg of milk twice daily, depends of the amount of concentrate fed- Has at least a 7% milk fat- Milk is whiter in color than cow's milk- Days in lactation per year:210-280- Average number of lactations: 7 Calving: age at first calving: 34-41months Housing. In isolated pens beside the house- In pens connected to the house- In fields (usually tied to the ground) Watering: Through pipes for those kept in houses.- From irrigation can also for those in a field feeding usually fed base emduring the day and concentrate (corn, bran, cotton seed, etc.) at night.

Behavior and physiology of water buffalo:

In general the husbandry of buffalo is more or less similar to that of cattle. Buffalo are generally docile and easy to handle & unless wounded or severely stressed. Breeding throughout the year and having a calf every year. They carry their calf for 10 months; twin calves and dystokia are very rare. Adult females may reach 350kg, in Himalayans, to 800 kgin Bulgaria and Italy Longevity: up to20 years old Buffalo love to wallow in water but it is not necessary.

De-horning is not recommended as the horns provide a mechanism for body heat loss. Well constructed house is preferable can be loose yard or cubicles Buffalo exceed cattle in their ability to convert poor quality forage to meat or milk. Buffalo consume 2.5% of its body weight as a daily dry matter intake. tocking rates for buffalo:10-20%higher than for cattle. Buffalo prefer to graze a shorter sward to cattle, nearer to that for sheep.

Comparison with Cattle: Cattle and water buffaloes are obviously different animals.

Genetics:Swamp buffalo has 48 chromosomes. The River buffalo, 50 chromosomes. The chromosomal material is however similar in the two types and they crossbreed to produce fertile hybrid progeny. Cattle have 60 chromosomes, hybrids from the union are unlikely to occur.

Egyptian buffaloes are considered one of the most important farm animals that kept for dual purposes (milk/meat production). There are nearly 4 million buffaloes, representing 44% of dairy animals in Egypt (FAOSTAT, which contribute 44 % (2,640,638 ton) of total milk production (5,960,102 ton) and 18 % (270,000 ton) of total meat production (1,528,789 ton) in Egypt (FAOSTAT, 2008). Egypt suffers from a huge production gap in milk and meat detected in annual imported milk and meat Production of buffalo in Egypt couldn't fill such a gap due to the absence of specialized breeds/lines for (meat/milk) and the need for national genetic improvement programs. Therefore trials for the introduction of foreign breeds of buffalo (crossbreeding with both Italian and Pakistani breeds) were performed with the aim to significantly improve the genetic makeup of the Egyptian buffaloes for economic traits, as in case of the native cattle crossbreeding. Pakistani buffaloes have the potential of producing over than 5,000 liters of milk per lactation under efficient breeding, feeding and health care program. Nili Ravi is the best breed at national and international level in terms of its production potentiality, reflected in average milk yield per lactation of 2,430 liters, while some high yielding Nili Ravi also produce 3000-5000 liters/lactation (Bilal *et al., 2006)*. In Italy there are 300,000 buffaloes, used to be found in the central and south of Italy. Due to the quota on cattle milk, buffaloes have moved towards the north and replaced a portion of dairy cows. The number of recorded Italian milking buffaloes is around 44,000 (one third of the total buffalo population). Average milk production is 2,250 kg/lactation. It has increased the last 17 years

Buffalo meat, like the milk is lower in cholesterol and higher in mineral content than that of cows. Lean buffalo meat has less than half (44%) the total fat content of lean beef and has less saturated fat. When cooked there is little noticeable difference in the two meats, either visually or in taste or texture.[15]

Table 1: Comparison between buffalo and cows parameters

	Buffalo	**Cows**
Butterfat %	8.0%	3.9%
Protein %	4.5%	3.3%
Cholesterol	8mg	14mg
Color	Pure white	Creamy
Texture	Smooth	Less smooth
Taste	Sweet	Salty
Cell Counts	Very low	Higher
Yield/Lact'nKg	1850	5500

Meat Production:

Buffalo meat production. In developing countries of Asia where meat from ruminants constitute only about 21.0% of the total meat production, buffalo meat is about 11.52% of the total ruminant meat, and about 2.7% of all meat produced in the region. The average annual growth rate in production was about 1.3%. Undoubtedly, majority of world's buffalo meat is Asian, representing 91.89% and with volume of 3.08M tons in 2008 [7], About 78.5% of Asian buffalo meat was produced in South and South West Asia with the greater bulk contributed by India and followed by Pakistan. This is easily explained by the fact that these two countries have 75% of the buffalo population in the region. Improvement in buffalo meat yield is contributed by the increasing usage of male calves which were not fully utilized. In the past in the greater part of India, farmers were not paying enough attention to rescue the young animals from high mortality before reaching 6 months of age. In recent years, however, the rising export of Indian buffalo meat have given enough incentives for small herd farmers to rear these animals and put additional weight prior to slaughter, thereby sustaining the growth in the meat harvest from the Indian buffalo sector. On the average, however, the extraction rate registered among Asian countries is highest in Pakistan, Nepal and China [1]

Genetic Improvement:
Select superior buffalo bulls and cows for breeding. Performance testing, leading to the mass selection of superior animals, deserves high priority. A massive selection program is needed to bring about genetic progress. For each breed, bulls and cows with the potential for improving production of meat and milk and increasing draft power should be identified and used for breed improvement Crossbreeding of Swamp and River buffaloes is a potentially important route to genetic improvement[5]. Infusing genes for high milk production into the Swamp buffalo, now used mainly for meat and work, creates the potential for atriple-purpose animal. The use of artificial insemination and deep-frozen semen should be a major help in upgrading the buffalo. Transfer of live embryos for implantation in the uterus of surrogate mothers could be important for water buffalo [14].

Asian Water Buffalo: 97% of the world's water buffaloes are located In Asia
There are two types
1- Swamp buffalo:
Indigenous to those parts of Asia which do not have a great culture from consuming milk & milk byproducts (Indonesia northwards to China) It is a dual purpose animal (meat and draught) 2- River buffalo:
It is a triple purpose animal (milk, meat and draught power), Found in those countries where milk plays a more important part in the human diet e.g India, Pakistan, middle east, Caucasus and Balkans[5].
In addition to its for meat and/or milk, Buffaloes are still used in Asian countries as Drought animals (transport, land Cultiv. and carriage). An old Chinese women say:
"To my family, the buffalo is more important than I am. When Idle, they'll weep for me; but if our buffalo dies, they may starve.
South Asian countries where the rice cultivation depends mainly on buffalo-workload
Water Buffalo:
Kingdom: Animalia,Phylum:Chordata
Class:Mammalia,Order:Artiodactyla
Family:Bovidae ,Subfamily:Bovinae
Tribe:Bovini , Genus:Bubalus
Species: B. bubalis

Water buffalo: an asset undervalued
Buffaloes - their distribution
There are about 185 million buffaloes in the world (FAO Statistics). Roughly 97 percent of them or 176 million heads are water buffaloes essentially found in the Asian Region
The overall buffalo numbers are increasing at about 1.3 percent annually, while on other contraries, the numbers are dropping dramatically.
The different types of water buffaloes. The water buffaloes are of two types; the RIVERINE type and the SWAMP type.
Features of riverine and swamp buffaloes: Riverine buffaloes are usually black and have long curled horns. The Swamp buffaloes are usually dark grey but may also be black, black and white, or even all white. They have long gently curved horns.
Unique features of buffalo contributions: Richer Milk: Buffalo milk contains higher Total Solids (protein, fat, minerals) of 18 – 23 percent as compared to 13 – 16 percent in cow milk. This confers advantage to the production of cheese and some other dairy products Leaner Meat: Buffalo meat is tasty and lean. It contains lower saturated fat than beef and pork, and hence is considered a meat of good dietary value. Efficient Converter of Low Quality Feed: Buffaloes can utilize less digestible feeds (eg. rice straw, maize stovers, sugar-cane wastes, etc.) better than cattle to grow. This makes buffaloes easy to maintain using locally available roughage and crop residues. Buffaloes' values increase day by day as they grow (in contrast to machinery!). They need no costly fuel, never rust, and they reproduce!
Best Draught Power for Wet Environments: Buffaloes are superior to other draught animals inwet or waterlogged conditions, such as in muddy paddy fields. They can also be used for car haulage, carrying heavier loads than cattle. Enrich Soil Fertility: Buffaloes improve soil structure and fertility while treading paddy fields. Each year, an adult buffalo produces 4 to 6 tonnes of wet manure plus additional urine as bio-fertilizer to the land. This reduces or eliminates the need for chemical fertilizers as well as provides essential soil humus which chemicals can not provide. Secure Socio-Economic Status of Farmers: Buffaloes are

often used as cash savings, can be sold when needs arise (school fees, marriage, crop failure, debts etc.). Thus, the animals ensure the farmers' socio-economic security [2].

What should be done? Should buffalo rearing be promoted? Or should cattle simply replace buffaloes?

Also in future there will be an important role for buffaloes in Asian farming systems. Buffaloes and cattle are complementary.

Buffaloes can utilize feeds, especially low-quality feeds, more efficiently than cattle. Buffaloes also have unique advantages over cattle: they are less susceptible to ticks and other ecto-parasites (due to their wallowing); they can better withstand wet conditions underfoot and are therefore more suitable in many areas; and they are more docile (children/old people can manage them relatively easily)[18].

Future strategies for survival of buffaloes:

Improve access of small farmers to buffaloes through the many "Royal Initiatives" such as provision of animals to small farmers through the "Buffalo Banks" scheme, boosting buffalo raising in line with the "New Theory" for self-sufficient and sustainable agricultural production collect and effectively disseminate more and better quality information on farming systems (particularly with regard to economical and sustainable production) to farmers and other concerned parties. Further develop "model buffalo raising villages" based on best-practices and promote the concept. Provide incentives for production of quality meat and meat products from buffaloes. Buffalo meat should not merely be regarded as a cheap source for meat products like meat bulls, but should be valued as high quality meat, especially if derived [3]. From young animals, develop breeding schemes to improve buffaloes for those traits of importance to the long-term sustainable use of the species[3]. The three main areas for buffalo R & D Develop appropriate biotechnology innovations specific to buffaloes. At present, developments in biotechnology occur in other species, and the same techniques may be tried with buffaloes. Possible areas are artificial insemination, embryo transfer, cloning, biotechnology to improve feed utilization and disease control[8].

Fig 4: Reproduction

Fig 5: Feed and management

Fig 6: Disease control

The importance of buffalo in the world.

There are only countries in Asia, Africa and Europe that have reported milk producing buffalos to FAO, and the USA has reported meat producing buffalos [1]. The superior amount of the buffalos is in Asia with 95.5% of the world buffalo population. In Africa, 4.2% are found, in Europe 0.2% and in America 0.1%. India has the biggest buffalo population with about 33 million dairy buffalos and 10.8 million meat producing buffalos. India also produces most buffalo milk and meat in the world and the quantity is about 52 million tonnes of milk and 1.5 million tonnes of meat [1] These statistics shows that it is in the South and South-East part of Asia were most of the buffalos can be found. In Africa, Egypt has reported about buffalos. Number of animals for milk production is 1.6 million and for meat production 1.5 million .Three countries in southern and eastern Europe have to FAO

reported to have milk and meat producing buffalos in 2006; Bulgaria, Greece and Italy. Italy has the biggest amount of these animals; about 154 000 dairy buffalos and 5000 meat producing buffalos. Thereafter is Bulgaria, but with a strikingly lower number of animals; about 4000 dairy buffalos and 2000 meat producing buffalos. Greece has 150 dairy buffalos and 450 buffalos for meat production. [14] reported that buffalos are well adapted to hot and humid climate and therefore makes a good production animal in the Tropic Zone. The milk is consumed both as fluid milk, cheese and other dairy products. In Egypt, the milk is mainly used to make a product called "Queshta Mosakhana" of the floating cream after boiling the milk. In Bulgaria and Greece, the buffalo milk is mainly processed into yoghurt and in Italy buffalo milk is used to make mozzarella cheese, both for the national and international market.

Role of FAO in buffalo development: FAO has always emphasized the important role that buffaloes play in overall agricultural production in Asia. FAO has worked over the years on various aspects of buffalo production and draught animal power.

In the feed sector, as amply demonstrated through FAO projects, the development of systems to improve ruminant feed quality from straw (urea treatment) and the use of mineral feed blocks (urea-molasses blocks) can greatly assist in the improved efficiency of buffalo production. FAO provides a forum for information about the importance of the species. Various FAO publications provide information and know-how on buffalo development. Workshops have been held addressing new technologies for buffalo production (e.g. multiple ovulation embryo transfer, nuclear techniques in buffalo breeding and disease diagnostic, etc.). FAO was also instrumental in the establishment of the Asian Buffalo Association (ABA) formed to foster further scientific exchanges within the major buffalo-keeping countries. FAO also has linkage with the World Buffalo Federation (WBF) which attempts to involve other various regional associations. The International Livestock Research Institute (ILRI), which has only recently assumed a global role, is involved in examining the genetic diversity found in buffaloes. FAO has close cooperation with ILRI. Donor countries to international agricultural development have been involved in efforts to carry out crossbreeding strategies to improve productivity of buffaloes. FAO has been coordinating many of these efforts. FAO has been developing a global strategy on Animal Genetic Resources and has established a global databank in which disappearing breeds are identified and the status designated as "risk of extinction". Two editions of the "World Watch List for Domestic Animal Diversity" have been produced (1993 and 1997) FAO has whenever possible provided assistance to the global effort of buffalo promotion. It is feared that what's happening in Thailand may soon also take place in other countries (Cambodia, Indonesia, Laos, Myanmar, the Philippines) where the swamp buffalo population is still stable. Increased agricultural mechanization in these countries especially in the small farming sector may induce the decline of buffalo numbers similar to the situation witnessed in Thailand.

Table 2:Vital Clinical And Laboratory values:

Animal	**Cattle**	**Buffaloe**
Normal Temperature °C	38.5	38.2
Pulse rate/min	50-80	40-60
Respiratory rate/m	10-20	8-20
RBCs (T/l)	5-10	6-8
Hb (G/dl)	8-14	11.5-15.5
WBCs (G/l)	4-12	7-9
PCV (%)	26-42	32-52

Buffalo blood parameters diseases susceptibility vs cattle: [15]

The greatest buffalo losses are often among calves. Newborn buffalo calves, like cattle calves, can die in large numbers due to viruses, bacteria, and poor nutrition.

Calves especially rarely suffer from pneumonia or non-nutritional scours. Poor management during the calf's first2months of life may attribute to these losses., e.g. depriving calves from their valuable mother milk to sell it. Proclivity of buffalo calves for wallowing exposes them to water borne diseases. I- Non infectious disease

1-Exposure to heat and direct sunlight
•Buffaloes suffer if forced to remain, even for a few hours, in direct sunlight.
•They have only one-tenth the density of sweat glands of cattle and their coating of hair is sparse, providing little protection from the sun. Accordingly, buffaloes must not be driven over long distances in the heat of the day. They must be allowed time for watering and, if possible, for wallowing. Driving under a hot sun for long hours will cause heat exhaustion and possibly death; losses can be very high and can occur suddenly Young calves are particularly affected by heat[17].
the sun is inadequate to ripen, Sudden drops in temperature and chill winds may lead to pneumonia and death[17].
2- Exposure to extreme cold Buffaloes are also sensitive to extreme cold and seem less able than cattle to adapt to truly cold climates, Buffaloes don't do well where
3- Lameness and clinical mastitis is also rare in adults.
4-Lymphangitis and Limb Abscesses were frequently noted on Egyptian Buffalo.
5- There has been no incidence of BSE in any buffalo anywhere in the world. Traumatic reticuloperiton it is and its.
6-Allied syndrome Traumatic pericarditis[17]
7- Phytobezoar obstructing The reticuloomasal orificecausing
ruminitis, regurgitation,aspiration pneumonia and deathin a she-buffalo 8- Frothy Tempany
9- Metabolic diseases- Hypophosphatemia It occurs mainly at late pregnancy, It is related closely to feeding with Barseen (Sweet Clover). In Cattle it occurs primarily at peak of lactation and related directly to heavy milk production Milk fever occurred in mild form and respond to ca therapy as well as Tail Tip Necrosis in buffaloes Zinc deficiency/or microfilaria[10].

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STUDY ABOUT THE PRODUCTIVE CHARACHTERISTICS OF QUAILS FROM THE ”BALOTEŞTI” POPULATION

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Abstract

Raising Japanese quails for eggs and meat production has seen great development in recent decades because on the one hand of quail eggs and meat quality (rich and well balanced nutritionally, good taste), and the other hand due to natural medicine recommendations to consume these products with proven therapeutic effect. The aim of this paper was to determine the current average characteristics of incubation, the medium productive performances of youth and hens quails of egg-meat mixed population "Baloteşti", important population for raising quails in Romania and in witch is applying consistently an amelioration program. The research has established the following production data: the average percentage of hatching is 66.34 ± 0.22%, average weight is 8.94 ± 0.56 g at 1 day of age, and 210.45 ± 2.29 g at the age of 42 days, average gain between 1 and 6 weeks of youth raising is 201.51 g / head / period, average daily consumption of fodder during 1-6 weeks of raising is 19.85 ± 1.23 g. The average laying between 1 and 40 weeks of hens is 74.45 ± 1.03%, the average feed consumption during 1 to 40 weeks of laying is 32.25 ± 0.78 g, while the average yield at slaughter is 73.43 ± 0.44%. Productive characteristics of Balotesti quails such as: high weight of the chick at one day of age, fast-growing to 42 days, high performance at slaughter, average percentage of laying up to the age of 40 weeks of laying make this quail population recommended both for the eggs and meat production. An advantageous feature is that males resulting from sexing in using for egg production can successfully valorised for meat as complementary production. Remain to be improved the incubation results of this population by applying proper egg storage conditions (temperature, humidity), using the incubation technology and incubators being constructed following studies strictly conducted on quail eggs.

Keywords: *quails, productive parameters, youth, hens*

MATERIAL AND METHOD

The results have been determined in experiments on biological material represented by the Balotesti quail performed in Bucharest IONIŢĂ T.LUCIAN Individual Entreprise, in the working point located in the of Gherghiţa commune, Ungureni village.

To establish the hatching results of the studied population was organized an experiment in 1500 introduced hatching eggs, eggs incubated from a breeding flock of 5 months age. Environmental conditions in the hatchery were those employed in the specialized literature provided. Expected results were: percentage of hatching, eclosionability, clear eggs, dried egg percentage, percentage of dead chicken in eggshell during hatching and the average water loss after 15 days incubation.

To establish productive parameters in young mixed population of quail egg-meat "of Balotesti" was organized an experiment on 995 chicken of one day age. Environmental conditions in which the experiment that took place were within those provided in the literature. Results from the experiment set refers to the evolution of live weight at ages of 1 day, 7 days, 14, 21, 28, 35 and 42 days to determine average daily gain of each week of growth and then average weekly gain, average daily combined feed on week consumption, average specific consumption and mortality rate for the entire period of growth.

To establish production parameters in laying quails of eggs - meat mixed population "of Balotesti" was organized an experiment on an initial number of 400 quail hens, sexed from the initial series of chicken. Environmental conditions in which the experiment took place were within those provided in the literature. Hatching eggs were 8 days old, preserved at a storage temperature of 15°C and a relative humidity of 65%.

The results set refers to the average laying rate for the 1- 40 weeks period of laying, per week and cumulative egg production, live weight during 1- 40 weeks of lay, egg weight between 1-35 weeks of laying, daily feed consumption for the period 1- 40 weeks of laying, specific consumption and mortality for the period 1-40 weeks of laying.
For setting results in the slaughter of quail egg-meat mixed population "of Balotesti", carcasses were analyzed in a number of 100 males sexed from the initial series of quail chicks of the mentioned population.
Expected results from the experiment were live weight before slaughter, the average proportion of blood, flakes, organs and intestines, the average proportion of the chest, legs, back, wings, the average weight of the breast, the average proportion of meat, skin and bone from the breast.

RESULTS AND DISCUSSIONS

1.The results obtained from incubation of quail eggs in egg-meat mixed population "of Balotesti"

Table 1. Results from incubation of quail eggs in egg-meat mixed population "Balotești"

Specifications	**X±Sx**
Hatching ,%	66.34 ± 0.22
Clear eggs, %	15.14 ± 0.15
Dry eggs, %	1.30 ± 0.21
Dead chicken in the shell during the eclosion, %	17.22 ± 0.11
Water loss after 15 days of incubation, %	14.25 ± 0.45
Proportion of shell after the eclosion	9.06 ± 0.23
Proportion of chicken weight compared to the egg weight	74.37 ± 1.34

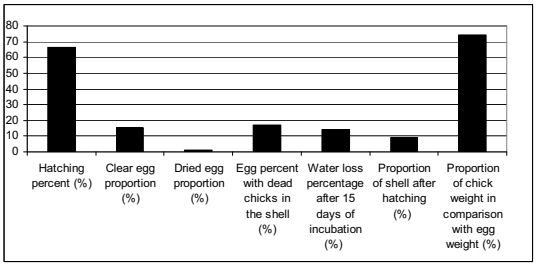

Fig. 1: The average results of hatching the analyzed batches of eggs from Balotesti population.

In a study conducted in Brazil, researchers Josue M. Romao et al. (2009, Brazil) set a weight ratio of 74.25% between the chick of one day old and the initial weight of egg if incubated in a humidity level of 36%. Weight loss during incubation in this case was 11.96 %. In addition, Soliman et al. (1994) showed a weight loss of 11.32% of the egg in Japanese quail eggs incubated at a temperature of 37.5° C and a relative humidity of 56%.
In a study by Garip M. and Dere S. (Turkey, 2011), were determined minimum percentages of hatching, between 35.2% in eggs stored for 15 days at 21°C and 54.2% in eggs stored for 10 days at a temperature of 27ºC. The maximum hatching percentages were between 79.4% in eggs stored for 15 days at 11ºC and 84.2% in those stored for one day at 11ºC.
Incubation results in this experiment are similar to those of Abdel-Azeem A., Abdel-Azeem F. (Egypt, 2009), who obtained 61.3% percent of hatching eggs stored for 2 days and 60.3% in eggs stored for 6 days.

2. Production parameters of young quails eggs - meat mixed population "of Balotesti" during raising between 1-6 weeks

Table 2. Production parameters of young quails eggs - meat mixed population "of Balotesti" during 1-6 weeks of growth

Specifications	**X±Sx**
Chicken weight at age of 1 day (g)	8.94 ± 0.56
Live weight at age of 42 days (g)	210.45 ± 2.29
Daily increase in the period of 1-6 weeks of growth (g/capita/period)	201.51
Daily consumption in the period of 1-6 weeks of growth (g combined fodder/capita/day)	19.85 ± 1.23
Cumulated daily consumption of fodder in the period of 1-42 days (g/capita)	834.00 ± 7.86
Specific consumption in the period growth of 1-6 weeks (g combined fodder/g gain)	4.14
Mortality in the period of 1-6 weeks of growth (%)	5.5

Researchers Abdel-Azeem A., Abdel-Azeem F. (Egypt, 2009) have established an average weight at age 1 day of 9.88 g (higher than that determined in this experiment) for eggs stored for 2 day. The same authors determined an average weight of the chicks at day one of 8.87 g in the case of eggs stored for 4 days and 8.30 g for eggs stored for 6 days, having similar weights to those in this experiment. Was established a gain of 186.42 g / 42 days

of raising period, a combined average fodder consumption of 536 g fodder/ capita/ period, specific fodder consumption of 2.87 kg feed / kg gain for chickens originated from eggs stored for 2 days and a mortality rate of 6.5% in 42 days for chickens that came from eggs stored for 6 days.

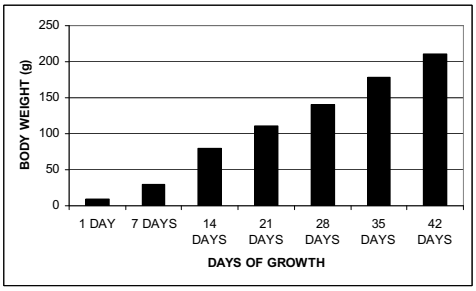

Fig. 2: Change in average live weight of young quail population Baloteşti analyzed during 1-42 days

3. Production parameters in laying quails eggs - meat mixed population "of Balotesti" in the period 1-40 weeks of laying

Table 3. Production parameters in laying quails eggs - meat mixed population "of Balotesti" in the period 1-40 weeks of laying

Specifications	**X±Sx**
Laying percentage (%)	74.45 % ± 1.03
Cumulated production per capita in the period of 1-40 weeks of egg laying (no. eggs/ capita)	208.46
Live weight (g/capita)	255.76 ± 1.58
Egg weight (g/egg)	11.67 ± 0.42
Combined fodder consumption (g /capita/day)	32.25 ± 0.78
Specific consumption (g/egg)	43.32 ± 0.93
Mortality rate (%)	0.15 ± 0.03

In a study conducted in Poland (Tarassewicz Z. et al., 2006) an average lay between 1-29 weeks of laying of 81.60% was established which is similar to that found in this study in population quail of Balotesti for the same period (78.68%).

To a flock of quail hens in Turkey some researchers (Okan F., 1999) have determined an average consumption of fodder (31.33 g fodder / capita) similar to that determined in the quail population of Balotesti (32.25g fodder / capita).

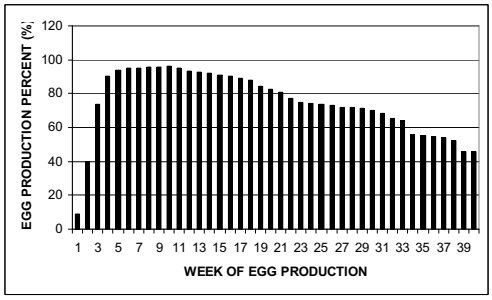

Fig. 3: Evolution of the average percentage of laying in the Balotesti quails from analyzed group between 1-40 weeks of laying.

4. Results of slaughtering egg-meat mixed quails population "of Balotesti" at 6 weeks of age

Table 4. Results of slaughtering egg-meat mixed quails population "of Balotesti" at 6 weeks of age

Specifications	**X±Sx**
Live weight at the age of 6 weeks (g)	210.45 ± 2.29
Proportion of blood (%)	5.34 ± 0.11
Proportion of feathers (%)	6.45 ± 0.34
Proportion of organs and intestines (%)	16.78 ± 0.65
Yield of carcass (%) (carcass weight / live weight)	73.43 ± 0.44
Breast proportion (breast weight / carcass weight) (%)	41.00 ± 0.41
Proportion of the thighs (thighs weight / carcass weight) (%)	24.12 ± 0.19
Proportion of the back (back weight / carcass weight) (%)	25.78 ± 0.62
Proportion of the wings (wings weight/carcass weight) (%)	9.76 ± 0.38
Breast weight (g)	58.65 ± 0.67
Proportion of breast meat (breast meat weight /breast weight) (%)	70.55 ± 0.44
Proportion of breast bones (breast bone weight / breast weight) (%)	16.65 ± 0.27
Proportion of breast skin (chest skin weight / breast weight) (%)	13.78 ± 0.54

In a study conducted in Nigeria by Raji A. O. (2006) on a flock of males Japanese quails, aged 10 weeks, he established a 67.82% return of the carcass, a proportion of 34.41% of the breast and of 24.02% legs, data similar to those found in population "Baloteşti" in this study. Live weight and average carcase weight were significantly lower than in population in this study (113.16 g and 76.91 g respectively).

A study conducted in Iran (Vali, N. et al., 2005) on youth quail indicated an average carcase weight of 121.89 g, a carcass yield of 70.58%, an average weight of the breast of 47.45 g, corresponding to a proportion of 38.98% and a weight of legs of 26.72 g,

corresponding to 22.94%. These features are similar to those determined in this experiment in Baloteşti population.

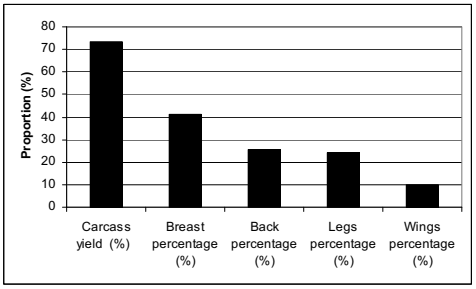

Fig. 4. The average yield of carcass and average proportions of breast, back, legs and wings of quail carcasses of the analyzed population Baloteşti

DISCUSSIONS

The results obtained in incubation of egg-meat mixed quail population "of Balotesti"

Weight loss of eggs during incubation is usually in the acceptable limits for hen eggs of 14.25 % in "Baloteşti" quails.

The proportion of clear eggs (without embryos or dead embryos in the first stage of incubation) in the quail population "of Balotesti" was 15.14 ± 0.15%.

The proportion of eggs with dead chicken in the shell in the quail population "Balotesti" was of 17.22 ± 0.11%.

Percentage of hatching registered in the quail eggs-meat population "of Balotesti" was in average of 66.34 ± 0.22%.

Production parameters of young quails eggs-meat mixed population "of Balotesti" during 1-6 weeks of raising

Average body weight at age 1 day of quail chicks of Balotesti was 8.94 ± 0.56 g and at age of 42 days was 210.45 ± 2.29 g.

Average gain of weight recorded was of 201.51 g/ head/ period of 6 weeks in Baloteşti quail rising.

Combined fodder average daily consumption was 19.85 ± 1.23 g / head / day during 6 weeks of growth.

The specific consumption was 4.14 kg fodder /kg gain in chicks during 0-6 weeks of Balotesti quails growth.

Viability of Balotesti quail during 0-6 weeks was 94.5%.

Production parameters in laying quails eggs – meat mixed population "of Balotesti" in the period 1-40 weeks of laying

Production performances recorded in the batch of egg-meat mixed quail population "of Balotesti" during 1-40 weeks of laying are:

The average percentage of laying for the period 1-40 weeks of laying was 74.45 % ± 1.03;

The average production of eggs cumulated during 1-40 weeks of laying was 208.46 eggs /head;

The average percentage of mortality during 1-40 weeks of laying was 0.15 ± 0.03% on week;

The average weight of egg during 1-40 weeks laying was 11.67 ± 0.42 g;

Average body weight registered in the period 1-40 weeks of laying was of 255.76 ± 1.78 g per capita;

Average consumption of mixed fodder recorded during 1-40 weeks of laying was 32.25 ± 0.78 g per capita and per day;

Specific average consumption recorded during 1-40 weeks of laying was 43.32 ± 0.93 g per capita and per egg.

Results from the slaughter of quail egg-meat mixed population "of Balotesti" at 6 weeks of age

The live weight and eviscerated carcass weight in quails "of Balotesti" at the age of 6 weeks

Average live weight at the age of 42 days was 210.45 ± 2.29 g.

The share of blood, flakes, organs and intestines in the carcass.

Average proportion of blood was of 5.34 ± 0.11%, the average proportion of flakes was 6.45% ± 0.34, while the average proportion of organs and intestines in total carcass was 16.78% ± 0.65.

Carcass yield of quails of "the Balotesti" population

Quail carcass yield at 6 weeks of age averaged 73.43 % ± 0.44.

The proportion of carcass parts in the analyzed groups "of Balotesti" population

The average proportion of parts of the carcass at the age of 6 weeks was as follows: 41.00 ± 0.41% breast, legs average proportion was

24.12 ± 0.19%, back 25.78 ± 0.62%, while the wings were 0.38 ± 9.76%.

Percentage share of parts of breast of the carcasses of quail in the population "of Balotesti"

From the average weight of the breast (58.65 ± 0.67 g), the breast meat has a high percentage share (70.55 ± 0.45%), the bones having a weight of 16.65 ± 0.27% and the skin 13.78 ± 0.54%.

CONCLUSSIONS

Following investigations, the best production features of Balotesti quail include:

- High weight at the age of 42 days, which recommend the population for meat production too;
- High viability in the raising period correlated with ensuring proper microclimate factors, under age category, taking into account the very high growth rate of chicks;
- High efficiency carcass (70%);
- High production of eggs, early entry into laying (first egg age at 36 to 42 days), good body weight during the laying period;
- High viability during the laying period correlated with ensuring microclimate factors and avoid possible stress factors (moving cages, regrouping birds in cages etc.);

The weaker features of Balotesti quails may be given by poor results in incubation, but it is more influenced by the technology incubation development in quails, which in our country is still poor (quail eggs incubation is carried out in most of the cases in incubators for hen eggs).

Particular importance in the incubation of quail eggs should be given to the oldness of the hatching eggs (it has to be minimized), and ensuring conditions for preserving eggs (low temperature, high humidity).

By conducting further studies on quail eggs hatching factors (temperature, humidity, turning, optimal carbon dioxide and oxygen levels, etc.), quail hatching technology can be greatly enhanced.

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PRELIMINARY RESULTS ABOUT THE EFFECT OF STORAGE PERIOD ON THE HATCHING PROCESS OF THE HEN EGGS

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Abstract

In the breeding and production of broilers, eggs for hatching are subject to different storage conditions. This work aims to show the effects of different levels of storage conditions and preheating of hen eggs from Ross 308 hybrid and to help establish a optimal program for storage and preheating of eggs. Hatching eggs had a different retention period of 3 days, 7 days or 14 days, then were preheated for 4, 8 or 12 hours at a temperature of 23 ° C, 25 ° C or 28 ° C. These eggs were weighed at the beginning of storage and the introduction to incubate and then followed during embryonic development, the mortality of different stages was established. Following the incubation process were determined proportion of viable chick, chick yield, hatchability and hatching percentage. It concludes that batches of eggs that were stored for 3 or 7 days and then incubated, have made the best values of the indices follow its incubation and hatched chick quality. The best choice to obtain maximum results in incubation of hybrid Ross 308 from the combination of duration and storage conditions, the duration of preheating and levels of preheating of the eggs were: storage 7 days, preheating for 8 or 12 hours at 23 °C or 25 °C. Elevated values of incubation parameters have appeared also to the eggs stored for 3 days. The embryonic mortality had also values below the average batch in all stages analyzed, at the group of eggs stored for 3 days and then preheated for 4 hours at 25 °C.

Key words**:** *egg storage period, preheating, egg incubation, hatchability, hatching percentage*

INTRODUCTION

In Romania, hen eggs placed in incubation, come mostly from fewer breeding units and is often brought from great distances, reaching a maximum storage of eggs included in "fresh" category, so it is necessary to undertake a study more complete and competent regarding to the conditions of storage and preheating of hatching eggs. This paper aims to determine the physical parameters of the optimal storage and preheating eggs from commercial hybrid of meat chicken "Ross 308", depending on their storage conditions. This paper aims also to support the improvement the performance of hatching chicken eggs, by establishing a optimal polifactorial program for storage and preheating egg when incubation begins.

The optimal duration of egg storage before incubation appears in the literature with different data:

- up to 21 days at 7.2 °C [10, 11], or at a temperature of 11.7 °C and relative humidity of 85% [4];
- up to 8 days at low temperature and high relative humidity [14]**;**
- up to 7 days under standard conditions (10-20 °C and 50-80% relative humidity) [1], up to a week at a temperature between 11-16 °C and moderate relative humidity [3], to 7 days at a temperature of 15 °C and air relative humidity of 85% [4], up to 7 days if the storage temperature of the eggs is 12.78 °C and relative humidity of 75% [12];
- up to 5 days to the ideal temperature for storage of 12.77-15.55 °C and a humidity of 75-80% [4];
- up to 5 days at a temperature of 16-20 °C and a relative humidity of 65-70% [9];
- up to 4 days at a storage temperature of egg incubation at 20 °C and the relative air humidity of 75-80% [4].

Following other authors eggs can be kept different time intervals, with good hatching results, if those are kept in optimum conditions (temperature and relative humidity); it is considered that the ideal storage temperature of chicken eggs for a period greater than 4 days is

on 10 °C, range between 8-12 °C [13]. When eggs are kept and stored between 1-3 days, they must be kept at 18.3 °C and the relative humidity of 75% and when the duration of storage increases, the temperature at which eggs are kept drops to 14.7 °C and the relative humidity must be between 75-80%. At an interval higher than one week of storage conditions, hatching eggs are subject to an average temperature of 12.2 °C and the relative humidity of 80%.

The optimal time of pre-heating seems to be different: 18-24 hours [8], 16 hours [10.11], 10 hours [2] or 6 hours [5]. Preheating period is conditioned also by the eggs age, their temperature and the possibilities of raising the temperature up to 23.9-26.7 °C [13].

In specialized literature are not complex experiments covering both storage and different preheating conditions of eggs.

MATERIAL AND METHOD

This paper aims to determine which is most advantageous combination of technology on three factors: duration of storage of eggs and the preheating time and level of eggs to achieve the best results of hatching chicken eggs.

The research was conducted in „Staţia de Incubaţie Nouă" at SC Avicola Tartasesti SA, part of Agroli Grup, using eggs and chicks from Ross 308 hybrid. The working method consisted in analyzing the results of the hatching eggs from hens aged between 25 and 35 weeks. Eggs had a different retention period of 3 days, 7 days or 14 days. Eggs stored in this way, were then preheated for 4, 8 or 12 hours at a temperature of 23 °C, 25 °C or 28 °C.

According to the experimental design were used 27 experimental groups (batches) and 9 control (batches). Each experimental group consisted by a number of 150 eggs and a total of 1377 eggs were analyzed. Eggs were weighed at the beginning and end of storage period and then placed in the incubator. After the preheating period, eggs were incubated and then transferred from day 18 of embryonic development at the hatcher. At the end of the hatching process, it was separated chicks from the hatching debris. Chicks were then sorted according to quality, were counted, vaccinated and placed in boxes for delivery to farms. Hatching residues were analyzed, eggs were broken and evaluated causes of embryonic mortality.

Finally the main hatching indices were calculated by assessing the activity of hatching eggs: hatchability and hatching percentage for each batch.

RESULTS AND DISCUSSIONS

Weight loss of incubated eggs in storage period. Eggs stored for 3 days decreased in weight with an average of 0.45%. Loss was accentuated with the increasing of the storage interval. Eggs stored for 7 days, have lost 1.04% and those who were the stored for 14 days, have lost 1.58% of there initially weight (Table 1).

Table 1. Weight loss of hatching eggs during storage period (%)

Preheating program		Storage period (days)			Average
		3	7	14	
4 hours	23ºC	0.37	1.84	2.17	1.46
	25ºC	0.24	1.34	1.86	1.15
	28ºC	0.38	0.47	1.53	0.80
8 hours	23ºC	0.19	1.41	1.17	0.92
	25ºC	0.17	0.50	1.20	0.62
	28ºC	0.41	1.25	1.04	0.90
12 hours	23ºC	0.35	1.52	2.46	1.44
	25ºC	0.48	0.67	1.23	0.80
	28ºC	1.49	0.35	1.56	1.13
Average of experimental batches		0.45	1.04	1.58	1.02
Control batch (3,7 or 14 days storage, no preheating)		0.11	1.46	1.04	0.87

Declines of the control batch (3,7 or 14 days storage, no preheating) were on average of 0.87%, with 0.15% less than the average of the experimental batches.

Dead embryos in phase I (1 – 8 days). Embryonic mortality increased by duration of storage of eggs (Table 2). From eggs stored for 3 days and then incubated embryonic mortality in phase I was an average of 2.67% at the experimental batches and up to 2.66% to the control batch (3,7 or 14 days storage, no preheating).

Table 2. Dead embryos in phase I (1 – 8 days) depending on the storage period of eggs (%)

Preheating program		Storage period (days)			Average
		3	7	14	
4 hours	23ºC	2.00	1.34	7.34	3.56
	25ºC	3.34	4.00	8.00	5.11
	28ºC	4.67	2.00	14.67	7.11
8 hours	23ºC	3.34	4.00	3.34	3.56
	25ºC	2.00	3.34	3.34	2.89
	28ºC	3.34	2.67	8.00	4.67
12 hours	23ºC	2.00	1.34	6.00	3.11
	25ºC	2.00	4.00	6.00	4.00
	28ºC	1.34	2.00	7.34	3.56
Average of experimental batches		2.67	2.74	7.11	4.17
Control batch (3,7 or 14 days storage, no preheating)		2.66	3.33	10.00	5.33

In batches of eggs that were stored for 7 days, the embryos were dead in phase I at a rate of 2.74% for experimental batches and 3.33% in the control batch, the difference between the two values was of 0,59%. When eggs were of 14 days old, embryonic mortality in phase I it was, on average of 7.11%, in experimental batches and 10.00% in control batch. These values are considerably higher than that of fresher eggs. Between experimental batches, differences between eggs stored 14 days and those stored for 3 days, were of 4.44% and 4.37% less than 7 days old. There were more than 1% difference between average values of experimental batches and control batch, the difference was of 1.16%.

Dead embryos in phase II (9 – 15 days). The proportion of dead embryos in phase II to all analyzed eggs was low (Table 3). The lowest proportion was recorded in eggs stored for 3 days, of 1.12% at the experimental variations and 1.33% in the control batch. Then came the eggs stored for 7 days, the dead embryos in phase II were at the rate of 1.19% and the highest percentage was recorded for batches of eggs stored for 14 days, of 2.00%. Overall the averages of the experimental batches and control batch had similar values, the difference between the two of them was of 0.11%.

Dead embryos in phase III (16 -21 days). As far as the proportion of dead embryos in phase III, it was higher than dead embryos from phase II, but less than those from the first phase.

At the eggs of three days old, embryonic mortality in phase III was of 2.22% in the experimental batches and 3.33% for the control batch, when the eggs were not preheated (Table 4). This result shows that the relatively fresh eggs, can reduce by more than 1% the late embryonic mortality (1.11%) if it is made the preheating of the eggs.

Table 3. Dead embryos in phase II (9 -15 days) depending on the storage period of eggs (%)

Preheating program		Storage period (days)			Average
		3	7	14	
4 hours	23ºC	0.67	0.67	0.67	0.67
	25ºC	2.00	2.00	1.34	1.78
	28ºC	0.67	0.67	1.33	0.89
8 hours	23ºC	0.67	0.67	2.00	1.11
	25ºC	2.00	1.34	1.34	1.56
	28ºC	1.37	2.00	3.33	2.23
12 hours	23ºC	0.67	0	2.67	1.11
	25ºC	1.34	0.67	4.67	2.23
	28ºC	0.67	2.67	0.67	1.34
Average of experimental batches		1.12	1.19	2.00	1.44
Control batch (3,7 or 14 days storage, no preheating)		1.33	0.67	2.00	1.33

At the eggs stored for 7 days, the proportion of dead embryos in phase III was of 1.78% at the experimental batches and 1.33% in control batch, preheating of the eggs had beneficial effects on the embryonic mortality.

Table 4. Dead embryos in phase III (16 -21 days) depending on the storage period of eggs (%)

Preheating program		Storage period (days)			Average
		3	7	14	
4 hours	23ºC	3.34	2.00	5.34	3.56
	25ºC	2.00	2.00	5.34	3.11
	28ºC	0	2.67	2.67	1.78
8 hours	23ºC	1.34	0.67	6.00	2.67
	25ºC	4.00	0	5.34	3.11
	28ºC	2.00	1.34	2.67	2.00
12 hours	23ºC	1.34	2.00	5.34	2.89
	25ºC	3.34	2.67	2.00	2.67
	28ºC	2.67	2.67	3.34	2.89
Average of experimental batches		2.22	1.78	4.23	2.74
Control batch (3,7 or 14 days storage, no preheating)		3.33	1.33	5.33	3.33

Instead, the old eggs, which were kept for 14 days before being placed in incubation, the percentage of dead embryos in phase III it was significantly higher in eggs without preheating, of 5.33% compared with the preheated ones, of 4.23, the differences from batches being of 1.10%.

Viability of hatched chicks. Viability of all batches of chicken have been considered high, regardless of the duration of storage eggs. Total average values were of 98.34% for the experimental batches and 99.21% for control batch, the difference was of 0.87% (Fig. 1).

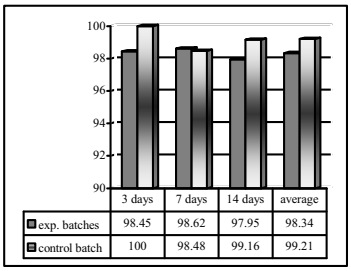

Fig. 1. Viability of hatched chicks according to the period of eggs storage (%)

From the data presented in Fig. 1 can be seen that close to 100% viability of hatched chicks were obtained from all periods of storage of eggs, which leads us to believe that the viability of chicken is not influenced by age but is more about the respect for incubation technology itself.

Optimum efficiency of the chicken is between 67-68%. In most variants presented experimental results were obtained falling within this range. Values greater than 69% were recorded in batches of chickens which came from eggs stored for 14 days (Fig. 2).

The lowest yield was recorded on chicks who came from eggs stored for a week, of 68.45%. Optimum efficiency of the chicken then increased to 68.79% in offspring derived from eggs stored for 3 days and reached up to 69.24% in chickens from two weeks old egg. In all cases it is considered that normal values are achieved. Return to the chicken, the optimum efficiency of the chicken is influenced by the duration of eggs storage. The manufacturer agrees that in studied hybrid, optimum efficiency of chicken increases by 0.5% per week of hatching egg storage, thus leading to the eggs stored for 2 weeks, resulting in optimum efficiency of chickens these eggs to be of 69%.

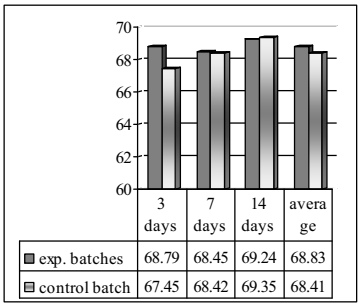

Fig. 2. Optimum efficiency of the chicken depending on the storage period of eggs (%)

Differences between control batches of eggs who had not been preheating and those from the experimental periods who have different levels of preheating, were very small, from 68.83% to 68.41%, being only 0.42%. This shows that optimum efficiency of the chicken performance is influenced in a higher proportion by the age and storage conditions of eggs and their only warm-applied treatment of hatching eggs.

Hatchability. Highest hatchability of incubated eggs from Ross 308 hybrid has been those batches who have been kept for seven days and have suffered treatment for preheating, of 94.11% (Table 5, Fig. 3). Eggs of the same age, but were not preheated, conducted a hatchability percentage of 93.62%, with 0.49% less. Batches stored for 3 days showed good values of hatchability, by 93.59% in experimental batches to 92.47% to the control batch. The lowest values of hatchability percentage were recorded at 14 days old eggs. They had an hatchability of 85.64% on the batches of eggs that were treated by preheating and 82.07% in the control batch. Noteworthy is the fact that hatchability decreased by 5.58% to average on eggs of two weeks old, where was performed preheating and by 7.32% in the control batch.

Hatching percentage. The averages values made by analyzed batches of the hatching percentage was of 87.83% for eggs with preheating treatment and 85.78% to those from the control batch.

Table 5. Hatchability eggs incubated according to their storage period (%)

Preheating program		Storage period (days)			Average
		3	7	14	
4 hours	23ºC	93,75	95,89	82,14	90,59
	25ºC	92,52	91,67	84,29	90,49
	28ºC	94,52	94,56	80,95	90,01
8 hours	23ºC	94,44	94,41	88,36	92,40
	25ºC	91,89	95,14	88,81	91,95
	28ºC	93,10	93,75	85,62	90,82
12 hours	23ºC	93,75	96,60	85,31	91,89
	25ºC	93,06	92,47	86,98	90,84
	28ºC	95,24	92,46	88,28	91,99
Average of experimental batches		93,59	94,11	85,64	91,22
Control batch (3,7 or 14 days storage, no preheating)		92,47	93,62	82,07	89,39

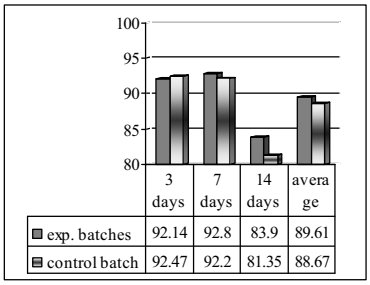

Fig. 3. Hatchability evolution of incubated eggs depending on their storage period (%)

Table 6. The hatching percentage of eggs incubated based on their storage period (%)

Preheating program		Storage period (days)			Average
		3	7	14	
4 hours	23ºC	90,00	93,33	76,67	86,67
	25ºC	90,67	88,00	78,67	85,78
	28ºC	92,00	92,67	79,33	88,00
8 hours	23ºC	90,67	90,00	86,00	88,89
	25ºC	90,67	91,33	79,33	87,11
	28ºC	90,00	90,00	83,33	87,78
12 hours	23ºC	90,00	94,67	81,34	88,67
	25ºC	89,33	90,00	84,67	88,00
	28ºC	93,33	90,00	85,33	89,55
Average of experimental batches		90,74	91,11	81,63	87,83
Control batch (3,7 or 14 days storage, no preheating)		90,00	88,00	79,33	85,78

What is remarkable is that the hatching percentage has the highest values when the eggs were stored for 7 days and then were preheated of 91.11%. When eggs were stored for 3 days and then were preheated, the hatching percentage was of 90.74% at the experimental batches and 90.00% in the control group (Table 6, Fig. 4).

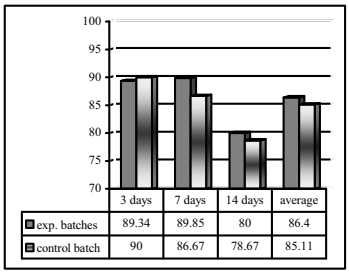

Fig. 4. Evolution of the hatching percentage of eggs incubated based on their storage period (%)

The lowest values of the hatching percentage of eggs were recorded from old ones.

Eggs stored for 14 days have had a hatching rate of 79.33% if was not done preheating and one of 81.63% when this treatment was applied. Both values are lower than the batches averages with 6.20% at the experimental batches and to 6.45% in the control batch.

Table 7. Hatching results of Ross 308 hybrid eggs depending on the studied variables

Storage period	Preheating program		Parameter (%)		
			Viability of hatching chicks	Hatchability	Hatching percentage
3 days	4 hours	23ºC	99.26	93.75	90.00
		25ºC	99.26	92.52	90.67
		28ºC	99.28	94.52	92.00
	8 hours	23ºC	97.06	94.44	90.67
		25ºC	97.79	91.89	90.67
		28ºC	99.26	93.10	90.00
	12 hours	23ºC	97.78	93.75	90.00
		25ºC	97.76	93.06	89.33
		28ºC	98.57	95.24	93.33
7 days	4 hours	23ºC	97.86	95.89	93.33
		25ºC	97.73	91.67	88.00
		28ºC	97.12	94.56	92.67
	8 hours	23ºC	98.52	94.41	90.00
		25ºC	99.27	95.14	91.33
		28ºC	97.78	93.75	90.00
	12 hours	23ºC	100.00	96.60	94.67
		25ºC	100.00	92.47	90.00
		28ºC	99.26	92.46	90.00
14 days	4 hours	23ºC	99.13	82.14	76.67
		25ºC	93.22	84.29	78.67
		28ºC	95.79	80.95	79.33
	8 hours	23ºC	100.00	88.36	86.00
		25ºC	96.64	88.81	79.33
		28ºC	99.20	85.62	83.33
	12 hours	23ºC	100.00	85.31	81.34
		25ºC	99.21	86.98	84.67
		28ºC	98.39	88.28	85.33
Average of experimental batches			98.34	91.22	87.83
Control batch (3,7 or 14 days storage, no preheating)			99.21	89.39	85.78

CONCLUSIONS

Following the researches made until now in terms of duration and effect of storage conditions on hatching results were obtained the following conclusions:

- Weight loss of incubation eggs during their storage period, was the highest in the eggs kept and stored for 14 days and then preheated for 12 hours at 23°C, of 2.46%. Minimum weight loss of eggs, only 0.17% were recorded in the batches of eggs kept and stored for 3 days, which were then heated for 8 hours at 25°C.
- The lowest embryonic mortality in phase I (1 – 8 days) was of 1.34% in the batches of eggs stored for 3 or 7 days, which were then preheated 4 or 12 hours at 23°C or 28°C. The highest value of dead embryos in phase I has been in the batch of eggs stored for 14 days and preheated for 4 hours at 28°C, of 14.67%.
- Embryonic mortality in phase II (9 -15 days) was nonexistent in the batch of eggs stored for 7 days preheated 12 hours at 23°C. Old eggs, those of two weeks old have been the highest rate of dead embryos in phase II when the preheating batch of eggs was made at 25°C for 12 hours and reached a value of 4.67%.
- Embryonic mortality in phase III (16 – 21 days) was null when the eggs were kept and stored for 3 or 7 days and then preheated to 25°C or 28°C for 4 or 8 hours. Maximum values of embryonic mortality was recorded on old eggs, which were stored for 14 days and then preheated to 23°C for 8 hours, of 6%.
- Optimum efficiency of the chicken had values that were located on all batches within the limits set by the manufacturer of the studying hybrid. Longer can see the fact that the temperature of preincubation did not affect the efficiency of the hatched chicken but the egg storage conditions and duration of the preheating treatment.
- Viability of hatched chicks was considered placed in all batches at high values.
- Hatchability of incubated eggs has been maximum when they were stored for 7 days and then preheated at 23°C for a duration of 12 hours, of 96.60% and have been minimum in the batches stored 14 days and heated then for 4 hours at 28°C, 80.35%. Clear difference of 1.83%, from the average of the experimental batches and the control batch shows that the preheating treatment of eggs positively influence their hatchability percentage. Duration of storage also influenced eggs hatchability, those batches who were kept and stored less time have been better results compared with those stored for 14 days.
- The hatching percentage of eggs was elevated if the chickens came from eggs which were stored for 7 days. The best combination of factors has emerged in the group of eggs stored 7 days and then preheated 12 hours at 23°C, of 94.67%. Batches of eggs of two weeks old have made small hatching percentage, under the average of the control and experimental batches. The worse combination of factors has been the in the batches of eggs that were kept and stored for 14 days and then preheated at 23°C for 4 hours. In this case fertility rate was of 76.67%.
- Finally, concludes that the batches of eggs that were stored for 3 or 7 days and then incubated, have made the best values of the indices follow its incubation and hatched the chicken quality. The best choice of combination of duration of storage, duration of preheating and the level of preheating of the eggs to obtain the best results in incubation of Ross 308 hybrid were: storage 7 days, preheating for 8 or 12 hours at 23°C or 25°C.
- Elevated incubation parameters have arisen also for the eggs stored for 3 days with the lowest weight loss, the lowest embryonic mortality with values below the batches average.

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QUALITY EVALUATION OF WELLS WATER FROM TELEORMAN COUNTY

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Abstract

The aim of present paper is quality evaluation of wells water from some operational sites located in Teleorman County. Were taken well water samples from 5 localities situated along Vedea river. Those samples were analyzed for determine nitrate, nitrite, inorganic nitrogen and phosphorus. The samples were taken in two seasons, spring and autumn. We observed that the well water from analyzed areal are vulnerable of pollution with nitrites came from agriculture and husbandry actions and from some surface aquiferes can appear the eutrophication problem. The presence of different forms of nitrites in well water is a major problem for a human health, for children under 6 months old, esspecially.

Key words**:** *pollution, well water.*

INTRODUCTION

The quality of surface water is a parameter which needs continuous and carefully monitoring, because the chemical, physical and biological process from water mass are dynamic, are inside in cycling compartments of nutrients and are most vulnerable compartments to nitrites pollution from agricultural and husbandry fields. The continuous monitoring of surface water is a necessity because their quality influences the quality of fresh and underground water. During the 2004-2007 period, the vulnerable zones to nitrites pollution represented perimeters of 251 localities from 34 counties and 10 hydrographical basins, which means 1.217.147 ha surface and 8.2% from total agriculture surface. [2].

MATERIAL AND METHOD

The samples taken were realised along altitudinal gradient, from west to south, using the quantitative methods, from wells existed in villages. The frequency of samples taken was by season, in spring and autumn, respectively. The conservation and working water samples were made by classical methods.

For determine the $N\text{-}NH_4$ was used the „indophenols blue" method. The method principle consist in reaction of phenol with ammonia in presence of oxidant agent (sodium hypochlorite) and formatting in alkaline conditions a colour compound which absorb the energy with $\lambda = 660$ nm.

For determine the nitrate ion was used a spectrophotometric method with salicylic acid as chromate agent. This method take a long time and is under influences of organic matter and $N\text{-}NO_2$ interferences, for water surface analyzed samples got good results due to small content of them in compound which can give interferences.

The method principle consists of aromatic compounds nitrating in environment with very small pH and measurement to 410 nm of new compound absorbance after NaOH adding. Nitrite ions determine by photocolourimetry.

The small values of reactive phosphorus dissolved in surface water, need choosing an analyze method more sensitive and free of interferences. For this purpose we applied a modified method of Hess and Derr which used malachite green to form a complex with PO_4 ion [4].

The results obtained were compared with maximum allowed concentrations of those ions: 0.4 mg/l for $N\text{-}NH_4$, 11,2 mg/l for $N\text{-}NO_3$, 0,15 mg/l for $N\text{-}NO_2$ and 0,01 mg/l for phosphorus [3].

RESULTS AND DISCUSSIONS

The results of chemical analyze for surface water samples from determine area, are presented in table 1, by season. At the first taken, in spring season, it's observed exceed to maximal limits for nitrates taken from localities situated up the river. This fact happened because those localities haven't sewage system and nutrients, including those came from agricultural and husbandry sources are washed from soil and taken in surface water.

The same trend happened to nitrites. The phosphorus concentration is in normal limits, and ammonia ion has a concentration above allowed limit in 4 from 14 studied localities (situated upstream of the river, too).

Table 1.Average concentration of biogene elements in well water for site 1

Indicator (U.M.)	Well water		
	Up	Middle	Down
N-NH_4 (mg N/l)	0.37	0.36	0.4
N-NO_2 (mg N/l)	0.14	0.0149	0.152
N-NO_3 (mg N/l)	9.7	9.7	9.9
P-PO_4 (mg P/l)	0.00	0.01	0.00

Table 2. Average concentration of biogene elements in well water for site 2

Indicator (U.M.)	Well water		
	Up	Middle	Down
N-NH_4 (mg N/l)	0.6	0.58	0.6
N-NO_2 (mg N/l)	0.189	0.188	0.19
N-NO_3 (mg N/l)	10.8	10.5	10.8
P-PO_4 (mg P/l)	0.02	0.02	0.024

Figure 1 shows that there is an increased ammonium ion concentration in well water, along the altitudinal gradient. This phenomenon is due to further accumulation of the Vedea river to fermentable materials (manure washed off the land and the pastures used for grazing domestic faeces percolating water and infiltrates into the soil, the area is not sewage, fertilizers natural or synthetic without strict rules apply and whose concentration was not monitored), etc.

Table 3. Average concentration of biogene elements in well water for site 3

Indicator (U.M.)	Well water		
	Up	Middle	Down
N-NH_4 (mg N/l)	0.87	0.95	1.02
N-NO_2 (mg N/l)	0.144	0.151	0.157
N-NO_3 (mg N/l)	10.0	10.5	10.8
P-PO_4 (mg P/l)	0.021	0.025	0.025

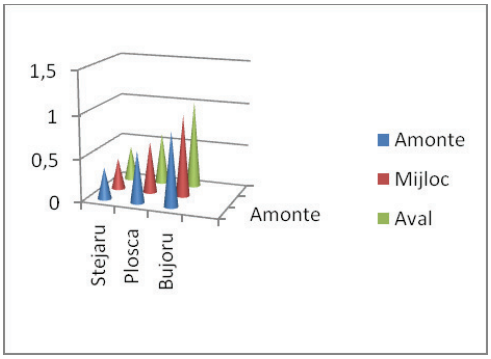

Fig. 1. Dynamic of N-NH_4 concentration in well water alonside of altitudinal gradient

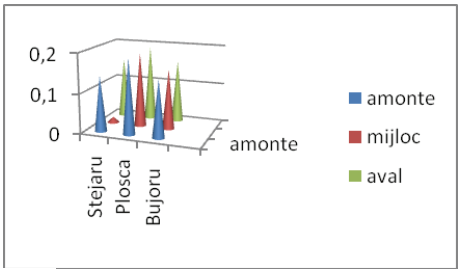

Fig. 2. Dynamic of N-NO_2 concentration in well water alonside of altitudinal gradient

Note that the highest concentration of nitrates in the water fountain located in the village flasks, probably because this area is currently and historically vegetable in primary and secondary livestock. This requires an intense fertilization of the soil throughout the year there is a supply of nutrients that migrate from the soil and surface water to groundwater. Nitrates - concomitant presence of ammonia and nitrates show a continuous pollution and inadequate disinfection. Small amounts of nitrates can be found in almost all waters. Nitrates comes of these sources of pollution: fertilizer used in large amounts in intensive agriculture,

pesticides with nitrogen fertilizer storage sites damaged or built too close to private wells. Even if an additional quantity of nitrate does not reach the earth will pass much time until the nitrate content of existing water will degrade. In surface water nitrate concentrations can range from 0 to 8 mg / l and in highly polluted waters can reach up to 50-150 mg / l or more. In 1988, 36% of wells in Romania had nitrate concentrations above 45 mg / l.

CONCLUSIONS

The nitrites presence in surface and well water asociated with nitrates and ammonia ion presences shown as an impurity of water with organic matters and a long presence of pollution process, because transformations of the organic matters in nitrites take long time (weeks) [1].

The Teleorman county area is predominance cereal, exist a great number of households where are exploited animals, without respect the minimal standards of surface water protection against pollution with nitrates and nitrites came from agricultural and husbandry sources. Those facts leads to nitrites appearance in surface water, infiltrations in water table and them consumption by peoples and animals; existing the risk to appearance “the blue disease” at small babies (0-6 months), oesophagus or stomach cancer, etc., at adult peoples [5].

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STUDY ON THE HOURLY FREQUENCY OF EXPLORING AND SOCIAL BEHAVIORAL MANIFESTATIONS IN DOGS HOUSED IN A SPECIALIZED SHELTER IN RELATIONSHIP WITH THE SEX PROPORTION AND ANIMALS AGE IN THE GROUP

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Abstract

The purpose of present investigations was to determine the hourly frequency of paddock exploring and social behavioral manifestations in dogs housed in a specialized shelter in relationship with the male-female proportion and animals mean age in the group, to improve the latter structure. Findings indicate that a lesser male number and a lower female mean age in the group significantly influence female paddock exploring behavior, in that where more females and fewer males were accommodated showed the highest hourly frequency of female paddock exploring behavioral manifestations (with an hourly average of 1.58 ± 0.19 of behavioral manifestations). Hourly frequencies of play and aggressive social behavioral manifestations of females were also affected, recording a female play behavior higher in the case of keeping them with fewer males, but also for a lower female mean age in the group (with an hourly average of 0.98 ± 0.12 behavioral manifestations). Also, a higher male number and a lesser female number in the group increase the hourly frequency of female aggressive behavioral manifestations towards others (with an hourly average of 1.10 ± 0.10 of behavioral manifestations). Also, it has been registered a higher hourly frequency of female food theft in groups where the male number was lower than female number, correlated with lower female mean age. Regarding the influence of female number on male paddock exploring and social behavioral manifestations, it is obvious that a higher female number in the group, also correlated with a higher male mean age, reduces male aggressive behavioral manifestations towards themselves but increases the hourly frequency of male aggressive behavioral manifestations towards others (with an hourly average of 1.50 ± 0.24 of behavioral manifestations).

Key words**:** *age, behavior, dogs, sex ratio.*

INTRODUCTION

The domestic dog, *Canis familiaris*, is considered one of the first domesticated species (Thorne, 1992). In general, when there is possible dogs live in groups of 2 ÷ 6 individuals (Boitani et al., 1995) and usually have a male in the top of the hierarchy.

The role of dogs in today human society is complex. S.C. Tătărucă (2011) from the Sibiu Canine Center affirms that dog is the only animal with special skills to protect humans, to find, identify and search for various objects or substances that are illegally consumed and sold (drugs) or different explosives and other odors emanating from different bodies (such as corpses). The same author states that dog can be used to search and identify criminal law breakers, to save people, to prevent potential attacks and to discourage them.

Animal protection strategy in our country, involving matters of preparation, planning and proper implementation must start from thorough knowledge of animal welfare requirements, based on correct scientific study of behavior in different species to whom this refers. One of the species utmostly found in the attention of public opinion regarding necessity and opportunity for protection is represented by dogs (Elena Popescu-Micloşanu, Carmena Şerbănoiu, 2009).

Setup and running a dog shelter must take into account several aspects, namely: health, age, sex and aggressiveness level [2]. Design, construction and use of specialized shelters also assume knowledge of diverse behavioral aspects of dog biology (feeding, drinking, urinating, defecating, resting in different postures (standing, sitting, lying, squatting, etc.), the manner how social hierarchy is established in these shelters (aggression and affection behavior) and which are the various aspects of abnormal behavior of these dogs (walking in circles around the paddock, turning

around, different stereotypes). Also, these shelters must allow animals observation and health determining, but also easily perform of various veterinary treatments (Carmena Şerbănoiu, 2009).

MATERIAL AND METHOD

The study was conducted in a private shelter for stray dogs belonging to the Association Pro Animals in Târgu-Jiu, on a total of 15 dogs, 6 males and 9 females, housed in three different groups, as follows: *Group a* (2 M + 3 F) in paddock no. 7, *Group b* (3 M + 2 F) in paddock no. 11 and *Group c* (1 M + 4 F) in paddock no. 23.

Females mean age (Fma) in *Group a* was 9.7 years, in *Group b* was 4.5 years and in *Group c* was 4.8 years.

Males mean age (Mma) in *Group a* was 9.5 years, in *Group b* was 6.5 years and the single male in *Group c* was 13 years old.

Animals were observed 10 days, 10 hours a day in the time interval 8:00 ÷ 18:00. The amount of behavioral manifestations for each dog was recorded at every hour. The hourly observations were classified in 3 types, namely:

1 - activity behavioral manifestations (feeding, drinking, defecation, urination, cleaning their own fur, scratching, exploring paddock and social behavior as play, aggression, fear, affection and theft of food),

2 - sleep behavioral manifestations (standing up, sitting back, lying down on abdomen and on one side and squat) and

3 - abnormal behavioral manifestations (walking around inside the paddock, turning around in circle and various stereotypes).

Data were processed using Microsoft Excell 2003, and the significance of differences between the averages of 3 groups of behavioral manifestations was *t*-tested using Student test.

RESULTS AND DISCUSSIONS

1. Hourly frequency of female paddock exploring and social behavioral manifestations in relationship with the male-female proportion and females mean age in the group

1.1 Hourly frequency of female paddock exploring behavior in relationship with the male-female proportion and females mean age in the group

In paddock no. 23, where a group of 1 M + 4 F with an adult Fma of 4.8 years was housed, females showed the highest hourly frequency of paddock exploring events (with an hourly average of 1.58 ± 0.19 behavioral manifestations), compared with females of paddock no. 11 (with an hourly average of 1.12 ± 0.14 behavioral manifestations)

Table 1. Hourly frequency and daily average total number of female behavioral manifestations in the 3 groups (paddocks) during the studied period

Specification	*Group a* Paddock no. 7 (2 M + 3 F)		*Group b* Paddock no. 11 (3 M + 2 F)		*Group c* Paddock no. 23 (1 M + 4 F)	
	Daily observation time interval 8:00 ÷ 18:00					
	X ± s ^{X}	Total	X ± s ^{X}	Total	X ± s ^{X}	Total
Paddock exploring	0.95 ± 0.16 $^{ans\ c}$	9.5	1.12 ± 0.14 $^{ans\ bns}$	11.2	1.58 ± 0.19 $^{bns\ c}$	15.8
Social behavior						
Play	0.6 ± 0.06 $^{ans\ c}$	6	0.66 ± 0.06 $^{ans\ b}$	6.6	0.98 ± 0.12 $^{b\ c}$	9.8
Aggression						
- towards itself	0.6 ± 0.13 $^{a\ c}$	6	0.68 ± 0.05 $^{a\ b}$	6.8	0.96 ± 0.13 $^{b\ c}$	9.6
- towards others	0.15 ± 0.17 $^{a\ c}$	1.5	1.1 ± 0.10 $^{a\ b}$	1.1	0.55 ± 0.08 $^{b\ c}$	5.5
-towards the environment	0	0	0.75 ± 0.10 ^{b}	7.5	0.4 ± 0.08 ^{b}	4
Fear	0.48 ± 0.13 $^{ans\ c}$	4.8	0.53 ± 0.10 $^{ans\ b}$	5.3	0.74 ± 0.12 $^{b\ c}$	7.4
Affection						
- muzzle contact	1.1 ± 0.12 $^{ans\ cns}$	11	0.9 ± 0.08 $^{ans\ bns}$	9	1.1 ± 0.12 $^{bns\ cns}$	11
- congener fur grooming	0.43 ± 0.09 $^{ans\ cns}$	4.3	0.65 ± 0.15 $^{ans\ bns}$	6.5	0.48 ± 0.06 $^{bns\ cns}$	4.8
Food theft	0.07 ± 0.06 $^{ans\ c}$	0.7	0.17 ± 0.10 $^{ans\ bns}$	1.7	0.24 ± 0.15 $^{bns\ c}$	2.4

Note: significant difference between averages are accompanied by the same letter and the insignificant ones by the notation "ns"

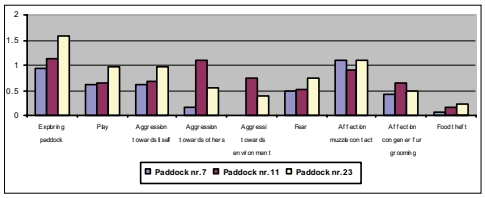

Fig. 1: Hourly frequency of female paddock exploring and social behavioral manifestations in the 3 groups (paddocks) during the studied period

where a group of 3 M + 2 F with similar adult Fma of 4.5 years was housed, and females of paddock no. 7 (with an hourly average of 0.95 ± 0.16 behavioral manifestations), where a

group of 2 M + 3 F with the oldest Fma of 9.7 years was housed.
The difference is significant only between the hourly averages of behavioral manifestations of females of paddocks no. 23 and no. 7.

1.2 Hourly frequency of female social behavior in relationship with the male-female proportion and females mean age in the group

1.2.1 Hourly frequency of female play behavior in relationship with the male-female proportion and the females mean age in the group

In paddock no. 23, where a group of 1 M + 4 F with an adult Fma of 4.8 years was housed, females showed the highest frequency of play events (with an hourly average of 0.98 ± 0.12 behavioral manifestations), compared with females of paddock no. 11 (with an hourly average of 0.66 ± 0.06 behavioral manifestations), where a group of 3 M + 2 F with similar adult Fma of 4.5 years was housed, and females of paddock no. 7 (with an hourly average of 0.60 ± 0.06 behavioral manifestations), where a group of 2 M + 3 F with the oldest Fma of 9.7 years was housed.
There are significant differences only between the hourly averages of behavioral manifestations of females of paddock no. 23 and females of paddocks no. 7 and no. 11.

1.3 Hourly frequency of female aggressive behavior in relationship with male-female proportion and females mean age in the group

1.3.1 Hourly frequency of female aggressive behavior towards itself in relationship with the male-female proportion and females mean age in the group

In paddock no. 23, where a group of 1 M + 4 F with an adult Fma of 4.8 years was housed, females showed the highest hourly frequency of behavioral manifestations of aggression towards themselves (with an hourly average of 0.96 ± 0.13 behavioral manifestations), compared with females of paddock no. 11 (with an hourly average of 0.68 ± 0.05 behavioral manifestations), where a group of 3 M + 2 F with similar adult Fma of 4.5 years was housed, and females of paddock no. 7 (with an hourly average of 0.60 ± 0.13 behavioral manifestations), where a group of 2 M + 3 F with the oldest Fma of 9.7 years was housed.
There are significant differences between the hourly averages of behavioral manifestations of the females in all 3 groups.

1.3.2 Hourly frequency of female aggressive behavior towards others in relationship with male-female proportion and females mean age in the group

In the paddock no. 11, where a group of 3 M + 2 F with an adult Fma of 4.5 years was housed, females showed the highest hourly frequency of aggression towards others (with an hourly average of 1.10 ± 0.10 behavioral manifestations), compared with females of paddock no. 23 (with an hourly average of 0.55 ± 0.08 behavioral manifestations), where a group of 1 M + 4 F with an adult Fma of 4.8 years was housed, and females of paddock no. 7 (with an hourly average of 0.15 ± 0.10 behavioral manifestations), where a group of 2 M + 3 F with the oldest Fma of 9.7 years was housed.
There are significant differences between the hourly averages of behavioral manifestations of the females in all 3 groups.

1.3.3 Hourly frequency of female aggressive behavior towards environment in relationship with male-female proportion and females mean age in the group

Females of paddock no. 7, where a group of 2 M + 3 F with the oldest Fma of 9.7 years was housed, did not show aggressive behavior towards the environment, while females of paddock no. 11, where a group of 3 M + 2 F with an adult Fma of 4.5 years was housed, showed the highest hourly frequency of aggressive events towards the environment (with an hourly average of 0.75 ± 0.10 behavioral manifestations). Females in paddock no. 23, where a group of 1 M + 4 F with an adult Fma of 4.8 years was housed, recorded the lowest hourly frequency of aggressive events towards the environment (with an hourly average of 0.40 ± 0.08 behavioral manifestations).
There is a significant difference only between the hourly averages of behavioral manifestations of females of paddocks no. 11 and no. 23.

1.4 Hourly frequency of female fear behavior in relationship with the male-female proportion and the females mean age in the group

In the paddock no. 23, where a group of 1 M + 4 F with an adult Fma of 4.8 years was housed, females showed the highest hourly frequency of fear events (with an hourly average of 0.74 ± 0.12 behavioral manifestations), compared with females of paddock no. 11 (with an hourly average of 0.53 ± 0.10 behavioral manifestations), where a group of 3 M + 2 F with similar adult Fma of 4.5 years was housed, and females of paddock no. 7 (with an hourly average of 0.48 ± 0.13 behavioral manifestations), where a group of 2 M + 3 F with the oldest Fma of 9.7 years was housed.
There are significant differences between the hourly averages of behavioral manifestations of females of paddock no. 23 and females of paddocks no. 7 and no. 11.

1.5 Hourly frequency of female affection behavior in relationship with the male-female proportion and the females mean age in the group

1.5.1 Hourly frequency of female affection behavior by muzzle contact in relationship with the male-female proportion and the females mean age in the group

Females of all 3 groups showed approximately the same hourly frequency of affection behavior by muzzle contact (1.10 ± 0.12 the females of paddock no. 7, 0.90 ± 0.08 the females of paddock no. 11 and 1.10 ± 0.12 the females of paddock no. 23).

There are no significant differences between the hourly averages of behavioral manifestations of females in all 3 groups.

1.5.2 Hourly frequency of female affection behavior by congener fur grooming in relationship with male-female proportion and females mean age in the group

Females of all 3 groups showed approximately the same hourly frequency of affection behavior by cleaning fur congeners (0.43 ± 0.10 the females of paddock no. 7, 0.65 ± 0.15 the females of paddock no. 11 and 0.48 ± 0.06 the females of paddock no.23).

There are no significant differences between the hourly averages of behavioral manifestations of females in all 3 groups.

1.6 Hourly frequency of female food theft behavior in relationship with the male-female proportion and the females mean age in the group

In the paddock no. 23, where a group of 1 M + 4 F with an adult Fma of 4.8 years was housed, females showed the highest hourly frequency of food theft events (with an hourly average of 0.24 ± 0.15 behavioral manifestations), compared with females of paddock no. 11 (with an hourly average of 0.17 ± 0.1 behavioral manifestations), where a group of 3 M + 2 F with similar adult Fma of 4.5 years was housed, and females of paddock no. 7 (with an hourly average of 0.07 ± 0.06 behavioral manifestations), where a group of 2 M + 3 F with the oldest Fma of 9.7 years was housed.

There is a significant difference only between the hourly averages of behavioral manifestations of females of paddocks no. 23 and no. 7.

2. Hourly frequency of male paddock exploring and social behavioral manifestations in relationship with the male-female proportion and the males mean age in the group

2.1 Hourly frequency of male paddock exploring behavior in relationship with the male-female proportion and males mean age in the group

The male paddock exploring behavior was affected by the male-female proportion in the group. The frequency of paddock exploring events recorded in males from paddocks no. 7 was of 0.94 ± 0.22 and 1.16 ± 0.14 in paddock no. 11). The single male in paddock no. 23 showed the highest frequency of paddock exploring events (1.43 ± 0.19), than males in other 2 paddocks.

There are significant differences between the hourly averages of behavioral manifestations of the males in all 3 groups.

2.2 Hourly frequency of male social behavior in relationship with the male-female proportion and males mean age in the group

2.2.1 Hourly frequency of male play behavior in relationship with the male-female proportion and the males mean age in the group

The highest hourly frequency of male play events (0.71 ± 0.22) occurred in males from paddock no. 7, where a group of 2 M + 3 F with the oldest Mma of 9.5 years was housed, compared with males in the paddock no. 11 (0.37 ± 0.08), where a group of 3 M + 2 F with an adult Mma of 6.5 years was housed. The single, 13 years old dog in paddock no. 23 showed no play event.

The difference is significant only between the hourly averages of behavioral manifestations of males of paddocks no. 7 and no. 11.

Table 2. Hourly frequency and daily average total number of male behavioral manifestations in the 3 groups (paddocks) during the studied period

Specification	*Group a* Paddock no. 7 (2 M + 3 F)		*Group b* Paddock no. 11 (3 M + 2 F)		*Group c* Padock no. 23 (1 M + 4 F)	
	Daily observation time interval 8:00 ÷ 18:00					
	X ± s ^{X}	Total	X ± s ^{X}	Total	X ± s ^{X}	Total
Paddock exploring	0.94 ± 0.22 $^{a\ c}$	9.4	1.16 ± 0.14 $^{a\ b}$	11.6	1.43 ± 0.19 $^{b\ c}$	14.3
Social behavior						
Play	0.71 ± 0.22 ^{a}	7.1	0.37 ± 0.08 ^{a}	3.7	0	0
Aggression						
- towards itself	0.55 ± 0.13 $^{ans\ c}$	5.5	0.4 ± 0.05 $^{ans\ cbns}$	4	0.2 ± 0.13 $^{bns\ c}$	2
- towards others	0.78 ± 0.24 $^{a\ c}$	7.8	1 ± 0.08 $^{a\ b}$	1	1.5 ± 0.24 $^{b\ c}$	15
-towards the environment	0.05 $^{ans\ cns}$	0.5	0.35 ± 0.10 $^{ans\ bns}$	3.5	0.5 ± 0.04 $^{bns\ cns}$	5
Fear	0	0	0.25 ± 0.03	2.5	0	0
Affection						

- muzzle contact	1.3 ± 0.1 *ans cns*	13	0.95 ± 0.10 *ans bns*	9.5	1.1 ± 0.12 *bns cns*	11
- congener fur grooming	0.49 ± 0.15 *ans cns*	4.9	0.45 ± 0.11 *ans bns*	4.5	0.3 ± 0.06 *bns cns*	3
Food theft	0.48 ± 0.16 *ans cns*	4.8	0.23 ± 0.10 *ans bns*	2.3	0.3 ± 0.07 *bns cns*	3

Note: significant differences between averages are accompanied by the same letter and the insignificant ones by the notation "ns"

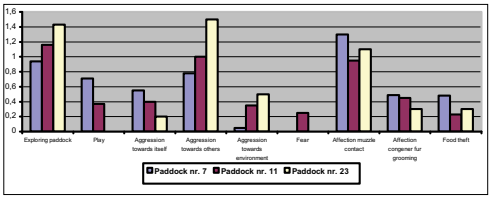

Fig. 2: Hourly frequency of male paddock exploring and social behavioral manifestations in the 3 groups (paddocks) during the studied period

2.3 Hourly frequency of male aggressive behavior in relationship with male-female proportion and males mean age in the group

2.3.1 Hourly frequency of male aggressive behavior towards itself in relationship with the male-female proportion and males mean age in the group

Males in paddock no. 7, where a group of 2 M + 3 F with the oldest Mma of 9.5 years was housed, showed the highest hourly frequency of behavioral manifestations of aggression towards themselves (0.55 ± 0.15) than males in paddock no. 11 (0.40 ± 0.05) with an adult Mma of 6.5 years and the single 13 years old male in the paddock no. 23 (0.20 ± 0.13).

There is a significant difference only between the hourly averages of behavioral manifestations of males of paddocks no. 7 and no. 23.

2.3.2 Hourly frequency of male aggressive behavior towards others in relationship with male-female proportion and males mean age in the group

The single, 13 years old male in paddock no. 23, housed with 4 F, showed the highest hourly frequency of aggressive behavior towards others (1.50 ± 0.24) than males in paddock no. 11 (1.00 ± 0.08) with a Mma of 6.5 years and housed with 2 F and males in paddock no. 7 (0.7 ± 0.24), with a Mma of 9.5 years and housed with 3 F.

There are significant differences between the hourly averages of behavioral manifestations of the males in all 3 groups.

2.3.3 Hourly frequency of male aggressive behavior towards environment in relationship with male-female proportion and males mean age in the group

The single, 13 years old male in paddock no. 23, housed with 4 F, showed the highest hourly frequency of aggressive behavior towards environment (0.5 ± 0.04) than males in paddock no. 11 (0.35 ± 0.10) with a Mma of 6.5 years and housed with 3 F and males in paddock no. 7 (0.05), with a Mma of 9.5 years.

There are no significant differences between the hourly averages of behavioral manifestations of the males in all 3 groups.

2.4 Hourly frequency of male fear behavior in relationship with the male-female proportion and the males mean age in the group

Male fear was manifested only in the paddock no. 11 (2.5 daily average manifestations) where there was the highest number of males (3 M) with an Mma of 6.5 years. In other 2 paddocks, with less number of males then females, males did not expressed fear.

2.5 Hourly frequency of male affection behavior in relationship with the male-female proportion and the males mean age in the group

2.5.1 Hourly frequency of male affection behavior by muzzle contact in relationship with the male-female proportion and the males mean age in the group

Males of the 3 groups showed approximately the same hourly frequency of affection behavior by muzzle contact (1.30 ± 0.10 paddock no. 7, 0.95 ± 0.10 paddock no. 11 and 1.10 ± 0.12 paddock no. 23).

There are no significant differences between the hourly averages of behavioral manifestations of the males in all 3 groups.

2.5.2 Hourly frequency of male affection behavior by congener fur grooming in relationship with male-female proportion and males mean age in the group

Males of the 3 groups showed approximately the same hourly frequency of affection behavior by congener fur grooming (0.49 ± 0.15 paddock no. 7, 0.45 ± 0.11 paddock no. 11 and 0.30 ± 0.06 paddock no. 23).

There are no significant differences between the hourly averages of behavioral manifestations of the males in all 3 groups.

2.6 Hourly frequency of male food theft behavior in relationship with the male-female proportion and the males mean age in the group

Male food theft was manifested in all 3 groups but there are no significant differences between the hourly averages of behavioral manifestations of males in all 3 groups.

CONCLUSIONS

A lesser number of males housed with females in the group favored an increased frequency of paddock exploring behavior manifestations in females (paddock no. 23, 1M + 4F). Regarding the mean age of females housed in the 3 paddocks, findings show that paddock exploring behavior manifestations in younger females (4.5 ÷ 4.8 years old) occurred at a higher frequency comparatively to the older ones (9.7 years old).

Play behavior manifestations frequency in females was higher both in the case of females housed with a lesser number of males (paddock no. 23 with 1M + 4F) and in the case of a lower mean age of females (4.8 years old, paddock no. 23).

A lesser number of males housed with females in group, and a lower female mean age led to a higher frequency of male aggressive manifestations towards themselves (paddock no. 23, 1M + 4F, 4.8 years mean age of females).

In paddocks where male number was relatively lesser than that of females findings show a higher frequency of food theft events, also correlated with lower female mean age (paddock no. 23).

The frequency of paddock exploring, affection and food theft events was not significant affected neither by the male-female proportion nor by the male mean age in the 3 groups (paddocks).

A higher female number housed with males in the group, correlated with an older male mean age led also to a reduced frequency of female aggressive events towards others and to an increased frequency of male aggressive events towards others (paddock no. 23).

A lower number of males housed with females in the group favoured a higher frequency of behavior manifestations of paddock exploring, play, aggression of females towards themselves, fear and food theft of females and a higher frequency of behavior manifestations of paddock exploring and aggression towards other of males. No influence has been reported on the affection behavior neither of males nor of females and on fear and food theft behavior manifestations of males.

For an exact establishment of the relatedness of social behavior manifestations in dogs housed in specialized shelters and their underlying causes, in order to ensure them a better boarding, it is necessary to fulfil further studies in this respect.

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COMPARATIVE STUDY ABOUT PRODUCTION AND SLAUGHTERING PERFORMANCES IN AN INDUSTRIAL COMPANY WITH ROSS 308 STANDARD CHICKEN HYBRID

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Abstract

The aim of this paper is to study production and slaughtering efficiency of a poultry flock up to 50 days of age by using technological indexes compared to a standard flock of the same age. Two groups of 50 birds each (both males and females) were randomly taken from production house in this purpose. Parameters followed were: live weight, carcass weight, slaughtering output, and also cut and of-boned pieces of significant weight when marketed as well defined products (breast and legs). Statistical data processing methods were used to process data from in slaughterhouse: media, media error and variability coefficient. During next phase a comparative analyze between data from standard and experimental group (Student test) and finding of phenotypic correlations between body weight and cut parts at both sexes were used. Obtained results led to conclusion that there are significant differences of average production performances between experimental and standard group and these differences are in favor of standard group. Slaughtering outputs have shown differences from standard.

Key words**:** *Ross308 hybrid, slaughtering, statistical calculation*

INTRODUCTION

Superior production and marketing of poultry meat is influenced by many different factors. Most important influence factors are the endogen factors (genetic potential of the biologic material used) and also some exogenous (technological) factors. Poultry meat production effectiveness is influenced by many factors such as live weight and carcass quality. Live weight is not limited by sex and it has direct influence on quantitative meat production. Main objective is best economical and biological efficiency of production process through a combination of factors involved in this process and poultry resources. Carcass quality assessment involves some stages as following: measuring or recording live animal conformation and finding slaughtering output yield, carcass exterior aspect assessment, determining percentage of different carcass parts and meat/bones outputs [3].

Poultry carcass cutting is performed differently, based on target market. The following pieces are generally cut: breast (with and without bones and with and without skin), superior legs, inferior legs, wings, backs, drumsticks. Carcass structure in 1- 1.5 kg broilers is generally as following: breast 20-25%, legs 32-35 %, back 30-32 %, wings 13-15 % from carcass weight and representing 75-80 % from live weight. [1,5].

Improvement companies are always at the start of production line and they are massively influencing the nutritional and sensorial attributes of poultry meat, including meat/bones share, breast percentage and fat percentage [4].

As genetics has an important role inside poultry meat production line, we are about to study ROSS 308 hybrid, one of the most productive and widespread hybrids both worldwide and in our country. Experimental results were compared with hybrid's standard results.

MATERIAL AND METHOD

Two groups of 50 birds (males and females) were formed for the experiment. Birds were randomly chosen from the same house of the production farm to fulfill an essential criterion namely an identical production environment. House flock was delivered to slaughterhouse al 50 days of age at 9% mortality and an average delivery weight of 2400 g.

At the slaughterhouse each experimental bird was individualized and weighted on an electronic weighing machine before hanging on the cutting conveyer and each individual value

was recorded. After slaughtering three were also registered: carcass weight, cuts (breast, legs) weight, and de-boned meat (legs and breast) weight. Experimental data were statistically processed and arithmetical media, media error and variability coefficient of carcass, cuts and de-boned pieces were calculated: [4]. Next experiment phases were as following: slaughtering output was calculated for each flock, comparative analyzes between standard hybrid and experimental group data (test Student), and finding phenotypic correlations between body weight and cuts weight in the two sexes.

RESULTS AND DISCUSSIONS

Live weight and carcass parts weight in males and female are shown in table 1.

Broiler live body weight performances in the experiment are below standard hybrid's performances at same age and testing age significance with Student test is revealing that difference between the two groups (experimental and standard), of 16.2 % in males and 16.9 % in females is statistically assured and very significant. Weight difference between sexes is similar in both groups with 16.5 % and 15.8 % in experimental and standard group respectively. Birds uniformity in experimental group is amazing with a variability coefficient of 8.20 and 7.84 in males and females respectively (table 2).

Slaughtering yield during the experiment (73.36 % and 73.32 % in males and females respectively) had values close to standard of 73.71 % and 72.19 % in males and females respectively with no difference by sex.

Test Student was used again to see if there are significant differences between cutting areas of legs and breast in standard and experimental group. Very significant differences between samples were found (tables 3 and 4).

Cut breast weight was bigger than standard in both sexes (848 ± 0,011 g and 694±0,010 in experimental group in males g and females respectively), with 14.6% bigger and 12.6% bigger in males and females respectively. Testing difference between the two groups is revealing a very significant difference in favor of standard group. Experimental group was less uniform than standard group with a variability coefficient of 9.28% and 11.20% in males and females respectively compared to 8% in both sexes in standard group.

In experimental group legs with bones had a smaller weight with 150 g and 81 g in males and females respectively compared to 849±0.010 g and 638±0.007 in standard males and females respectively. Tests are revealing a very significant difference between the two studied groups in favor of standard group.

Cuts weight reported to la live weight were 27.83 % and 27.28 % for breast weight in males and females respectively and legs weight 22.94 % and 21.89 % in males and females respectively. Compared to average data of 16 % for breast percentage and 25 % for legs percentage reported by other authors [5], breast percentage is 11-12 % bigger and percentage of legs which are in a smaller demand from consumers is 2-35 % smaller.

The same procedure as that for cuts was followed for de-boned meat. Field data from legs and breast de-boning are shown in table 5.

Average meat percentage of breast is 22.64 % from live weight and 21.89 % from live weight in males and females respectively. Meat percentage of legs is 17.72 % from live weight and 16.7 % in males in females. Student test was used again to verify if there are significant differences between experimental and standard group.

Boneless breast weight (table 7) obtained during the experiment was 4.75 % smaller than standard in males and almost equal with standard in females. Testing difference significance is revealing that there are no significant differences between the two studied groups in none of the two sexes. Experimental female group had very low uniformity with variability coefficient of 12.74% and experimental male group had a variability coefficient of 9.28%.

Boneless legs weight was 7.22% below standard or 540±0.007 g and 13.3% below standard or 425 ±0.006 g in males and females respectively compared to 582 ±0.006 g in males and 490 ±0.005 g in females respectively in standard group. Testing differences is revealing a significant difference in males and a distinct significant difference in females in favor of standard group.

Next point of the trial was phonotypical correlation between body weight and cuts in males and females. Phonotypical correlations between live weight and cuts in males (table 8), is showing a positive correlation (r = + 0.175 ± 0.117) between live weight and breast weight. Correlation between live weight and cuts is negative.

Phonotypical correlation between carcass weight and cuts in males (table 9), is showing a slightly negative but close to zero correlation in males (r = -0.004 ± 0.142). Excepting the slightly positive but close to zero correlation between carcass weight and legs weight in males (r = + 0.029 ± 0.138), all the other correlations between carcass weight and cuts are negative.

There is also a positive correlation (r = + 0.244 ± 0.108) between live weight and breast weight in females (table 10). Correlation between live weight and cuts is also negative in females.

Phonotypical correlation between carcass weight and cuts is showing a positive correlation between carcass weight and breast weight (r = + 0,310 ± 0,098) in females (table 11). All the other correlations between carcass weight and cuts are negative.

Positive correlation between live weight and breast weight is showing that when live weight has been increasing breast weight has been increasing as well and this is a justification for efforts of hybrid's producer company to improve breast percentage from carcass.

Table 1. Live weight and weight of carcass parts (experimental group) in kg

Specification	Live weight	Breast	Legs	Wings	Back + neck	Carcass
Males	3.047	0.848	0.609	0.246	0.537	2.33
Females	2.554	0.694	0.557	0.208	0.474	1.938

Table 2. Comparative analyses for live weight at 50 days of age

Specification	Males		Females	
	Average + error (g)	Variability coefficient (%)	Average + error (g)	Variability coefficient (%)
Standard	3634±0.028	8.00	3061±0.021	8.00
Experiment	3047±0.035	8.20	2544±0.028	7.84
Student Test (t)	10.8		11.5	

[1] $t > t_\alpha$, for $P<0.001$, differences are very significant

Table 3. Comparative analyses for breast with bones, at 50 days of age

Specification	Males		Females	
	Average + error (g)	Variability coefficient (%)	Average + error (g)	Variability coefficient (%)
Standard	724±0.008	8.00	606±0.006	8.00
Experiment	848±0.011	9.28	694±0.010	11.20
Student Test (t)	1.15		7.5	

[1]In males, $t < t_\alpha$, at the level of $P < 0.05$, differences are not significant

[2]In females, $t > t_\alpha$, at the level of $P< 0.001$, differences are very significant

Table 4. Comparative analyses for bone-in legs at 50 days of age.

Specification	Males		Females	
	Average + error (g)	Variability coefficient (%)	Average + error (g)	Variability coefficient (%)
Standard	849±0.010	8.00	638±0.007	8.00
Experiment	699±0.009	8.97	557±0.007	8.90
Student Test (t)	11.5		8.1	

[1]In males, $t > t\alpha$ is showing that differences are very significant

[2]In females, $t > t\alpha$ at the level of $P<0.001$, differences are very significant

Table 5. De-boned meat weight (experiment) (kg/%)

Males				Females			
Live weight	Breast (Gr./ %)	Legs (Gr. / %)	Carcass (Gr. / %)	Live weight	Breast (Gr. / %)	Legs (Gr. / %)	Carcass (Gr / %)
3.047 100	0.690 22.64	0.540 17.72	1.230 40.36	2.544 100	0.557 21.89	0.425 16.70	0.982 38.60

Table 6. Comparative analyse for de-boned breast weight at 50 days of age

Males			Females		
	Average + error (g)	Variability coefficient (%)		Average + error (g)	Variability coefficient (%)
Standard	724±0.008	8.00	Standard	555±0.006	8.00
Experiment	690±0.011	9.28	Experiment	557±0.010	12.74
Student Test (t) = 2.7			Student Test (t) = 0.169		

[1]In both males and female differences are not significant

Table7. Comparative analyse for de-boned leg weight at 50 days of age

Males			Females		
	Average + error (g)	Variability coefficient (%)		Average + error (g)	Variability coefficient (%)
Standard	582±0.006	8.00	Standard	490±0.005	8.00
Experiment	540±0.007	9.78	Experiment	425±0.006	10.11
Student Test (t) = 4.2			Student Test (t) = 7.7		

[1]In males differences are significant and in female they are distinctly significant

Table 8. Phonotypical correlations between live weight and cuts weight in males

LIVE WEIGHT	x Wings weight	- 0.131 ± 0.124
	x Legs weight	- 0.082 ± 0.131
	x Breast weight	+ 0.175 ± 0.117
	x Back + Neck weight	- 0.103 ± 0.128

Table 9**.** Phonotypical correlations between carcass weight and cuts weight in males

CARCASS WEIGHT	x Wings weight	- 0.183 ± 0.116
	x Legs weight	+ 0.029 ± 0.138
	x Breast weight	- 0.004 ± 0.142
	x Back + Neck weight	- 0.001 ± 0.142

Table 10. Phonotypical correlations between live weight and cuts weight in females

LIVE WEIGHT	x Wings weight	- 0.398 ± 0.086
	x Legs weight	- 0.184 ± 0.116
	x Breast weight	+ 0.244 ± 0.108
	x Back + Neck weight	- 0.091 ± 0.129

Table 11. Phonotypical correlations between carcass weight and cuts weight in females

CARCASS WEIGHT	x Wings weight	- 0.447 ± 0.079
	x Legs weight	- 0.245 ± 0.107
	x Breast weight	+ 0.310 ± 0.098
	x Back + Neck weight	- 0.129 ± 0.124

CONCLUSIONS

During experiment average broiler production performances were below hybrid's standard performances at same age (50 days of age) of 3047 ± 0.035 g and 2544 ± 0.028 g in males and females respectively. Testing significance of differences with Student test is re3vealing that there are very significant differences between the two groups (standard and experimental) with 16.2 % and 16.9 % in males and females respectively. Weight difference between sexes is similar in both groups with 16.5 % and of 15.8 % in experimental and standard group respectively. There is a remarkable uniformity of experimental groups with a variability coefficient of 8.2% and 7.84% in males and females respectively. Difference of live weight between sexes is similar in both groups with 16.5 % and 15.8 % in experimental and standard group respectively.

Slaughtering output during trial was close to standard figure at same age with no differences by sex.

Weight of bone-in breast from cutting produced during trial was 848 ± 0.011 g in males and 18.2% smaller with 694 ±0.010 g in females and it was very significantly higher compared to standard group with 14.6% and 12.6% in males and females respectively. Experimental group was less uniform compared to standard group with a variability coefficient of 9.28% and 11.20% in males and females respectively compared to 8% in males and females in standard group.

Bone-in legs in experimental group were of 699 g ± 0.009 and 557 g ± 0.07 in males and females respectively with very significant differences in favor of standard group between the two studied groups.

Percentage of cuts weight from live weight in the studied hybrid were 11-12 % higher for breast percentage and percentage of legs which are in a smaller demand from consumers is 2-35 % smaller.

Boneless breast weight obtained during the experiment was 690 g ± 0.011 and 557 g ± 0.010 in males and females respectively with insignificant differences compared to standard group. Experimental female group had very low uniformity with variability coefficient of 12.74% and experimental male group had a variability coefficient of 9.28%.

Boneless legs weight was 7.22% below standard or 540 ± 0.007 g and 13.3% below standard or 425 ± 0.006 g in males and females respectively compared to 582 ± 0.006 g in males and 490 ± 0.005 g in females respectively in standard group. Testing differences is revealing a significant difference in males and a distinct significant difference in females in favor of standard group.

Average meat percentage of breast is 22.64 % from live weight and 21.89 % from live weight in males and females respectively. Meat percentage of legs is 17.72 % from live weight and 16.7 % in males in females.

Phonotypical correlations between live weight and cuts weight is showing a positive correlation between live weight and breast weight both in males (r = + 0.175 ± 0.117) and in females (0.244 ± 0.108). This positive correlation between live weight and breast weight is showing that when live weight has been increasing breast weight has been increasing as well and this is a justification for efforts of hybrid's producer company to improve breast percentage from carcass. Correlation between live weight and cuts weight are negative both in males and in females.

Phonotypical correlation between carcass weight and cuts, is also showing a positive correlation between carcass weight and breast weight in females (r = + 0.310 ± 0.098) and a slightly negative but close to zero correlation in males (r = - 0.004 ± 0.142). Excepting the slightly positive but close to zero (r = + 0.029 ± 0.138) correlation between carcass weight and legs weight in males all the other correlations between carcass weight and cuts are negative both in males and in females.

Following these results it is recommended a much careful observance of poultry production technology.

Considering also the high proportion of breast from carcass revealed by hybrid's standard and also the trial results farmers and especially processors of this hybrid are advise to market the poultry meat especially as de-boned and processed products which are offering them a higher economical revenue.

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RESEARCH ON THE SHEEP BREEDING IN ORGANIC FARMING SYSTEM IN ROMANIA

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Abstract

Organic livestock has grown in Romania in recent years, especially after 2000, because animal breeding in organic farming system represent a niche activity for farmers. The purpose of this paper was to make a radiograph at national level as regards the sheep breeding in organic farming system. The share of organic production within total production varies according to the different animal sectors. The statistic data showed that cattle and sheep are the most popular species reared using organic production methods. The highest share is found in the sheep sector due to lower difficulties to convert to organic production. Indeed the productive system of sheep farming in the hill and mountainous areas of Romania is considered to be very close to that of organic sheep farming and this fact, gives a competitive advantage to the Romanian sheep farmers. Organic sheep breeding in Romania is based on native breeds, which are well adapted to their life environment. Most of these organic sheep farms are situated in the hill and mountains areas, places where the prevalent breed is Tsurcana. The sheep livestock farmed organically in 2010 was 58,372 heads and in present 0.70% from the sheep livestock are reared organically. Sheep breeding into organic farming system has a strong growing tendency in our country in the future and this is confirmed by the fact that in 2011 the number of sheep and goats farmed organically reached to 168,593 heads.

Key words: *sheep breeding, organic farming, livestock, farms*

INTRODUCTION

Organic farming is an agricultural sector which increased steadily in recent years worldwide and especially in the EU [5, 2]. Also, our country has seen a continuous growth of this sector and especially after 2000.

In our country there are great opportunities for practicing organic farming, due to favourable natural conditions, such as: a large area occupied by pastures and natural meadows, use of a quantity of fertilizers and pesticides significantly lower than in other countries and a reduced pollution of water and soil compared to other countries [1].

Organic livestock has grown also in Romania in recent years because animal breeding in organic farming system represent a niche activity for farmers.

Animals are considered to be an essential component of organic farms because represents an efficient way to use organic plant products, ensure the integration of plant production with animal production and provides natural fertilizer on plant culture.

Also the presence of animals in an organic farm provides stability, biodiversity and ecological sustainability for farm.

Romania has very favorable conditions for animal breeding in organic farming and a special perspective in European context for the development in this direction [2].

The productive system of sheep farming in the hill and mountainous areas of Romania is considered to be very close to that of organic sheep farming and this fact, gives a competitive advantage to the Romanian sheep farmers.

Sheep breeding in ecological system has the main aim to produce milk and meat organic from which may result many dairy and meat products, with high demand both internally and especially externally and from this point of view our country can become a major supplier of organic products to cover the

growing demands of this market, which is satisfied in a very small proportion.
The purpose of this paper was to make a radiograph at national level as regards the sheep breeding in organic farming system.

MATERIAL AND METHOD

The analyze of sheep breeding in organic farming system was made with special reference to organic sheep number at national and districts level, the number of organic sheep farms at districts level, the sheep breeds which are farmed organically, the land area exploited by the organic sheep farms, the organic conversion situation of animals and land areas, the organic certification bodies involved in organic certifying of sheep farms. To achieve these objectives we processed the official statistical data provided by different institutions (Eurostat, Ministry of Agriculture and Rural Development), we statistically processed the raw data received from responsibles with organic agriculture from the county agricultural directions and we interpreted the obtained data.

RESULTS AND DISCUSSIONS

The share of organic production within total production varies according to the different animal sectors in Romania.
Livestock farmed organically in 2010 were as follows: 12,761 heads of cattle, 58,372 heads of sheep, 2,320 heads of goats, 537 heads of pigs and 23,740 heads of poultry [8].
The statistic data showed that cattle and sheep (with a share of 0.51% and respectively 0.70% of total livestock) are the most popular species reared using organic production methods in our country (Table 1) [6, 7].
Not surprisingly it is for the pork sector that the sector has the lowest weight. This stems partly from the difficulties posed by the provision of organic animal feed (compound feed).

Table 1. Organic livestock 2010 in Romania (heads)

Specification	Cattle	Sheep	Goats	Poultry	Pigs
Total livestock	2,512,300	8,386,000	917,300	93,343,000	5,783,400
Organic livestock	12,761	58,372	2,320	23,740	537
% of total livestock	0.51	0.70	0.25	0.03	0.01

The certified organic livestock is presented in Table 2 [6]. From these data we can remark also that cattle and sheep have the highest share of total certified organic animals.

Table 2. Dynamics of certified organic livestock (heads)

Specification	2006	2008	2010	% changes 2006-2010
Bovine	11,365	7,567	5,358	-52.8
Sheep	86,180	121,175	23,029	-73.3
Goat	117	4,296	1,093	834.2
Pigs	1,652	416	320	-80,6
Poultry	4,300	6,080	21,580	401.9

The highest share is found in the sheep sector due to lower difficulties to convert to organic production (well identified products, feed based mainly on grass and hay).
The sheep livestock farmed organically in 2010 was 58,372 heads and in present 0.70% from the sheep livestock are reared organically (Table 3 and Figure 1) [6, 9].
From the data in Table 3 we can see that the total number of sheep increased by 10.2% in 2006-2010, and the number of sheep reared organically decreased by 32.3% in the same period.

Table 3. The evolution of sheep reared organically and their share from total sheep livestock (heads)

Specification	2006	2008	2010	% changes 2006-2010
Total sheep livestock	7,611,000	8,469,000	8,386,000	10.2
Sheep reared organically	86,180	121,175	58,372	-32.3
Share from total (%)	1.13	1.43	0.70	-38.1

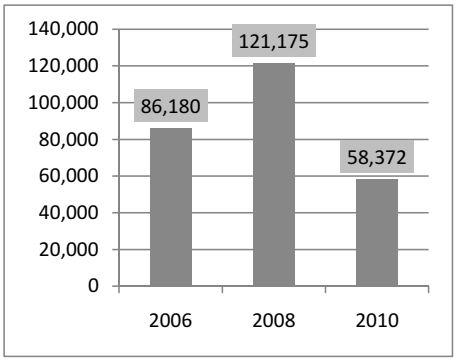

Fig. 1 Dynamics of organic sheep livestock (heads)

Romania recorded a large increase in the number of organically farmed sheep, especially in 2008 (1.43% share of total livestock), respectively after the integration of our country in the EU, but after this year their number decreased and mainly the number of organic certified livestock.
In Table 4 is presented the situation of sheep breeding in organic farming system at counties level [8].
In the year 2010 we can note that from the total of 58,372 heads of organic sheep 35,343 heads were in conversion and 23,029 heads were ecological certified.

Table 4. The situation of sheep breeding in organic farming system in 2010 at counties level (heads)

No.	District	Conversion	Certified	Total
1	ALBA	152	406	558
2	ARAD	22,473	-	22,473
3	ARGES	-	-	-
4	BACAU	-	-	-
5	BIHOR	-	50	50
6	BISTRITA N.	6,088	760	6,848
7	BOTOSANI	-	-	-
8	BRASOV	-	-	-
9	BRAILA	-	-	-
10	BUZAU	-	-	-
11	CARAS S.	170	-	170
12	CALARASI	-	-	-
13	CLUJ	1,389	467	1,856
14	CONSTANTA	-	115	115
15	COVASNA	18	-	18
16	DAMBOVITA	-	-	-
17	DOLJ	-	-	-
18	GALATI	-	-	-
19	GIURGIU	-	-	-
20	GORJ	-	-	-
21	HARGHITA	25	77	102
22	HUNEDOARA	1,337	-	1,337
23	IALOMITA	-	207	207
24	IASI	-	-	-
25	MARAMURES	-	37	37
26	MEHEDINTI	-	-	-
27	MURES	1,523	354	1,877
28	NEAMT	-	-	-
29	OLT	-	-	-
30	PRAHOVA	-	-	-
31	SATU MARE	498	-	498
32	SALAJ	228	-	228
33	SIBIU	-	19,904	19,904
34	SUCEAVA	413	411	824
35	TELEORMAN	-	-	-
36	TIMIS	265	241	506
37	TULCEA	-	-	-
38	VASLUI	-	-	-
39	VALCEA	750	-	750
40	VRANCEA	-	-	-
41	ILFOV	-	-	-
42	BUCURESTI	14	-	14
TOTAL		**35,343**	**23,029**	**58,372**

The number of organic sheep farms at national level is very low. Unfortunately, their exact number it is unknown, but from the data provided by the responsibles with organic farming at the county level seems it is up to 100 such farms.
The counties with the most holdings and thus with the largest number of sheep exploited in organic system in 2010 (Figure 2) were Arad (22,473 heads), Sibiu (19,904 heads) and Bistrita Nasaud (6,848 heads).

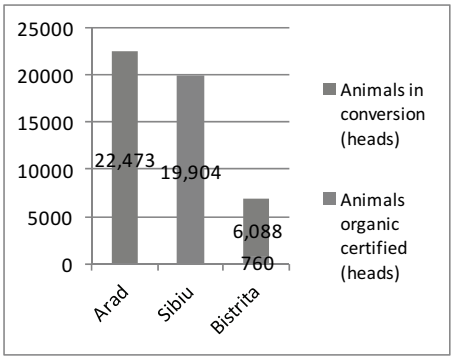

Fig. 2 Distribution of organic sheep livestock in the main counties with the largest number of organic sheep (heads)

Also we can remark that there are 19 counties where doesn't exist organic sheep. Conversely, in Sibiu county the total sheep livestock registered in organic farming system are organic certified and represent nearly 86% of total organic certified sheep number in Romania [3].
As regards the size of organic sheep exploitations this study revealed that it is different from a county to another and range from 8 heads/farm and 1750 heads/farm. In Sibiu County the size of organic sheep farms is higher compared with other counties, respectively the lowest size is 122 heads/farm, and the highest is 1750 heads/farm. Conversely, in Suceava County, the size of organic sheep farms is very small (the lowest is 8 heads/farm and the biggest 65 heads/farm).
The land surface per organic sheep farm is also different from a county to another and range from 3.4 ha/farm and 350.0 ha/farm. In Sibiu County the agricultural land area exploited by the organic sheep farms is higher compared with other counties, respectively

the lowest is 60 ha/farm, and the highest is 350 ha/farm. Conversely, in Suceava County, the agricultural land exploited by organic sheep farms is very small (the lowest is 4.9 ha/farm and the biggest 17.5 ha/farm).
Regarding the legal status of farmers that have organic sheep farms, this study highlights the fact that most of them are individuals, a small part are authorized individuals and just few are companies with legal personality.
Of the 14 certification bodies working in organic farming in Romania and are accredited by Ministry of Agriculture and Rural Development, in the organic sheep breeding sector are involved only 7. By far the largest number of sheep organic farms at national level is inspected by SC Ecoinspect SRL.
Organic sheep breeding in Romania is based on native breeds, which are well adapted to their environment. The choice of breeds and breeding strategies used in the organic sheep livestock sector needs to ensure farm profitability, safeguard animal health and welfare, focus on conserving genetic diversity and promote human health.
Most of Romanian organic sheep farms are situated in the hill and mountains areas, places where the prevalent breed is Tsurcana [4].
The present study reveal that in all counties where there are organic sheep farms (with one exception, respectively in Ialomita County the breed raised organically is Tsigai breed) the breed which is exploited in organic farming is Tsurcana.
Sheep breeding into organic farming system has a strong growing tendency in our country in the future and this is confirmed by the fact that in 2011 the number of sheep and goats farmed organically reached to 168,593 heads.

CONCLUSIONS

The sheep livestock farmed organically in 2010 was 58,372 heads, from which 35,343 heads were in conversion and 23,029 heads were ecological certified.
In present 0.70% from the total sheep livestock are reared organically in Romania. The size of organic sheep exploitations is different from a county to another and range from 8 heads/farm and 1750 heads/farm.
The counties with the most holdings and thus with the largest number of sheep exploited in organic system in 2010 were Arad (22,473 heads), Sibiu (19,904 heads) and Bistrita Nasaud (6,848 heads).
Organic sheep breeding in Romania is based on native breeds. Most of Romanian organic sheep farms are situated in the hill and mountains areas, places where the prevalent breed is Tsurcana.

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THE EVOLUTION AND CURRENT SITUATION OF GOAT BREEDING IN ROMANIA

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Abstract

After the integration of our country within the EU have occurred major changes in many areas of activity and including in the goat breeding sector. The purpose of this paper is to investigate the evolution and situation of goat breeding sector in Romania in relation with the goat livestock, the number and size of goat exploitations and goat 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 goat livestock has increasing with 135.6% in 2010 compared with 2002 and with 80.1% compared with 2006. In terms of number and size of goat exploitations, the results showed that in the year 2010 there were 176,353 exploitations of goat of which 88.6% are individual exploitations that have an average number of 2.4 heads goats per unit and which hold these animals exclusively for self-consumption of family and only 3% of total exploitations have more than 100 heads, as average size of goat farms existing in the western countries of EU. However, compared with the existing situation in 2002, the number of exploitations in the year 2010 decreased with about 25%, especially based by reducing of small exploitations, respectively those which are framed in class less than 10 heads.

Key words*: goat breeding, evolution, goat livestock, exploitations*

INTRODUCTION

Goat is an animal that uses very well the cheap feed such as grass, roughage and woody plants, with relatively low costs compared with other species, and which provide valuable foods for the human consumption.

Goat breeding is a traditional activity in our country because we have a large area of natural grassland, spread in all forms of relief, and which cannot be operated more efficiently than through the rearing of sheep and goats.

After the integration of our country within the EU have occurred major changes in many areas of activity and including in the goat breeding sector. The goats rearing sector has grown continuously in recent years against other ruminant species, sheep and cattle respectively [3].

Unlike cow's milk, goat milk has no quota limit required by the European Union because the demand is much higher than supply, so that the market is ensured both internally and especially externally. Goat milk and goat cheese became popular both in Romania and abroad because of their nutritional and dietary quality [2].

For these reasons goat breeding represents an investment opportunity and a profitable business for Romanian market. The purpose of this paper is to investigate the evolution and situation of goat breeding sector in Romania after the integration in EU.

MATERIAL AND METHOD

In order to characterize the evolution and current situation of goat breeding in Romania, the following indicators were used: number of goat stocks, the number and size of goat farms, the milk and meat production. The period analyzed in this study was 2002-2010. The data, collected from FAO and National Institute of Statistics, have been processed and interpreted.

RESULTS AND DISCUSSIONS

Romania is one of the first five EU goat producing countries (Table 1) [4]. Thus, the first five goat producing countries of EU

(Figure 1) are Greece (33.6%), Spain (23.5%), France (10.8%), Italy (7.7%) and Romania (7.3%).

From the Table 1 data we can note that France and Romania recorded an increasing of the goat population in the period 2002-2010, while on the whole EU 27 and in the rest of other three major producing goat countries (Greece, Spain and Italy) the goat population has decreased.

Table 1. The evolution of the goat livestock in the first five countries of the EU goat producing during 2002-2010 (thousand heads)

Specification	2002	2010	% changes 2002-2010
Greece	5,180	4,200	-18.9
Spain	3,047	2,934	-3.7
France	1,232	1,349	9.5
Italy	1,025	961	-6.7
Romania	525	917	74.7
EU 27	13,636	12,488	-8.4

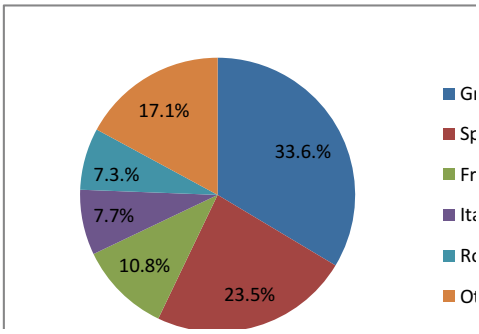

Fig. 1 Distribution of the goat population in the EU in 2010

According to the statistical data provided by FAO, the goat livestock in Romania was situated in 2010 at 917,300 heads (Table 2) [4].

Unlike data provided from FAO, the data provided by National Institute of Statistics (NIS) from the General Agricultural Census conducted in 2010 show that the goat livestock was situated in 2010 to 1,237,000 heads (Table 3) [5].

The difference between the two provided data is very significantly, respectively nearly to 35% or 320,000 heads, which affect the data interpretation.

We believe that this difference is inadmissible and there are necessary corrective measures which must be taken by the Romanian authorities responsible for transmitting statistical data to FAO.

Table 2. The evolution of the goat livestock in Romania during 2002-2010 (heads)

Specification	2002	2010	% changes 2002-2010
Goat livestock	525,100	917,300	74.7

Table 3. The evolution of the goat livestock in Romania during 2002-2010 (thousand heads)

Specification	2002	2010	% changes 2002-2010
Cattle	2,871	1,985	-30.9
Sheep	7,238	8,386	15.9
Goats	744	1,237	66.3
Pigs	8,260	5,387	-34.8
Poultry	82,407	78,867	-4.3

Regarding the evolution of the goat livestock in the period 2002-2010, the data presented in Tables 2 and 3 show that this had a significant increase, regardless of data source.

According to data provided by FAO the goat livestock registered an increasing with 74.7% in the analyzed period 2002-2010 and according to the statistical data provided by NIS an increasing with about 66.3% during the same period.

As shown by statistical data, the increasing of the goat livestock is very significant in the analyzed period, fact which shows that the interest of breeders for this species is very high.

The same conclusion follows from the data of Table 3, where we can see that compared to other species goats had the highest increase in livestock in the analyzed period, especially that the cattle, pigs and birds have declined regarding the livestock number.

According to data from the National Institute of Statistics the number of goats and sheep per 100 hectares of agricultural land in 2010 increased by 27% compared to 2002, respectively from 59 heads in 2002 to 75 heads in 2010 (Table 4) [5].

Table 4. The evolution of the sheep and goat number at 100 ha agricultural land during 2002-2010 (heads)

Specification	2002	2010	% changes 2002-2010
Sheep and goat	59	75	27.1

In terms of size and structure of goat farms in our country, the data provided by National Institute of Statistics show that in the year

2002 there were 234,705 of goat farms of which 95.56% are holdings that have under 10 heads per unit which hold these animals exclusively for self-consumption of family and only 0.50% of total holdings had more than 50 heads, as average size of goat holdings existing in the western countries of EU. (Table 5) [1].

Table 5. The structure and size of goat holdings in Romania in the year 2002

Specification	Number of exploitations	% from total	Agricultural individual exploitations	Commercial societies
1-2 heads	169,615	72.77	169,589	26
3-9 heads	53,496	22.79	53,465	31
10-19 heads	7,034	3.00	7,010	24
20-49 heads	3,384	1.44	3,358	26
50-99 heads	858	0.37	843	15
100-199 heads	282	0.12	274	8
200-499 heads	32	0.01	32	-
over 500 heads	4	0.001	2	2
Total	234,705	100	234,573	132

Unlike the situation in 2002, the number of goat holdings fell in 2010, reaching to 176,353 holdings, respectively a reduction of about 25% (Table 6) [5].

Table 6. The structure and size of goat holdings in Romania in the year 2010

Specification	Number of exploitations	% from total	Stocks -heads-	% from total	Average no. per holding
1-2 heads	106,407	60.3	159,294	12.9	1.5
3-9 heads	49,840	28.3	216,489	17.5	4.3
10-19 heads	7,975	4.5	97,958	7.9	12.3
20-49 heads	6,874	3.9	206,595	16.7	30.1
50-99 heads	3,325	1.9	215,636	17.4	64.8
100-199 heads	1,454	0.8	185,574	15.0	127.6
200-499 heads	425	0.25	112,091	9.1	263.7
over 500 heads	53	0.03	43,340	3.5	817.7
Total	176,353	100	1,236,947	100	7.0

Compared with the situation in the year 2002, the number of goat holdings decreased with about 25% in the year 2010, especially based on decreasing of individual holdings, respectively farms framed under 10 heads class (156,247 in 2010 versus 223,111 in 2002).

From the data presented in Table 6 we can see that the average number of animals per farm was 7 heads in 2010, which represents an increase of 2.33 times compared to the situation existing in 2002 (7 heads/farm in 2010 versus 3 heads/farm in 2002).

Also, from the data presented in Table 6 we can see that the percentage of farms which hold more than 50 heads reached almost 3% of total holdings, against 0.5% as represented in 2002.

This is encouraging for the future of goat breeding sector in our country. Thus in 2010 there were 5,257 of goat holdings which have the size over 50 heads, compared to 1,176 as were of that size in 2002. The 5,257 holdings have 556,641 heads of goats, which represents about 45% of the total number of goats in the country. It is also worth noting that in 2010 in our country were 53 of big farms which have over 500 heads of goats per holding, compared with 4 farms were of this size in 2002.

Concentration of a greater number of animals per farm and the increasing of average production per animal are the tendencies which are in attention of countries with a tradition in goat breeding, especially in countries which were in the European Union before the first wave of accession in 2004 (15 countries) [1].

Regarding the goat milk production, from data presented in Table 7 it can be noted that at the whole EU level this remained relatively stable in the analyzed period (a small increasing of 2.7%).

Romania has a good goat milk production (161 thousand tonnes) being classed at fourth place in EU from this point of view (8.1% from EU goat milk production) and registered the greatest increasing in the analyzed period (75.0%) [4].

Table 7. Evolution of goat milk production in the top five largest EU producer countries in the period 2002-2010 (thousand tonnes)

Specification	2002	2010	% changes 2002-2010
France	536	645	20.3
Spain	513	602	17.4
Greece	523	470	-10.1
Romania	92	161	75.0
Bulgaria	105	61	-41.9
EU 27	1,935	1,987	2.7

The highest production of goat milk in EU (Fig. 2) is produced in France (32.5), followed by Spain (30.3%) and Greece

(23.7%). It is noteworthy that in these three countries is produced about 86.5% of goat milk production from the EU 27.

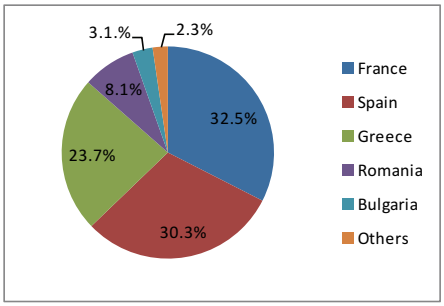

Fig. 2 Distribution of the goat milk production in the EU in 2010

Concerning the goat meat production, from data presented in Table 8 it can be noted that at the whole EU level this remained relatively stable in the analyzed period (a small increasing of 1.8%) [4].

Table 8. Evolution of goat meat production in the top five largest EU producer countries in the period 2002-2010 (tonnes)

Specification	2002	2010	% changes 2002-2010
Greece	44,890	53,700	19.6
France	6,700	12,053	79.9
Spain	15,072	9,000	-40.3
Romania	3,454	7,355	112.9
Bulgaria	5,358	3,699	-31.0
EU 27	92,608	94,291	1.8

Romania has a good goat meat production (7,355 tonnes) being classed at fourth place in EU from this point of view (7.8% from EU goat meat production) and registered the greatest increasing in the analyzed period (112.9%).

The highest production of goat meat in EU (Fig. 3) is produced in Greece, which produces more than half of goat meat production in the EU (56.9%), being followed by France (12.8%) and Spain (9.5%).

It is noteworthy that in Bulgaria and especially in Spain the goat meat production decreased very significantly in the analyzed period (31.9% and respectively 40.3%).

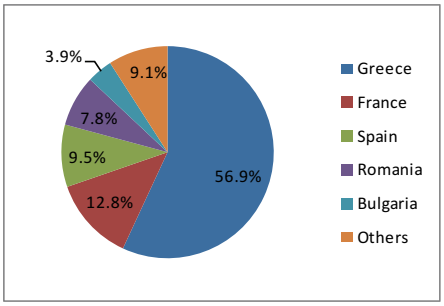

Fig. 3 Distribution of the goat meat production in the EU in 2010

CONCLUSIONS

The goat livestock in Romania was situated in 2010 at 917,300 heads, recording an increase of 74.7% in the period 2002-2010.

Compared with the situation in the year 2002, the number of goat holdings decreased with about 25% in the year 2010, especially based on decreasing of individual holdings, respectively farms framed under 10 heads class.

Romania has good productions of goat milk and goat meat, our country being classed on fourth place in EU for both productions. However, the potential of our country is much higher and especially regarding the goat milk production.

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HYDROLYSIS OF SAGO (METROXYLON SAGO ROTTB.) PITH POWDER BY SULFURIC ACID AND ENZYME AND FERMENTATION OF ITS HYDROLYZATE BY *PICHIA STIPITIS* CBS 5773, *SACCHAROMYCES CEREVISIAE* D1/P3GI, AND *ZYMOMONAS MOBILIS* FNCC 0056 INTO BIOETHANOL

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Abstract

The purpose of this study was to determine hydolysis efficiency of sago pith powder and fermentation efficiency by P. Stipitis CBS 5773, S. cerevisiae D1/P3GI, and Z. mobilis FNCC 0056.. This research was experimentally and consist of hydrolisis process sago pith powder and fermentation of its hydrolyzate. Sago pith powder has content 77,5% starch, 4,63% cellulose, 4,86% hemicelluose, lignin 3,07%, dan water 10,12%. In gelatination process in temperature at 120˚C, the yield of sugar concentration 0,62% and dextrose equivalent (DE) 0,89% obtained from the size of 100 mesh. Sago gelatine hydolized by sulfuric acid acid, α-amylase, hemicellulase, cellulase, dan amyloglukosidase produce sugar concentration 53,28% and DE 68,52%. At fermentation process in sugar concentration 5% with content of glucose 4.17 %, fermentation by Pichia stipitis, Saccharomyces cerevisiae, and Zymomonas mobilis provide ethanol fermentation efficiency 23,5%, 37,65%, dan 32,13% respectively. At fermentation process with sugar concentration 10% with content of glucose 8,28 %, fermentation by Pichia stipitis, Saccharomyces cerevisiae, and Zymomonas mobilis yielding ethanol fermentation efficiency 9,30%, 50,85% and 20,65% respectively.

Key words: Hydrolysis, Sago Pith Powder, fermentation*, Pichia stipitis CBS 5773, Saccharomyces cerevisiae D1/P3GI, Zymomonas mobilis FNCC 0056*

INTRODUCTION

Indonesia currently has a high dependence on fossil fuels and has at least three serious problems that the depletion of petroleum reserves, the instability of fuel prices due to high demand and rising greenhouse gas (CO2) from burning fossil fuels so that Indonesia needs alternative and renewable energy sources. On the other side of Indonesia as a tropical country that has many resources, especially carbohydrates and cellulose from agricultural waste. Especially for Eastern Indonesia has vast forest sago has great potential for renewable energy sources and energy independence.

Bio-ethanol is ethanol produced by fermentation by various microorganisms (yeasts and bacteria) [1]. Currently ethanol is used as 'fuel additive' (fuel mixture), it can even replace non-renewable fuel conventionally.

One of substrate which is potential and environmentally friendly to produce ethanol is a sago plant. Although Sago need long age harvest is about 6 years but can produce starch in large quantities. Sago is Indonesia plant native, can grow on various soil types, such as: dry soil, clay, swamp or soil flooded. Sago palm acreage in Indonesia is very wide which is about 1.128 million ha [2]. Seedling density of about 1480 trees / ha when the crop yield and 125-140 trees / year [3]. (Each of the sago tree containing an average of 200-400 kg sago starch, so can obtain 30-60 tonnes of sago starch / ha [4]. (Bintoro, 2003). Trunk of sago pith without peel has weight an average 850 kg with starch content 29 percent, water content 50 percent and fibers ranged 21% [5]. Overall utilization of sago for ethanol production is perceived to be more effective because of the stem consists of the sago pith which is containing starch and fiber. Sago starch content about 84.7 to 85.9% with the percentage of amylose 27 % and amylopectin 73%[2], while the sago fiber composed of cellulose and hemicellulose, when hydrolyzed

completely into simple sugars, especially glucose[2].

In this study uses Sago (Sago Metroxylon Rottb.) obtained from Kibin, Banten. Sago is processed (self-preparative) sago flour. Based on proximate test, the pith of sago contain of starch about 77.50%,and fiber 12:56%, water 10.12%. Total amount of sugar contained in the pith flour equal to the amount of carbohydrate. Total sugars of pith flour in size 50 mesh is equal to 60.47% (w / w), it meansthat every 1 g of the pith flour contained 0.604 g of carbohydrates. While the total sugar contained in the pith flour 100 mesh size is equal to 77.76% (w / w) means that in every 1 g of the pith of flour contained 0.778 g of carbohydrates. With high carbohydrate content means sago is very potential as a major source of sugar for ethanol fermentation.

Several groups of microorganisms have the ability to produce ethanol from carbohydrates through the fermentation process. Some microorganisms such as Pichia stipitis can ferment xylosa . Saccharomyces cerevisiae, and Z. mobilis has the ability to ferment hexose (C6) [6]. While Z.mobilis is a type of bacteria that can produce ethanol from hexose sugars and fermentation capabilities faster than S. cerevisiae [7].

On lignocellulose biomass is necessary to pre-treatment such as desizing of the pith flour and temperature of gelatinization. Optimum temperature of the pre-treatment can increase the acid and enzyme -hydrolysis which is expected to produce sugars from cellulose then fermented into ethanol. Concentration of ethanol produced is influenced by the type and concentration of sugar. Because microorganisms have different preferences and tolerances to concentration of sugar.

Pre-treatment. Pre-treatment of lignoselulose needs to be done prior to enzymatic or acid hydrolysis. Pretreatment aims to solve, dissolve, hydrolyze and separate components of cellulose, hemicellulose, and lignin [8]. (Saha, 2003). Various methods for biomass pretreatment methods such lignoselulosik autohydrolisis,treatment with organic solvent, thermo mechanical; milling, refining, cutting, extortion, acid treatment; acid solution (H_2SO_4, HCl), concentrated acids (H_2SO_4, HCl),treatment with alkali; Sodium hydroxide, ammonia, hydrogen and peroxide alkaline [8]. The use of acid for the initial treatment materials can lower the cost for enzyme.

Gelatinization and Saccharification. The conversion process of starch, cellulose, and hemicellulose into sugars through the stages of gelatinization and hydrolysis. Stages of gelatinization facilitate the action of the enzyme hydrolyzes the substrate. Gelatinization is influenced by temperature, substrate type and concentration of the substrate itself. The smaller of gelatin concentration the higher of reducing sugar content. Because acid is difficult to diffuse to the high starch concentrations. Hydrolysis is the decomposition of polysaccharides into monosakarida. Hydrolysis carried out by crushing the raw material of sago mixed with water, acids or enzymes such as termamyl, at pH 6.5 and heated at a temperature of 90oC. Further saccharification process using dextrozim for 24 hours at pH 4.5 and temperature 60 ° C[9] (Bujang, Ishizakhi, and Goh Ping Yau, 2006).

Hydrolysis can be performed using acids, enzymes, or mixtures of acids and enzymes. The acid used can be either concentrated acid or dilute acid. Acid will break down the starch molecules randomly and sugars produced mostly reducing sugars. At the optimum conditions for starch molecules will produce 88% glucose. However, in practice, using the acid hydrolysis of starch to glucose would only result in the value of DE (Dextrose Equivalent) 55%. If the value of DE (Dextrose Equivalent) above 55%, it will produce furfural compounds and acid hydroximethyl levulinat which can inhibit the fermentation process.

Hydrolysis of polysaccharides into monosaccharides using enzyme carbohydrase, namely: α-amylase, hemiselulase, cellulase, and amiloglukosidase. The amount of enzyme amiloglukosidase at full dose (0.56 μ l / g) were used to hydrolyze starch (100%), whereas the amount of cellulase enzymes on the full dose (0.83 μ l / g) were used to hydrolyze pure cellulose Hydrolysis of starch and cellulose will produce glucose and additional products in the form of sugar that is hydroximethyl furfural derivatives. Hemicellulose hydrolysis would produce two types of sugar, pentose and

hexose. This research will study the influence of particle size reduction in pith flour of sago, gelatinization temperature, hydrolysis effectiveness of combination of acid and enzyme in hydrolysis and fermentation capabilities of *Pichia stipitis Sacchromyces cerevisiae and Zymomonas mobilis* in hydrolyzate results of sulfuric acid hydrolysis 6 M followed by the enzyme in hydrolysis.

MATERIAL AND METHOD

Pre-treatment and Hydrolysis. The research was carried out experimentally in the laboratory and conducted in three stages: (1) Pre-treatment: consists of optimization of particle size reduction (50 mesh and 100 mesh) of pith flour of sago, optimization of temperature in gelatinization which are 90°C, 100°C, 110°C, dan 120°C C for 20 min in 1 atm pressure (2). Acid and enzymatic hydrolysis in Gelatin pith flour. In the hydrolyzate of the pre treatment that produces the highest reducing sugar content was added 6 M sulfuric acid, hereinafter incubated for 1 hour at 120^{o}C in this hydrolizate (I) was . measured the concentration of reducing sugar and Dextrose equivalent (DE). Once hydrolyzed by 6 M sulfuric acid hidrolisate (I) is cooled to 25^{0}C and adjusted to pH 6.0, then α-amylase enzyme is added as much as 0.17 ml / g (volume enzyme/ g substrate), incubated at 104^{0C} for 60 minutes with a pressure of 1 atm (HII). The hydrolyzate II measured the concentration of reducing sugar and dextrose equivalent (DE). Hydrolyzate II is heated at a temperature of 121^{0}C with a pressure of 1 atm for 10 minutes. Then added with hemiselulase as much as 1/3 dose. Hydrolysed further incubated at 55°C with agitation of 150 rpm for 270 minutes (hydrolyzate III) hydrolyzate III had cooled 25^{0}C. Then the pH was adjusted to 4.8. After that cellulase enzymes are added as much as 0.55 ml / g and amyloglucosidase 0.37 ul g (volume enzyme / g substrate). Subsequently incubated at 60^{0}C for 48 h with agitation 130 rpm. At this stage, concentration of reducing sugar and DE is measured.(Gerhartz,1990).

Fermentation by Pichia stipitis CBS 5773, Saccharomyces cerevisiae D1/P3GI, and Zymomonas mobilis FNCC0056 a single culture to ferment sugars hydrolizate pith of sago starch hydrolyzate.

The reducing sugar content measurement method done by DNS. Sago as much as 15 g gelatin dissolved in distilled water until the volume is 25 ml. The next sample is introduced into a centrifuge tube and centrifuged at 3500 rpm for 20 minutes. Clear sample solution of 1 ml pipetted into a test tube inserted. Then into the test tube was added 3 ml of DNS (3.5 Dinitrosalicylic acyd). After that, samples were homogenized with a vortex and heated in a boiling water bath for 5 minutes. After five minutes of heated, then cooled in an ice bath. After the absorbance was measured using a spectrophotometer at a wavelength of 550 nm. Calculation of Dextrose Equivalent (DE) using the formulaDextrose Equivalent (DE) = reducing sugar concentration of the sample (%) x 100%/ Total sugar concentration (%).

Preparation of Substrate Fermentation and fermentation. The hydrolyzate which is having highest DE (Dextrose Equivalent) and reducing sugars content. then adjusted to 5% and 10% ofreducing sugar content.. Each hydrolyzate is added with fermentation medium containing (per liter): yeast extract 4g, KH_2PO_4 2g, $(NH4)_2SO_4$ 3g, MgSO4.7H2O1g, and pepton 3, 6 g (Sanchez et al., 2002). pH was adjusted to pH 7. For fermentation by P. stipitis CBS 5773 and S. Cerevisiae D1/P3GI pH medium was adjust to pH 5, and medium for Z. mobilis FNCC 0056 pH adjusted to ph 7. Fermentation medium were sterilized. For fermentation every cultures (Pichia stipitis CBS 5773 S. Cerevisiae D1/P3GI and Z. mobilis FNCC 0056) as much as 10% put into a fermentation substrate, which is containing hydrolyzate with concentration sugar are 5% and 10%. Then cultures in medium shake-incubated at 30^{o} C for 72 h with agitation 150 rpm. During incubation, samples were taken at 0, 6, 12, 18, 24, 30, 36, 48, 60, and 72 hours for measurement of ethanol and reducing sugar content.

RESULTS AND DISCUSSIONS

Optimization of desizing of partikel of pith flour and temperature incubation in gelatinized Pith flour used in this study had a starch content 77.5%, cellulose 4.63%,

hemicellulose 4.86%, lignin 3.07% and water 10.12%. Prior to the hydrolysis step, the substrate made gelatinization. Gelatinization is a mechanism of entry of water into the starch granules so as to facilitate the action of the enzyme hydrolyzes the substrate. Gelatinization is influenced by temperature, substrate type and concentration of the substrate itself [10]. Treatment with temperature 90˚C, 100˚C, 110˚C, and 120˚C of the pith flour of in 50 and 100 mesh resulted a reducing sugar concentrations were as follows.

Table 1. Duncan's multiple range test analysis of Concentration of reducing sugar in size of pith flour 50 mesh and 100 mesh.

Temp of Gelatinization (˚C)	Cons. Of Red. sugar (%) in 50 mesh flour	Cons. Of Red.sugar (%) in 100 mesh
90	0,28a	0,25a
100	0,35b	0,27a
110	0,42c	0,40b
120	0,53d	0,62c

From the results of Duncan's multiple range test analysis can be seen that the increase in gelatinization temperature, the greater the concentration of sugar produced. The results of starch gelatinization of sago pith 50 mesh at 120˚C is a reducing sugar concentration of 0.53%. While on 100 mesh at 120˚C obtained 0.62%. Concentration of sugar produced from the pith of sago in 100 mesh size larger than 50 mesh. 100 mesh size, mean grain size of pith starch particles is 175µ. While the 50 mesh size, mean grain size of pith starch particles is 350µ.. The smaller a particle, the greater the absorption area [11].

Table 2. Duncan's multiple range test analysis of Dextrose equivalent (DE) at various temp. on 50 mesh and 100 mesh

Temp of Gel. (˚C)	Dextrose equivalent (DE) (%) in size 50 mesh	Dextrose equivalent (DE) (%) in size 100 mesh
90	0,51a	0,31a
100	0,57b	0,32b
110	0,69c	0,52c
120	0,85d	0,89d

Grain flour 100 mesh has a smaller particle size will absorb more heat and water, therefore the more that comes out of the starch amylose so that the concentration of sugar produced more than 50 mesh size. Dextrose equivalent (DE) indicates the number of starch polymers that have been cut into glucose molecules are much simpler. Dextrose equivalent can be obtained from the ratio of the concentration of sugar samples with a total sugar concentration.

From the analysis of Duncan's multiple range test t is known that increasing the temperature of gelatinization, the greater the dextrose equivalent (DE) is generated. From starch gelatinization of sago pith flour 50 mesh obtained the highest DE 0.85% at 120˚C and DE 89% at 100 mesh. This is due to the high temperature will facilitate the breaking of carbon-hydrogen bond, so that more polymer is cut starch into glucose[12]. Gelatin then is hydrolyzed. chemically and enzymatically, Chemical hydrolysis using sulfuric acid 6M and the pH was adjusted to 2.

Tabel 3: Conc. of red. sugar and (DE) of substrat is hydrolyzed by sulfuric acid 6 M and amylase, hemicellulase, cellulase and amyloglucosidase

Treatment of Hydrolysis	Cons. Of red. sugar.	DE (%)
Sulfuric acid 6 M	22,6	28,63
α-amylase	33,02	42,47
Hemicellulase	33,94	43,65
Cellulase and Amyloglucosidase	53,28	68,52

DE = *dextrose equivalent*

Concentration of reducing sugar produced in the pith flour of sago is hydrolyzed by sulfuric acid is measured by DNS method. Hydrolysis using sulfuric acid 6M produce concentration of reducing sugars 22.26% and DE2 8.63% . On the acid hydrolysis, acid breaks the bond glycosidic pith flour of sago at random but consecutive to the smallest molecule called glucose. However, the results of a reducing sugar and DE obtained indicates that not all pith flour of sago can be hydrolyzed by acid. This is in accordance with the statement [13] that the conversion of starch using acid will only generate a maximum of DE 55%.

Hydrolysis by enzymes

Hydrolysates I generated at acid hydrolysis using sulfuric acid, and then hydrolyzed by the enzyme α-amylase with doses 0,17 ul/g, which lasted for 60 minutes at a temperature of 104ºC and pH 6.0, hydrolysis using α-amylase enzyme, obtained hydrolyzate II ith reducing

sugars obtained amounted to 33.02%, and DE 42.47%. Increased concentrations of reducing sugars due to the breakdown amylose and amylopectin in starch by α-amylase which does not occur at optimum in the acid hydrolysis. α-amylase is an endo-enzyme that can break the bond of alpha-(1,4) glycosidic randomly. Break down of amylose by α-amylase into maltose and maltotriosa that occur at random. Then form of glucose and maltose as the final result. While working of α-amylase on the amylopectin would produce glucose, maltose, and various types of oligosaccharide [15]. Hydrolysates II produced by α-amylase then hydrolyzed by hemicellulase. Hydrolysis by hemicellulase for 27^0 minutes at a temperature of 55ºC and pH 6.0. Concentrations of enzyme were added at 1/3 of the recommended enzyme concentration is equal to 0.001 g / g substrate. Hydrolysis by hemicellulase obtained hydrolyzate III.

The main purpose hemicellulase, fibers which is containing hemicellulase will produce simple sugar such as hexose and pentose. The use of hemicellulase is able to raise of a reducing sugar concentration and DE, reducing sugar from 33.02% to 33.94% and DE from 42.47% to 43.65%.. The use of hemicellulase not only able to contribute of increase in reducing sugar concentration of the hydrolysis of hemicellulose but also helps the action of the enzyme cellulase..

Hydrolysis by cellulase enzymes and Amyloglucosidase. The addition of cellulase done because in the previous stage, the hydrolysis of cellulose was not optimal. While the addition amiloglukosidase made to hydrolyze dextrins obtained from the previous stage so as to produce glucose. Provision of both types of enzyme was carried out simultaneously as cellulase and amiloglukosidase have synergistic activity.Hydrolysis by cellulase enzymes and amiloglukosidase (saccharification) was held at a temperature of 60 º C and pH 4.8. DX dextrozyme enzyme dosage was 0.37 ml / g (dose of 2/3), while the number of Celluclast 1.5 L is inserted is 0.55 ml / g (dose of 2/3). Saccharification carried out for 48 hours. Hydrolysis using dextrozyme DX and Celluclast 1.5 L produces a reducing sugar concentration [% (w / w)] 53.28% and DE 68.52% after 48 hours of incubation.

Cellulase enzymes play a role at the beginning of the process is made starch granules more open so that amyloglucosidase a chance to hydrolyze starch granules inside which is have higher of sensitivity towards amiloglukosidase activity[15]. Reducing sugar concentration increased, this is due to cellulase enzymes break down cellulose into selobiosa. Selobiosa is then split into glucose. In addition, DX dextrozyme breaking glycosidic bond α-1, 4 and α-1, 6 produce monomers of glucose. Bond of α-1 ,4-glycosidic found in maltose and maltotriosa which is the result of a process of amylose and amylopectin liquefaction while bonds α-1 ,6-glycosidic which is the dextrins present in the process liquefaction of amylopectin.

At this stage of the hydrolysis of the resulting concentration of DE for 68.52%. This is consistent with the statement Langlois and Dale (1940) in Tjokroadikoesoemo [16] that the hydrolysis process using a combination of acid and enzymes can increase the value of DE. Initially carried out by acid hydrolysis process to DE 55%, then uses the enzyme hydrolysis followed by amilolitik to DE 61-65%. In this study, DE values greater than 65% can be caused by the addition of the cellulolytic enzyme hydrolysis process, the cellulase and hemicellulase so that the amount of glucose produced more. Hydrolyzates of sugar which is the result of hydrolysis, then set the concentration to 10% and 5%. HPLC analysis of the results obtained that 10% of sugar hydrolizate consists of glucose 8.28% and maltotetraosa 1.72%. Sugar concentration of 5% consisted of glucose 4.17% and maltotetraosa 0.83%. Sugar is then fermented by *P.stipitis, S.cerevisiae, and Z.mobilis* a single culture.

Efficiency fermentation of *Pichia stipitis, Zymomonas mobilis and Sacchromyces cerevisiae* in fermentation of reducing sugars of hydrolyzate of pith of sago starch. Concentration of sugar and ethanol at the end of the process of ethanol fermentation of hydrolyzate sugar by *Pichia stipitis*. Hydrolyzate fermentation medium sugar

concentration of 5%, containing glucose 4,17% glucose and maltotetraosa 0.83%. While the concentration of sugar hydrolyzate medium 10% containing glucose 8.28% glucose and 1.76% maltotetrosa.

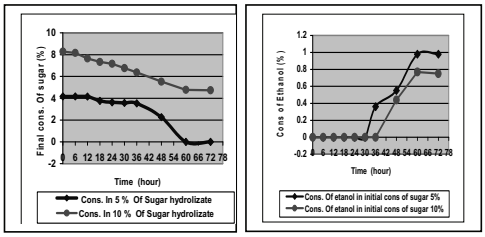

Fig 1. Reducing sugar and ethanol concentration changes during fermentation hydrolyzate of pith of sago starch by *P.stipitis*

During the fermentation process takes place, the concentration of sugar hydrolyzate 5% containing glucose 4,17% is used up by *P.stipitis* at the 60 hours. While the initial sugar hydrolyzate concentration 10%, after 72 hours as much as 8.28% glucose used by P.stipitis and leaving glucose 4.73%, this means only 3.55% used by *P.stipitis*. This suggests that *P.stipitis* less tolerant to sugar concentration 10%. Sugar is used as a nutrient and for ethanol fermentation. On medium hydrolyzate sugar 5% containing glucose 4,17% produces ethanol only 0.98% which began to form after 36 hours, meaning that up to 36 hours glucose used for nutrition. From the fermentation is well known efficiency of glucose fermentation by Pichia stipitis only 23.5%, while according to theory, 100% sugar when fermented will produce 50% ethanol [12].

Concentration of ethanol in the fermentation by *P.stipitis* sugar in a medium of sugar hydrolyzate 10% containing glucose 8.28%, produces ethanol only as much as 0.77% for 72 hours and ethanol is formed at 48, it means up to 36 hours, glucose used as a nutrient. Concentration of ethanol produced in the hydrolyzate 10% less than the ethanol produced in the sugar hydrolyzate 5%. thus the efficiency of fermentation by *P.stipitis* on glucose medium containing 8.28% only 9.30%.

Efficiency of fermentation by *Pichia stipitis* is very low, it is presumed *P.stipitis* less able to ferment hexose sugars. The best ability to ferment pentose sugars. More glucose is used to increase the cell biomass. Also *P.stipitis* have a low tolerance to high sugar concentration. This can be seen in 10% glucose medium, *P.stipitis* lag phase longer than the medium of 5%.

Concentration of reducing sugar and ethanol at the end of the process of ethanol fermentation of hydrolyzate sugar by *Saccharomyces cerevisiae*

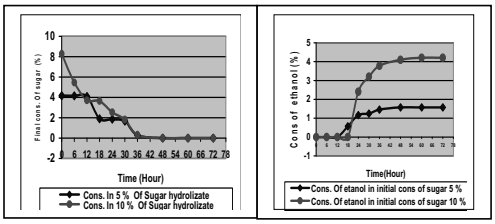

Fig 2. Reducing sugar and ethanol concentration changes during fermentation hydrolyzate of pith of sago starch by S. cerevisiae

Hydrolyzate sugar concentration both at 5% and 10%, during the fermentation process, glucose is used up by the S.cerevisiae for 36 and 48 hours. This shows S.cerevisiae has the ability to ferment hexose sugars, particularly glucose very well. S.cerevisiae has invertase enzyme that acts to convert glucose into ethanol[17] . Sugar medium consisting of glucose 4.17%, fermentation by S.cerevisiae for 72 hours produced 1.57% ethanol and ethanol have started to form after 18 hours. Efficiency of fermentation in sugar hydrolyzate 5% by S.cerevisiae as much as 37.6%. While the concentration of ethanol produced by S.cerevisiae on fermentation of sugar hydrolyzate 10% in medium containing glucose 8.28%, producing ethanol as much as 4.21% for 72 hours. Fermentation efficiency of 49.2% reaching almost 50%, this corresponds to the theory that 100% of sugar when fermented will produce 50% ethanol [12].

Concentration of reducing sugar and ethanol at the end of the process of ethanol fermentation of hydrolyzate sugar by *Zymomonas mobilis*.

During the fermentation process, hydrolyzate sugar concentration 5% containing glucose

4.17% is used up by Z.mobilis after 36 hours and the ethanol yield of 1.34%.

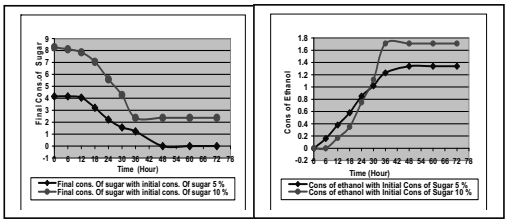

Fig 3. Reducing sugar and ethanol concentration changes during fermentation hydrolyzate of pith of sago starch by *Z.mobilis.*

Glucose is used for cell growth and ethanol fermentation substrate. Whereas in sugar hydrolysates 10% containing glucose 8, 28% still remaining glucose. .37%. This is due to bacterial cells die after 36 hours, while ethanol produced as much as 1.34% for 72 hours, the ethanol begins to form after 6 hours. Based on amount of ethanol that is formed is known that the sugar hydrolyzate 5% fermentation efficiency Z.mobilis reachs 32.13% while on the sugar hydrolyzate 10%, ethanol fermentation efficiency reach 20.65%. According to[18]. Z.mobilis has a good ability to ferment glucose, the results are even greater than the S.cerevisiae due to the formation of ethanol by the Entner Doudoroff. In this study the fermentation of sugars hydrolyzate b Z.mobilis obtained a little amount of ethanol, because the medium pH dropped to 3:37 is so low that bacterial cell death occurred.

CONCLUSIONS

1. Desizing of pith of sago starch particles in the size of 100 mesh, and gelatinization temperature at 120 ˚ C, can increase the reducing sugar and dextrose equivalent (DE).
2. Hydrolysis by acids and enzymes can be applied in synergy so as to increase the activity on the hydrolysis of lignocellulosic materials.
3. Efficiency of fermentation in the sugar hydrolyzate sugar concentration of 5% containing of glucose 4.17% *Pichia stipitis, Sacchromyces cerevisiae* and *Zymomonas mobilis*, respectively are 23.5%, 37.65% and 32.13%. While in the hydrolyzate sugar concentration10%of sugar with glucose content 8.28%, fermentation efficiency of *Pichia stipitis, Sacchromyces cerevisiae* and *Zymomonas mobilis* respectively are 9.3%, 50.85% and 20.65%.

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STUDY OF MAGNETIC FIELD AND ULTRAVIOLET ACTIVATION IN GEESE EGGS HATCHING

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Abstract
The hatching eggs disinfection it is a very important process of eggs hatching technology. The results of hatching process as well depend on the method of eggs disinfection before the incubation starts. Method of hatching eggs disinfection needs to be chosen in that way to improve the hatching eggs without harming pre and post development of poultry youth.Using of ultraviolet activation and different systems of magnetic fieldled to increase hatching index of geese eggs, better results of hatchability had the geese eggs after using magnetic field in system of permanent activation during 15 minutes and it was 75.3% in last experience or higher by 1.7% comparing with control group.Studding the embryo death during the hatching period there was established that the magnetic field used in permanent system during 15 minutes had a positive influence on this index and there was noticed the difference between first and control groups of 1.7 % for first group.Studding the number of received goslings of first quality there was noticed that it was higher in the second group wheresystem of split magnetic field was used during 10 minutes and it was higher comparing with control group by 4.2%.

Key words: *eggs hatching, magnetic field, ultraviolet activating, goslings*

INTRODUCTION

Eggs incubation technologies provide various methods to increase the eggs hatching. One of the factors influencing the outcome of hatching process is the method of hatching egg sanitizing before their incubation. Currently in eggs incubation technology there are used two methods, namely chemical and physical. The chemical method of eggs processing provides using different chemical preparations to combat bactericidal load on the mineral egg shell, one of the most commonly used preparation is formaldehyde and other solutions some of which may have negative effects. Formaldehyde has been recognized as a preparation with negative effects on human health and being used in the eggs incubation may have a negative effect on the future of the chickens. Chemical method is not perfect and can be replaced by physical method of eggs processing. The most frequently used physical methods for processing the eggs, but also to stimulate embryonic development are ultraviolet radiation, laser, ionizing and lately for egg processing there is used the method by using magnetic field [1,2,3,4, 5, 6,7].

The experimental results obtained are talking about positive action of artificial sources of eggs radiation of different species.

The positive influence of ultraviolet rays is not explained only by their bactericidal capacity, and ability to change physical and chemical properties of the eggs white and eggs yolk in the usefulness of their assimilation by the embryo [6].

Researches on mechanism of action of magnetic field on different systems and its use in poultry have been taken less compared with researches on other forms of activation.

The mechanism of biological action of the magnetic field is very compound due to its capacity of penetration and specific structural and functional features of different cells and tissues of the body.

There has been investigated the mechanism of action of magnetic field on the nervous, blood and muscle systems. Increasing of the body resistance firstlings more of all is related to nervous activity and endocrine system, which

in turn draw in this process and other body systems.
Using ultraviolet and magnetic field activations in technology incubation of poultry eggs serve as a subject in carried out experiments.
Using optimal activation systems in hatching of eggs may influence the increasing of eggs hatchability index.

MATERIAL AND METHOD

The aim of present study was studding the influence of magnetic field and ultraviolet activation on geese eggs hatching index.
The studding object had served geese eggs of Holmogor breed, magnetic field generated by apparatus UEM 3and ultraviolet raysgenerated by ultraviolet bulbs with registration numbers ICDR 4246 and ICDR 644182.
Apparatus UEM 3provides four modes of activation: continuous, frequent, impulsive 8 Hz and impulsive 16 Hz during different activation time.
Apparatus UEM 3 is provided to ensure therapeutic action on the human body using magnetic waves.
In the experiments were used geese eggs kept no more than nine days under proper technological conditions of temperature and humidity.
Before incubation there were determined the indices of hatching eggs.

Table 1. Experimental scheme

Experimental series									
First				Second			Third		
Groups	Number of eggs	Activation system	Length of activation	Groups	Activation system	Length of activation	Groups	Activation system	Length of activation
Control	600	-	-	Control	-	-	Control	-	-
I	600	permanent	5,10,15, 20	I	permanent	15	I	permanent	15
II	600	split	5,10,15, 20	II	split	10	II	split	10
III	600	impulsive 8Hz	5,10,15, 20	III	impulsive 8Hz	10	III	impulsive 8Hz	10
IV	600	impulsive 16Hz	5,10,15, 20	IV	impulsive 16Hz	10	IV	ultraviolet	15
V	600	ultraviolet	5,10,15, 20	V	ultraviolet	15	-	-	-

Activation of eggs with ultraviolet rays and magnetic field was made directly in the incubation boxes before eggs being placed in the incubator. Incubation was carried in the incubator of ИУП- Ф-3М type.
The experiments were performed in three experimental series. Experimental scheme is presented in Table 1.
The eggs of experimental group were exposed to activation with magnetic field and ultraviolet systems. There were not activated the eggs of control group. After the incubation process was finished the hatching index were calculated. All hatched goslings of each group were divided into quality class.

RESULTS AND DISCUSSIONS

At the end of the first series of carried out experiments in geese eggs hatching with use of ultraviolet activation and magnetic field with different activation times were determined the regimes that had the positive effect on analyzed hatching indices, also there was noticed a comparative decrease of embryo death and increasing the quality of goslings of first quality. In table 2 there are presents the results of incubation of geese eggs activated with ultraviolet activation and magnetic field in the first series of experiments.

Table 2. The highest results of hatching index(first experience), %

Groups	Hatchability	± to control group	Eggs hatching	± to control group	Embryo death	± to control group
Control	74.6	-	62.7	-	25.4	-
I, 15 min.	81.9	+7.3	69.0	+6.3	18.0	-7.4
II,10 min.	74.4	+2.8	64.5	+1.8	22.6	-2.8
III,10 min.	83.8	+8.8	69.8	+7.1	16.8	-8.6
IV,10 min.	79.2	+4.6	66.0	+3.3	20.8	-4.6
V, 15 min.	76.4	+1.8	63.7	+1.0	23.6	-1.8

According to the data presented in table 2it is necessary to mention that the activation system of gees eggs by magnetic field having the highest influence on hatching index was impulsive 8 Hz system with the length of activation 10 minutes and the level of goslings hatchability was 83.8% or by 8.8% higher comparing with the control group, where this index was 74.6%.

It is necessary to mention that in experimental group nr.V where ultraviolet activation during 15 minutes was used the best result had been received. There was recorded the hatchability at the level of 76.4%.Studing the embryo death as well the lowest level was noticed in experimental group nr. III being 16.8% or lower comparing with control group by 8.6%.

During the experience there was studied the number of received goslings of first, second and third quality. The results of this study are presented in Fig. 1. There is presented the number of goslings of first quality and the difference between control group.

Data presented in Fig. 1 showed that the highest number of goslings of first quality had been received in second experimental group, at the same time there was noticed that in two experimental groups this index was lower comparing with control group, so it is necessary to mention that the activation had different influence on this index.

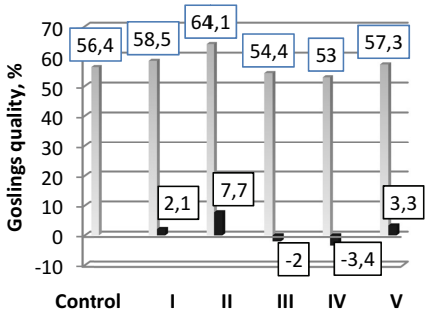

Fig. 1 Goslings quality,% (first quality)

The lowest result of hatching index was received using permanent system during 5 minutes, and hatchability was 66.4% or lower comparing with control group by 8.2% and embryo death was higher by 8.2%.

In second experience there were used the activation systems that had showed good results in first experience. The results of the second experience are shown in table 3.

Table 3. The highest results of hatching index in second experience, %

Groups	Hatchability	±to control group	Eggs hatching	± to control group	Embryo death	± to control group	Goslings quality	± to control group
Control	71.6	-	63.2	-	28.4	-	40.9	-
I	75.9	+4.3	66.7	+3.5	24.1	-4.3	42.5	+1.6
II	74.2	+2.6	65.8	+2.6	25.8	-2.6	47.3	+6.4
III	73.8	+2.2	65.2	+2.0	26.2	-2.2	44.8	+3.9
IV	73.1	+1.5	64.7	+1.5	26.9	-1.5	38.4	-2.5
V	74.5	+2.9	66.3	+3.1	25.5	-2.9	47.4	+6.8

Presented results showed in experimental groups the goslings hatchability was higher compare with control group but it is necessary to mention that the highest index was noticed in first experimental group where permanent system of magnetic field was used during 15 minutes and it was 75.9 % and in the control group this index registered 71.6% as well as the embryo death was lower in all experimental groups but the lowest was noticed in the same experimental group with the difference between control group - 4.3%

According to the data the highest number of goslings of first quality was received in experimental group nr. V where ultraviolet activation was used and this index registered 47.4% to 40.9 % in control group.

Feather there was held third experience. For this experience there was chosen the activation systems which showed better results during two previous experiences. There are shown the results of third experience in Fig. 2.

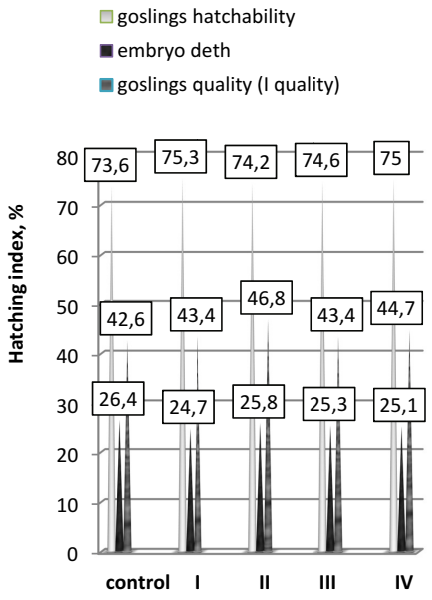

Fig.2.Hatching index, %(third experience)

The results of third experience showed that studied index of hatchability were higher in experimental group but better results were received in group I where permanent magnetic system was used during 15 minutes.

CONCLUSIONS

After the experience has been finished it is possible to make next conclusions:

1. Using of ultraviolet activation and different systems of magnetic field led to increase hatching index of geese eggs, better results of hatchability had the geese eggs under the influence of magnetic field in system of permanent activation during 15 minutes and it was 75.3% in third experience or higher by 1.7% comparing with control group.
2. Studding the embryo death during the hatching period there was established that the magnetic field used in permanent system during 15 minutes had a positive influence on this index and there was noticed the difference between first and control groups of 1.7 % for first group.
3. Studding the number of received goslings of first quality there was noticed that it was higher in the second group where system of split magnetic field was used during 10 minutes and it was higher comparing with control group by 4.2%.

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RESEARCHES CONCERNING THE ORGANIZATION OF A CRAFT MANUFACTORY FOR PROCESSING UNREELING SILK COCOONS

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Abstract

In the Romanian village the artisanal crafts were always a cultural-artistic and economic activity, which material and spiritual filled in the rural community. The existence of textile raw materials and some of other sources in the sericultural (family) microfarm requires only a correct stimulation of the crafts in the direction of activity development on the basis of economic and aesthetic orientation, of what specialists call Romanian "ethnodesign". The paper aims to present the organization of a craft manufactory for processing the unreeling silk cocoons from wastes of the technological process in the obtaining the reproduction biologic material, Bombyx mori L. Species. Such a technological model has been omologated and it works in the sericultural microfarm of Niculescu family from Stoenesti – Valcea. Its completion assumed the selection of some ways of processing the textile raw materials into fabrics and handicraft objects under conditions of simple technologies, as well as the documentary study on a minimum machinery inventory for a textile manufactory, structural, technological and economic adapted and integrated in the management of the reproduction sericultural microfarm. The activities carried out in the frame of craft manufactory are based on textile techniques and technologies of processing, dyeing, weaving, wickerwork, sewing, node making and finishing the raw materials from wastes and the sericultural by-products. By processing the textile material in the craft manufactory, the researchers have established a flow based on two technologies and six techniques of treatment, by which three types of yarns were obtained (by hand-made reeling) with 15 chromatic variants. By using the obtained yarns three prototypes of "basse-lisse" weavings have been created (thin fabrics type designed for spring-summer garments). Following the applicative researches carried out in the laboratories of National University of Arts Bucharest, for the craft manufactory two technologies and six techniques of textile material processing have been selected. Four types of yarn (by hand-made reeling) in 24 chromatic variants have been achieved. There have been elaborated the technological processes of three weaving prototypes, "basse-lisse" texture having the technical parameters in the category of thin abrics designed for spring-summer garments.

Key words**:** *craft manufactory, silk yarn, unreeling silk cocoons, loom*

INTRODUCTION

In Romania the rural civilization preserved an ancient traditional culture in which the sericulture gradually was inserted starting from the XIVth century, representing a well developed activity in the economy of the peasant household and connected with the other regional or local activities (agriculture, fruit growing, livestock breeding, fishing, raising honey bees). The geographic location in the continental-temperate zone and the pedoclimatic conditions promoted the mulberry cultivation, a tree which today is large spread from the plains area, podiums, till the slopes of the high subcarpathian hills. The village creation of material and cultural assets by practicing different textile crafts has been boosted by the changes in the Romanian society starting from the end of the XIXth century, along with the foundation of the modern European state, Romania.

A particular interest is manifesting in the last years in the majority of sericultural countries

for the recovery of wastes and sericultural by-products (unreeling cocoons, wastes from cocoon reeling, chrysalis) reflected in the scientific research [1], [2], [3], [4], [5], and [9].
The paper aims to present the organization of a craft manufactory for processing the unreeling silk cocoons from wastes of the technological process in the obtaining the reproduction biologic material, Bombyx mori L. Species [8].

MATERIAL AND METHOD

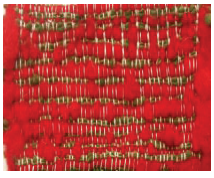
The principles which formed the basis for the organization of the pilot textile craft manufactory were: technological simplicity, coherency in the tradition continuing, accessibility in the learning of operating specific tools and machines, originality and variety of aesthetic solutions, compatibility with the core utility destination, commercial attractivity characteristic of the ecologic and hand-made textile production.
The determinants factors in the choice, positioning and flow of the textile process are:
a. The space organized in a minimum perimeter, multifunction fitted and equipped with utilities (electricity, current water, access to endowments).
b. Mechanical and manual technologies easily reproducible.
c. Simple machines with multiple possibilities of operating and positioning in certain processes of production and storage during breaks.
The labour initial composed the members of the farmers' family with minimum knowledge of "hand work".
The production composed of two textile technologies: manual reeling and weaving in multiple variants, following prototypes technical and aesthetical balanced, with different utilities and easily reproducible. The prototypes experimented and implemented in production have possibilities of creative evolution in the future.
The raw material on which is based the craft textile production is represented by the unreeling silk cocoons and different types of silk yarns.

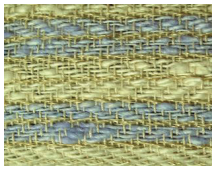
RESULTS AND DISCUSSIONS

Utilising the two core textile technologies in the craft manufactory there are accomplished mainly silk yarns and fabrics from natural silk or from mixtures of yarns specific to the ethnogeographic zone in which the microfarm is located.
In accordance with the number of yarns which doubles and with the torsion degree, more types of silk yarns result, with specific names and different uses in fabric structure.

Table 1. Different types of silk yarns obtained in the sericultural micro-farm

No	Commercial name	No.of doubled yarns	Cocoon no.for one yarn	Simple yarns torsion - twistings/m	Twisted yarns torsion – twistings/m	Yarns' destination
1.	"Organzin"	2-3	3-8 reeling	About 700	About 600	Warp fabrics
2.	"Grenadin"	2-3	3-12 reeling	About 700	About 1400	Warp fabrics
3.	"Trama"	2-4	3-12 reeling	About 700	8-120	Weft fabrics
4.	"Crepe"	6-8	3-12 mixture	About 700	800-3200	Crepe fabrics
5.	Yarns for knitwear	2-3	3-12 reeling	` About 500	About 400	Knitwear
6.	"Marabaut"	2-3	3-12 unreeling	8-120	1000-2000	Weft fabrics
7.	Sewing yarns	In accordance with the needs of creating textile assembly creation, mechanic or manual				Sewing yarns






The yarns obtained by spinning in the textile craft from unreeling cocoons are found in Table at positions 3, 4 and 6 and they presented a part of the raw material used in weaving [11]. The ornamental weavings and fabrics are made in the same types of horizontal (basse-lisse) and vertical (haute-lisse) looms, but the raw material, the warp preparation and the material quality, which requires compliance with the standards for utility and aesthetic products, are different. The weaving method is the most simple: weaving in two or four yarns. The 15 chromatic variants of silk and the 52 weaving prototypes for clothing represented two qualities: one thick for the autumn-winter season and another thin for the spring-summer season, in different drawings and chromatic variants [5].

All the processes and technological operations mentioned in table 2 were performed in the technological flow of the craft from the sericultural micro-farm [10].

A. Primary operations – preparation techniques as follows: dry cleaning of impurities and powder, sorting, sizing after type, cutting for easier processing, wet cleaning, washing, gradually boil-off (variable being the time, temperature and concentration % of basic solution of calcined soda), centrifuge, dyeing in different conditions (dry form, boiled-off in different stages, fleece, yarn, finished fabric, finished textile products), preparation for twisting (fluffing, teasing - shredding), preparation for continuing the technological process (reeling).

B. Secondary operations – technologies of product forming (yarn obtaining by spinning with fork and spindle), obtaining the yarn combination (twisting), flow vertical loom (warping spotted, looming with yarns, weaving itself), flow horizontal loom (warping spotted, loomed, pulled back, weaving itself), manual knitting with knitting, finishing rough fabric (mechanical, thermic, chemical, tailoring on shape, assemblying by manual sewing or with the sewing machine), guided trimming (cutting, snipping, unravelling, steaming, ironing on mold), assembling component parts and accessories by sewing, lamination, putting on wire, wickerwork, binding, node making.

C. Finishing operations – in order to give a commercial shape and presentation (surface chemical and thermic treatments, doubling, packing and storing).

Table 2. Endowments and machines of the textile craft manufactory for silk cocoons processing

No.	Textile technology	Machine or endowment name	No. of pieces	Technical characteristics	Position in craft
1.	Weaving	Horizontal (basse-lisse) loom	1	Manual machine weaving in 2 and yarns Maximum width 90cm Maximum length 2500cm.	Fixed positioning Work surface 3m^2
2.		Vertical (haute-lisse) loom	1	Manual machine thick weaving in 2 yarn Maximum width 100cm Maximum length 200cm.	Folding Mobil Work surface 1.5m^2
3.		Accessories for basse-lisse loom	different	Metalic yarns/ Metalic reed 2 piece Shuttles 2 pieces	Folding Mobil Storable
4.	Preparation	Vertical warping	1	Support to stretch the warp at comple fabrics till 24 yarn Size 150cm./200cm.	Folding Mobil Work surface 1.5m^2
5.	Weaving	Coil supports	1	Support for coils yarn warp max. 2 yarns	Multifunctional Storable Mobile
6.		Coiling shuttle	1	Manual equipment / Yarns preparation	Storable Mobile
7.		Reeling device / Manual reeling	1	Manual tool	Storable
7.	Reeling/	Fork	1	Reeling support	Storable Mobile
8.	Spinning	Spindle	2	Yarn twisting	Storable
9.		Electrical sewing machine	1	Sewing, scalloping, quilting, embroidery	Storable Mobile
10.	Finishing	Steaming iron	1	Finishing the fabric surface	Storable
11.		Ironing table	1	Ironing support	Storable Mobile
12.		Automatic washing machine	1	Attenuation, washing, centrifuge dyeing	Fixed positionin water/sewer source
13.	Treatments	Electrical dryer	1	Drying with jet of cold and hot air	Storable Mobile
14.	Dyeings	Drying support	1	Treatments	Storable Mobile
15.		Hose withdifferent feetings	25ml.	Treatments	Storable Mobile
16.		Stainless steel containers/ warn treatments	6	Dyeings and treatments	Storable
17.		Plastics containers / cold treatments	3	Treatments	Storable

Table 3. The evaluation of income from processing 100 kg unreeling silk cocoons

No.	Name of product from unreeling silk cocoons	Raw material weight per product	Number of products realized from 100 kg cocoons	Unit price	Production value at 100 kg cocoons
1.	Silk yarns of different colours – "shantung" type	113g/100g yarns	88.50 kg yarns	33.50 lei/100g 335 lei/1kg	29 647.50 lei
2.	Thick fabric type cloth – autumn/winter season	321.37 g./ml.	311 ml	135.51 lei/ml	42 143.61 lei
3.	Thin fabric type cloth – spring/summer season	295.66 g./ml.	338.22 ml	127.78 lei/ml.	43 217.75 lei

The most important for the craft technological flow are the achievement of the effective treatments for silk cocoons and the classic techniques of reeling and weaving as basis of the craft manufactory textile production. During 2007-2008 the experimental textile craft from the sericultural micro-farm processed reeling and unreeling cocoons from the own production under the coordination of the specialists which have worked at the research project CEEX 40/2005 [6], [7]. Today they continue freelance this activity recoverying in the Romanian market the ecologic and hand-made textile products from natural silk.

CONCLUSIONS

Such a craft manufactory of processing the unreeling silk cocoons has been approved and it works in the sericultural micro-farm of the Niculescu family from Stoenesti, Valcea district. Its achievement assumed the selection of some methods of processing the textile raw materials in fabrics and craft objects under the conditions of some simple technologies, as well as the documentary study on a minimum machine inventory for a textile manufactory, structural, technological and economical adapted and integrated into the management of reproductive sericultural micro-farm. The production with simple work methods of a wide range of yarns and fabrics. The economical optimization of the silkworm rearing micro-farm, by using the unreeling silk cocoons.

The supplement of the seasonal activity from the silkworm rearing field with an economic activity which increase the economic profitability of farm and provide a secondary activity during the cold season. The continuing of manufactory tradition and of cultural specificity, in the rural area.

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HORSE HEART RATE VALUES AT DIFFERENT TIMES OF TRAINING, RECORDED IMMEDIATELY AFTER EXERCISE AND 10 MINUTES AFTER EXERCISE

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Abstract

Generally, cardiovascular adaptations that occur in increase transport capacity and oxygen consumption in muscle during exercise in the horse are: increase of cardiac output (CO) by increasing heart rate (HR) and less systolic ejection volume (SV), redistribution of blood flow from inactive to the active territories, associated with increased venous return through muscle and respiratory pumps, increase the capacity of blood oxygen transporting with improved extraction and its retention in the muscles. A standard way of sport horses to respond to physical exercise is to increase cardiac output, possibly by increasing the HR and SV. The present paper analyzes the heart rate evolution and dynamic at 15 sport horses, during 3 periods of training. Assessment of heart rate was effectively executed indirectly (via stethoscope). Appreciated immediately after exercise heart rate increased in all 3 months of training proportional to speed, reaching a maximum of 81,86±1,14 cardiac movements / minute in the third month, at a speed of 550 m / minute and decreased at 10 minutes after effort, in all 3 months of training, reaching a minimum of 53,40±0,74 cardiac beats / minute in the third month at speed of 350 m / minute. Statistically the differences were insignificant in most cases were traced.

Key words**:** *Heart rate, sport horse, training*

INTRODUCTION

Flawless operation of metabolic pathways providing ATP, but dependent on oxygen, is inextricably linked with the capacity of some organs and systems to ensure a continuous flow of O2 to the muscle during labor [2].

This flow starts when air enters in the respiratory ways and until the oxygen gets to be used in aerobic processes run in the mitochondria. From the alveolar-capillary membrane O2 coupled with hemoglobin is transported by blood vessels to the muscle in exercise.

This physiological phenomenon of transport and distribution of precursors, including oxygen, has in center the vital effector - heart – which during the effort and training is adjusting the activity depending on their intensity and duration.

Generally, cardiovascular adaptations that occur in increase transport capacity and oxygen consumption in muscle during exercise in the horse are:

- increase of cardiac output (CO) by increasing heart rate (HR) and less systolic ejection volume (SV).
- redistribution of blood flow from inactive to the active territories, associated with increased venous return through muscle and respiratory pumps.
- increase the capacity of blood oxygen transporting with improved extraction and its retention in the muscles.

A standard way of sport horses to respond to physical exercise is to increase cardiac output, possibly by increasing the HR and SV [3].

MATERIAL AND METHOD

The present paper analyzes the heart rate evolution and dynamic at 15 sport horses, during 3 periods of training.

Assessment of heart rate was effectively executed indirectly (via stethoscope). Indirect

listening offers the examiner a comfortable position, without having to manipulate the animal. The chest-pieces of the stethoscope is inserted between the olecranon and axilla and the examiner sits sideways, on the left side of the horse. The hand that is free rest on the horse. The sounds will be: cardiac beat, pericardial sounds or/and the extra-cardiac ones. The main normal heart sounds are: first heart sound or systole, second heart sound or diastole [4].

RESULTS AND DISCUSSIONS

At speed of 350 m/min., immediately after effort in the three periods, the recorded values were very close (60.7 beats/min., 62,2 beats/minute, 64,6 beats/minute).
Values obtained after 10 minutes were also very close to normal resting values (54,8, 55 and respectively 53,4 beats / minute).

Table 1. Average values of heart rate recorded immediately after exercise and 10 minutes after exercise during 3 periods of training

Speed (m/min)	After exercise (distance 3000 m)					
	1st month		2nd month		3rd month	
	Right away	after 10'	Right away	after 10'	Right away	after 10'
350	60,70 ± 1,63	54,80 ± 1,20	62,20 ± 1,20	55,00 ± 1,25	64,60 ± 0,85	53,40 ± 0,74
450	66,60 ± 1,22	54,60 ± 1,54	67,06 ± 1,22	54,73 ± 1,35	72,40 ± 0,97	57,73 ± 0,71
550	79,13 ± 1,10	60,13 ± 2,42	80,20 ± 0,92	53,00 ± 1,14	81,86 ± 1,14	57,06 ± 0,70

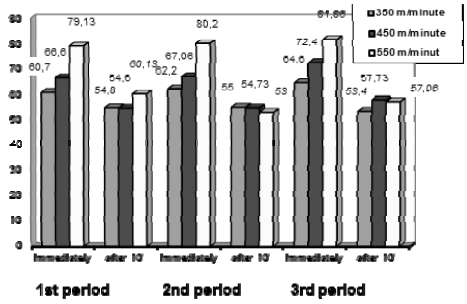

Fig. 1. Average values of heart rate immediately after exercise and 10 minutes after exercise in the 3 periods of training

At speed of 450 m/min., immediately after effort, the recorded values were slightly higher than those recorded at speed of 350 m/minute. Also in the 3rd training period at speed of 450 m/min the average value was a little higher than those recorded at the first 2 training period (66,6 beats/min., 67,06 beats/min., 72,4 beats/minute).
The average value of 1st and 2nd period were very close to each other
After 10 minutes of effort values recorded were very close: 54,6 beats/min., 54,73 beats/min., 57,73 beats / min).
At speed of 550 m/min., immediately after effort, the recorded values in the three training periods were very close to each other and the value in the 3rd period was also higher compared with the first two periods (79,13 beats / min, 80,20 beats / minute and 81,86 beats / minute).
Testing the significance of differences at speed of 350 m/min immediately after exercise, the differences between the three periods are not significant (Table 2).

Table 2. The significance of differences between the values of heart rate to 350 m / min, immediately after exercise

Period	350 m/min. immediately after effort					Significance
	$\bar{x}_1$	$\bar{x}_2$	d	Calculated t	Table t (t α) p<0,05	
1st-2nd	60,7	62,2	1,5	-0,72	2,04	NS
1st-3rd	60,7	64,6	3,9	-0,99	2,04	NS
2nd-3rd	62,2	64,6	2,4	-1,62	2,04	NS

The same can be observed at 10 minutes after exercise, the differences were not significant between the three training periods (Table 3).

Table 3. The significance of differences between the values of heart rate at 350 m / minute at 10 minutes after effort

Period	350 m/min. immediately after effort					Significance
	$\bar{x}_1$	$\bar{x}_2$	d	Calculated t	Table t (t α) p<0,05	
1st-2nd	54,8	55,0	0,2	-0,11	2,04	NS
1st-3rd	54,8	53,4	-1,4	0,98	2,04	NS
2nd-3rd	55,0	53,4	-1,6	1,09	2,04	NS

At speed of 450 m / min, immediately after effort, there were distinct differences between 1st-3rd period and 2nd-3rd period. Between 1st and 2nd period the differences were insignificant (Table 4).

Table 4. The significance of differences between the values of heart rate at 450 m / min, immediately after exercise

Period	450 m/min. - immediately after effort							
	$\bar{x}_1$	$\bar{x}_2$	d	t	Table t (t α)			
					p<0,05	p<0,01	p<0,001	
1st-2nd	66,60	67,06	0,46	-0,23	2,04	-	-	NS
1st-3rd	66,60	72,40	5,80	-3,66	-	2,76	3,67	**
2nd-3rd	67,06	72,40	5,34	-3,42	-	2,76	3,67	**

Also at speed of 450 m / min, but 10 minutes after exercise, the test of significance differences resulted insignificant differences, like the speed of 350 m / min (Table 5)

Table 5. The significance of differences between the values of heart rate to 450 m / minute at 10 minutes after effort

b	350 m/min. immediately after effort					Significance
	$\bar{x}_1$	$\bar{x}_2$	d	Calculated t	Table t (t α)	
					p<0,05	
1st-2nd	54,60	54,73	0,13	-0,06	2,04	NS
1st-3rd	54,60	72,40	17,80	-1,83	2,04	NS
2nd-3rd	54,73	72,40	17,67	-1,96	2,04	NS

At speed of 550 m / min immediately after exercise situation are no different, resulting insignificant differences (Table 6).

Table 6. The significance of differences between the values of heart rate at 550 m / min, immediately after exercise

Period	350 m/min. immediately after effort					Significance
	$\bar{x}_1$	$\bar{x}_2$	d	Calculated t	Table t (t α)	
					p<0,05	
1st-2nd	79,13	80,20	1,07	-0,74	2,04	NS
1st-3rd	79,13	81,86	2,73	-1,71	2,04	NS
2nd-3rd	80,20	81,86	1,66	-1,13	2,04	NS

At 10 minutes after exercise, at speed of 550 m / minute situation is different resulting significant differences between 1st and 2nd period, insignificant differences between 1st and 3rd period and between 2nd and 3rd period the differences are statistically distinct (Table 7).

Table 7. The significance of differences between the values of heart rate at 550 m / minute at 10 minutes after effort

Period	450 m/min. - immediately after effort							
	$\bar{x}_1$	$\bar{x}_2$	d	t	Table t (t α)			
					p<0,05	p<0,01	p<0,001	
1st-2nd	60,13	53,00	-7,13	2,56	2,04	-2,76	-	*
1st-3rd	60,13	57,06	-3,07	1,21	2,04		-	NS
2nd-3rd	53,00	57,06	4,06	-2,83	-	2,76	3,67	**

CONCLUSIONS

Appreciated immediately after exercise heart rate increased in all 3 months of training proportional to speed, reaching a maximum of 81,86 ± 1,14 cardiac movements / minute in the third month, at a speed of 550 m / minute and decreased at 10 minutes after effort, in all 3 months of training, reaching a minimum of 53,40±0,74 cardiac beats / minute in the third month at speed of 350 m / minute.

Statistically the differences were insignificant in most cases were traced.

Evolution of heart rate during the three training periods shows that regardless of horse training level, after 10 minutes the heart rate returns to values slightly above normal at rest (40 beats per minute).

Recovery heart rate after 10 minutes from exercise cessation in all three periods and at different speeds reveals high horse training level

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THE TECHNO-ECONOMY DYNAMIC SYSTEM ON BROILER FARMING INDUSTRIES IN WEST JAVA REGION

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Abstract

The basic research concept about positive feedback interaction between transformation process of technology and economy path was done with a study case approach on broiler farming industries in West Java Region. Technology path transformation as an internal aspect on micro level serves to promote the effectiveness optimality output toward, while economy path as an external aspect on the macro level serves functions to push efficiency leading of production going to input and output price stability. The aim of the research is to seek a systematic and holistic interpretation in determining problem solving model through mechanism of dynamics method system. Furthermore, this information can be used by the decision makers to formulate with appropriate strategies in facing changes of internal and external aspects. The research conclusions: Firstly, for knowledge contribution; the combination of technology and economy concept with regard to the positive interaction feedback is a contribution concept of techno economy knowledge. The mutual interaction is an issue that should be internalized in policy making process. Secondly, for policy contribution; i). in internal aspect, profile improvement of production resources competency among stakeholders need to be a part of policy on micro level, especially technology factor, ii). in external aspect, the price components of chicken meat as an output value, then raw materials and chicken feed as an input value, are macro level policy in economy factor on broiler farming industries determining.

Key words: *techno-ekonomy, broiler farming, West Java Region, dynamic system*

INTRODUCTION

The basic concept of this research is the positive feedback interaction between transformation process of technological path and economy, with a study case approach in broiler farming industries in West Java Region. Technological transformation as an internal aspect on the micro level serves to promote effectiveness toward output optimality, while economy as an external aspect on the macro level functions to push production efficiency leading to the stability of input and output price. The interaction of both factors can reflected maximal profit, in the (Fig 1, 2 and 3). (1,2)
Generally, the system dynamics of industries is determined by the interaction between internal and external factors.

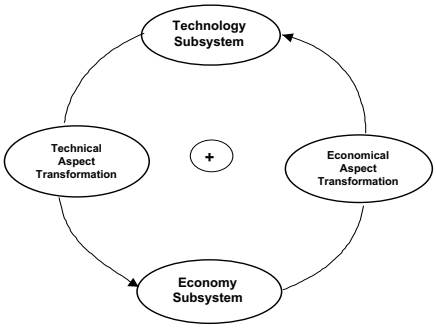


Fig. 1. Techno-Economy System

The internal factors of broiler farming are factors directly generated within the industrial system and controllable by the businessmen using technological approach, such as the process of chicken rearing, prevention of chicken mortality, and supply of chicken meat. (4,5)

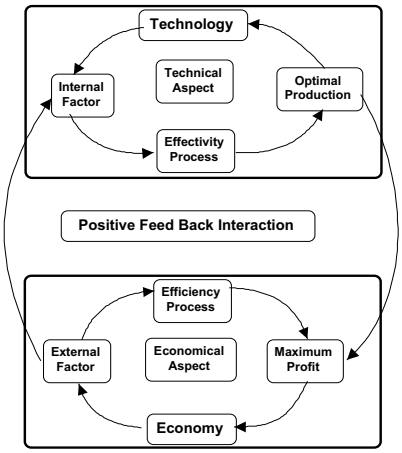


Fig. 2. Techno-Economy Transformation

The external factors consist of factors which are directly uncontrollable by mechanism within business units, but they directly affected performance of business units, were included the demand of chicken meat, substitution goods, population, income, chicken meat price, Day Old Chick price, feed price, corn price, soybean meal price, and fish meal price. (3,4)

THE RESULT OF RESEARCH

The increasing of chicken meat demand in West Java region was potential for dealing on broiler chicks, development, even for smallholder, medium or large farmer, as look as on these (Graphics 1,2,3 and Table 1). (6,7)

THE OBJECTIVE OF RESEARCH

This research aims to seek a systematical and holistic interpretation in determining problem solving model through system dynamics method. Further, the information can be used by decision makers to formulate appropriate strategies in facing changes of internal and external aspects.

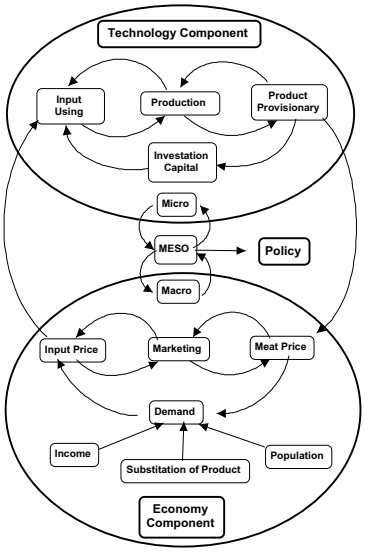
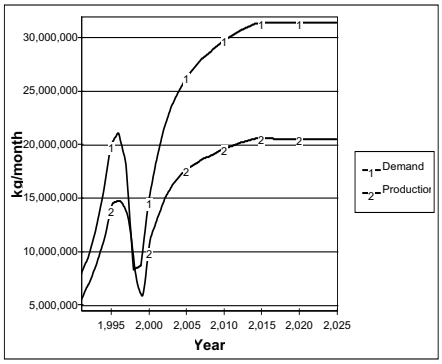
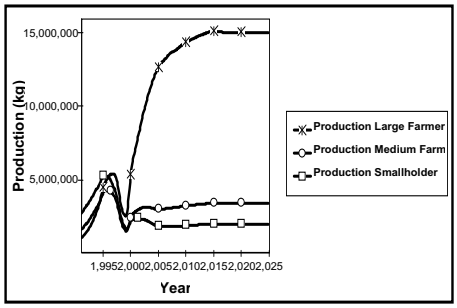


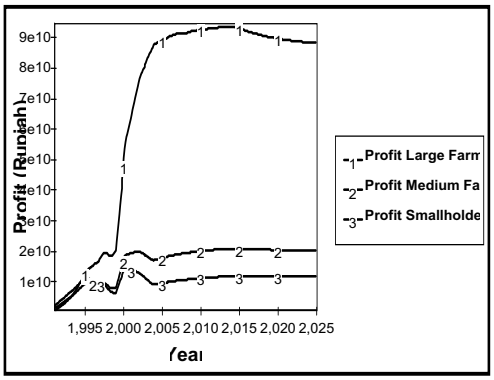
Fig. 3. Diagram of Techno-Economy Sympal Causal



Graphic 1. Simulation Product of Basic Model



Graphic 2. Broiler Meat Production



Graphic 3. The Profit of Broiler Farming Industries in West Java Region

Table 1. The Analysis of Broiler Meat Demand

Year Phase	1990-1996	1997 – 1999	2000–2003	2004-2025
Demand	Medium Increasing (8-20) jt kg	Decreasing (16-10) jt kg	Slowly Increasing (14-24) jt kg	Medium Increasing (25-31)jt kg
Production	(5-15) jt kg	(14-6) jt kg	(10-16) jt kg	(17-21) jt kg
	RP	RP	RP	RP
Chicken price	(1700-3050)	(3400-7850)	(9300-8425)	(8200-8175)
Ration price	(300-750)	(900-2150)	(2275-2100)	(2050-1975)
DOC price	(500-750)	(1000-2300)	(2200-1775)	(1775-1900)

CONCLUSION AND IMPLICATIONS

The results of this research come to conclusions as follow:

Firstly, the contribution to knowledge.

The combination of path of technological concept and economy with regard to positive feedback interaction is a contribution to concept of knowledge techno-economy of Economy as well as Technology i.e. the mutual interaction between both of them. The mutual interaction is an issue that should be internalized in the process of policy making.

Secondly, the contribution to policy i.e.:

i) From internal aspect, improvement in profile of production resources competencies among stakeholders need to be a part of policies on the micro level, especially technological factors,

ii) From external aspect, the components of price of chicken meat as an output value as well as raw materials and feed as input values are macro level policies of economy which are determinant of success in broiler farming industries.

Thirdly, the contributions to operations are:

a. Within the domain of knowledge, the development of the model of positive feedback with system dynamics method based on the concept of technology and economy approach is possible to be applied in industrial business,

b. The profile of production resources competency in broiler farming industries needs to be improved through a training system using Training Centre and Teaching Farm method,

c. Production process agricultural industry that serves as the supplier of chicken meat according to consumers preferences is able to be a standard of price control, and

d. Stability of raw material price needs to be controlled by the government through efforts to boost domestic commodity in order to reduce import dependency. Therefore, the boiler farming industries should be local resource-based industries in the long run.

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STUDY ON UNIT COST OF CERTIFICATE-TYPE BROILERS

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Abstract

A production cost means all costs for inputs consumption performed by the enterprise for goods and services produced and offered. Production costs are very meaningful about business quality and it is a decision-making condition for every producer; lowest cost level is the standard in choosing the right option. These researches were intended to give an overview about these problems. Objectives were first to find unit costs for feeds and kg live weight to broilers type Certificate and second to reduce unit costs by changing energy and protein content of feeds for these broilers. Experiment was performed with Ross 308 chickens, raised according to the technology to produce ecological poultry meat type Certificate. Three experimental variants were used; respectively three treatments/each variant and experiment design was in pens. Experimental period was of 56 days of age; feeding technology used was bi-phase, as following: group CM, with constant energy and protein level, group C1, with constant energy and variable protein level and group C2, with variable energy and constant protein level. Major production performances were checked and slaughtering was followed by cutting and finally all data were processed and read statistically. Finally unit costs per kg processed feed (1,308 – 1,362 lei) and per kg live weight (5,532 – 5,667 lei) were analyzed.

Key words: *certificate, cost per live weight, unit cost, weekly average gain*

INTRODUCTION

Cost is an extremely useful economical tool for decision making about resource usage, production amount and structure, increasing or decreasing product range, technological innovation, etc., in market oriented economies. Input consume for making such goods and services is found in their prices. Production cost in included in price and it must be calculated because: resources are limited; a smaller production cost makes a higher income possible; a smaller production cost keeps your clients and satisfies your share partners, administration board and employees [1].

A production cost means all costs for inputs consumption performed by the enterprise for goods and services produced and offered. Production costs are very meaningful about business quality and it is a decision-making condition for every producer; lowest cost level is the standard in choosing the right option [5].

These researches were intended to give an overview about these problems. Objectives were first to find unit costs for feeds and kg live weight to broilers type Certificate and second to reduce unit costs by changing energy and protein content of feeds for these broilers.

MATERIAL AND METHOD

Experiment was performed in S.D.E. Avicola Moara Domnească, experimental station of the University of Agriculture and Veterinary Science Bucharest, for Certificate type broilers, on three pen trials with even body weight and proportion of sexes in block trials. Chickens were Ross 308 and they were raised according to standard technology for this breed and in the same conditions of management, feeding and watering [3].

There were performed three treatments for every experimental flock to find bird's qualitative and quantitative performances and experiments were performed in the same time and with the same biological material and in the same unit.

Trial schedule was designed for Certificate chickens and it was as follows:

- treatment I (M): even energy and protein level;
- treatment II (E_1): variable protein level and even energy level;

- treatment III (E_2): even protein level and variable energy level;

In all the three treatments there were used 5 groups with 10 birds each were used by treatment (table 1).

Groups were formed with chickens from the same hatchery at day one. Chick's parents were of the same age to diminish genetic influence over results. Trial period was 56 days and feeding technology was biphasic. During trials it was used a processed feed produced in I.B.N.A. – Baloteşti according to the nutritional needs of chicks and based on the trial design.

Chickens live weight, feed intake and livability were the performance parameters established and checked weekly for every treatment and group during the trial.

Body weight was checked and registered weekly by individual weighting. Average daily weight gains, average weekly weight gains and average weight gains for whole trial period was calculated based on weight gain progression.

Processed feeds consumption was assessed by daily weighting of birds taking into account feeds left in feeders at the end of each week. From these data average feed consumptions were calculated.

Weekly and whole specific consumptions were calculated based on data about average weight gains and processed feed consumptions.

Mortality was registered each day and weekly mortality and mortality for whole raising period were assessed.

Table 1. Work schedule for Certificate type broilers

Specification	U.M.	Phase		
		Rising		
		T_1	T_2	T_3
Time	days	28	28	28
Flock	birds	50	50	50
Pens	no.	5	5	5
ME	MJ/kg	100	100	93.92
Protein	%	100	95.36	100
Specification	**U.M.**	**Phase**		
		Finishing		
		T_1	T_2	T_3
Time	days	56	56	56
Flock	birds	50	50	50
Pens	no.	5	5	5
ME	MJ/kg	100	100	93.06
Protein	%	100	95.30	100

Slaughtering performances were assessed at 56 days of age by slaughtering 25 % of flock. Chicks were scaled before slaughtering and chicks representing average weight of the group were slaughtered.

After slaughtering by neck breaking chicks were plucked, scaled and cut and weights of carcass, breast, legs, wing, internal organs and the rest of the carcass were assessed.

Resulting data were registered and statistically processed and for every experimental group there were assessed cost by product unit for analyzed broiler types based on results obtained.

RESULTS AND DISCUSSIONS

Cost is a value expression for a consumption of lucrative factors. Expense became cost through consumption and cost is preceded by consumption. Reducing production costs is a priority and so there have to be analyzed in details expenses included in costs, their efficiency study and the study of relationship between production costs and production outcome.

Unit costs (fix, variable, total) are calculated by referring the global costs to products quantity. Conversely, cost size for whole production in one industry or another is given by the quantity of products produces and the unit cost.

If price for acquiring production factors is decreasing at a given level of consumption of production factors for product unit cost is decreasing and opposite. If production factors price is constant and their consumption for product unit is decreasing unit cost is also decreasing. Cost increase for product unit is also influenced by change of product characteristics, product quality, etc. Limited resources of raw materials and energy are asking for more scientifically knowledge about value engineering which essentially means obtaining a minimum cost with no compromise on friability and performance etc.

Unit costs by product were assessed based on structure and cost of combined feeds used, consumption and cost of other resources and final production performances of Certificate type broilers by experimental groups [2, 4].

Table 2. Final production performances of Certificate type broilers

Specification	UM	Group		
		CM	C1	C2
Live weight	g	2384,86	2224,20	2269,60
Feed intake	kg	2,54	2,65	2,70
Live ability	%	92,00	89,60	89,80
Slaughtering output	%	81,40	76,80	78,90
Carcass weight	g	1941,27	1708,18	1790,71

Final production performances of Certificate type broilers are shown in table 2 and figure 1. Average weight at 8 weeks of age is between 2384,86 g for group CM and 2224,2 g for C1. Protein or energy variations had no influence on results and there were no statistically assured differences between results. Most favorable specific consumption is in CM, with constant protein and energy level, and the less favorable one is in C2, with variable protein and energy level, between 2,54 – 2,70. All differences between groups are statistically assured. Chickens live ability is also better in CM (mortality 8,0 %) and higher in C2 (mortality 10,2 %), but differences are not statistically assured. In conclusion, best results in production of Certificate type broilers are those of variant CM, with feed consumption significantly lower, compared to the other variants, and slaughtering performances are showing that the best efficiency of Cerificate type broilers, of 81,40%, is obtained at variant CM, with constant protein and energy.

FEED UNIT COST ANALYZE

Aims of experimental plans were both revealing unit cost by product at Certificate type broilers and a possible reduction of production costs by decreasing feed unit cost, because when a rise of feedstuffs cost brings a rise of feed cost first instinct is to find a solution to stop financial impact on our business, which usually is simply decreasing recommended nutritional parameters in feeds, to reduce feed cost by tone [6].

For this reason, average unit price of feeding for every experimental group was found based on processed feed consumption by production phase and production cost for every feed combination (Table 3 and Fig. 2).

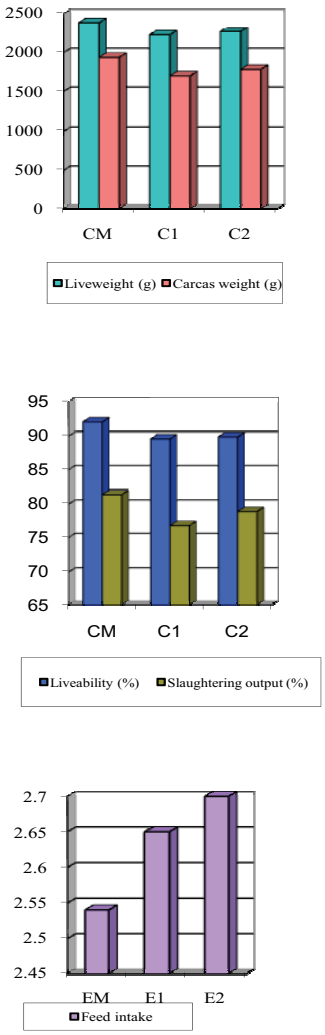

Fig. 1. Final production performances of Certificate type broilers

Table 3. Average cost of processed feed used for broilers type Certificat

Specification		Time (days)	Processed feed consum-ption (grams)	Production cost (lei/kg)	Average cost (lei/kg)
CM	Starter	0 - 28	1766,70	1,39	1,362
	Finisher	29 - 84	4290,84	1,35	
C1	Starter	0 - 28	1547,88	1,35	1,328
	Finisher	29 - 84	4346,25	1,32	
C2	Starter	0 - 28	1735,68	1,33	1,308
	Finisher	29 - 84	4392,24	1,30	

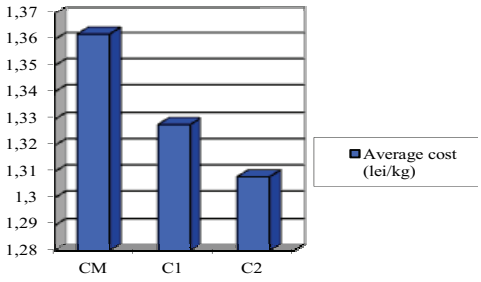

Fig. 2. Average cost of processed feed used for Certificate type broilers

Presented data are showing that at broiler type Bio production cost for combined feeds are varying by experimental group and unit cost are varying between 1,308 lei/kg to C2 and 1,362 lei/kg, to group CM.

UNIT COST BY KG LIVE WEIGHT ANALYZE

Desire to reduce feed cost per ton as much as possible should always be in agreement with maintaining or increasing profit. It is very important to understand the difference between reducing feed cost by bird and reducing feed cost by kg live weight or carcass parts. Feed cost by bird would be little diminished by reducing nutritional parameters of feeds. Performances would be reduced and results about live weight would mean rising production costs [6].

Unit cost for live weight meat production for every experimental group assessed were measured based on performances obtained in experiment (average daily weight gain, feed intake and livability), prices and resources consumption. These are between 5532,92 lei/ton in group CM, 5658,80 lei/ton in C1 and 5667,56 lei/ton in C2.

There are obvious differences about unit costs due to expenses for biological material, mortality losses, bigger or smaller compared to control group, and feed costs, due to both price differences between feeds used for the three experimental groups and higher or smaller feed intake compared to control group. So there are differences between Certificate type broilers (table 4 and figure 3) between +125,88 lei/ton in group C2 and +133,64 lei/ton in group C1.

Analyze of results reveals that diminishing nutritive composition of processed feeds are leading to lower feed costs but also to lower production performances (body weight, slaughtering output). These effects are telling that if we are dealing with a rise of feed cost reducing nutritive levels in feeds would be an answer but financial impact on whole business should be evaluated before taking such a decision.

Table 4. Cost difference structure for live meat production in Certificate type broiler

Specification		Total difference	Biological material	Feed intake	Processed feed cost
C1	Value - lei	+133,64	+73,92	+146,08	-86,36
	Structure - %	100	55,31	109,31	64,62
C2	Value - lei	+125,88	+53,76	+209,29	-137,16
	Structure - %	100	42,71	166,26	108,97

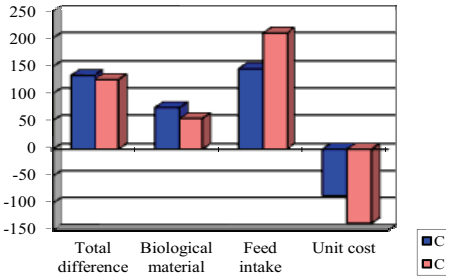

Figure 3. Cost difference structure (variants C1 and C2 compared to CM) for live meat production in Certificate type broilers

CONCLUSIONS

Researches described in this paper are leading to following conclusions:

- There are different production performances (average daily weight gain, feed intake, live ability) between experimental groups and best production performances are usually in control group CM;
- average cost of processed feed is different by experimental group and is between 1,308 lei/kg in C2 and 1,362 lei/kg in group CM;

- cost of product „live meat production" is between 5532,92 lei/ton in group CM and 5667,56 lei in C2;
- decreasing nutritive composition of processed feeds conduce is leading to lower feed costs but also to lower production performances;
- these effects are telling that if we are dealing with a rise of feed cost reducing nutritive levels in feeds would be an answer but financial impact on whole business should be evaluated before taking such a decision.

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RABBIT GENERAL ANESTHESIA FOR CATARACT SURGERY USING CISATRACURIUM AS NEUROMUSCULAR BLOCKING DRUG. CASE STUDY

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Abstract

Surgeries on rabbits are more and more frequently as they are used as pet animals and the owner is interested in their welfare. Cataract surgery is already a routine intervention in small animals as dogs and cats. This is why the aim of this study is to present a case study of a rabbit anesthesia for cataract surgery. Our subject was a three year old female laboratory rabbit which weight 2.07 kg. We premedicated him using butorphanol 0.4 mg/kg and midazolam 1 mg/kg subcutaneous 30 minutes before induction. To prevent sever bradicardia,we administered glycopyrrolate 0.01 mg/kg. Induction was realized using ketamine 10% and midazolam 0.5% mixed in the same volume and administered at a dose of 2 ml/kg. After induction the rabbit was intubated using blind technique and isoflurane was administered by the endotracheal tube. The rabbit was perfused at a rate of 20 ml/kg/h with Hartman solution, colloids and glucose all along anesthesia. To minimize the risk of cataract surgery we administered cisatracurium at a dose of 0,05 mg/kg as multiple boluses. Neuromuscular blockade was assessed using a standard peripheral nerve stimulator. Postoperative we administered enrofloxacine 5 mg/kg, meloxicam 0.2 mg/kg for postoperative analgesia and metoclopramide 0.5 mg/kg for digestive stimulation. Even if there is no dose given in literature for cisatracurium in rabbits, we concluded that it can be used with great success and with no incidence at a dose used for dogs and cats.

Key words: *rabbit, cisatracurium, cataract, neuromuscular block.*

INTRODUCTION

Rabbit's anesthesia have evolved much in the past years. The number of rabbit's owners increased as much as the demands of more specialized surgeries. Cataract surgery is a routine procedure in small pet animals like dogs and cats. The first steps in developing new cataract surgery techniques for rabbits starts with enrichment of particular anesthetic techniques, regarding a good myorelaxation. Our case study on rabbit research the use of cisantracurium to obtain a good myorelaxation. Cisantracurium is a 1R'-cis isomer of antracurium, four times more potent and has much less potential for histamine release. It is regular used as myorelaxant for dogs and cats surgeries [2].
The aim of this case study is to adapt cisantracurium use for a cataract surgery in rabbit.

Photo 1. Peripheral nerve stimulator (original)

MATERIAL AND METHOD

The subject was a two years male rabbit who was brought by the owner at CVU-ULG Liege for a cataract surgery (Photo 3. Our case study).

The rabbit was premedicated with glycopyrrolatev 0.01 mg/kg, butorphanol 0.4 mg/kg and midazolam 1 mg/kg, all administrated subcutaneous.

40 minutes from premedication, the rabbit was induced using ketamine 10% and midazolam 0.5% mixed equally and administrated intravenous as needed for intubation. The rabbit was then intubated with a 3 mm endotracheal tube using a 1.9 mm rigid endoscope.

Anesthesia was maintained with isoflurane and respiration was assisted using a closed circuit. During anesthesia the patient received intravenous a mixture of Heartmann 20 mL, colloid 20 mL and glucose 5% 5 mL solution at a rate of 20 mL/kg/hour using a syringe pump.

Other drugs administrated during anesthesia were: meloxicam 0.2 mg/kg intravenous and enrofloxacine 5 mg/kg. postoperative we administered metoclopramide 0.5 mg/kg to stimulate digestion and to reduce postoperative anorexic period.

Cisatracurium was administered at a dose of 50 µg/kg intravenous before surgery. Neuromuscular blockade was monitored using a peripheral nerve stimulator applied over the proximal lateral aspect of tibia, to stimulate superficial peroneal nerve (Photo 1. Peripheral nerve stimulator, original). We used cisatracurium 0.2% (Photo 2. Cisatracurium used in the study, original). Dose was repeated each time the neuromuscular blockade diminishes.

During anesthesia cardiorespiratory functions were assessed using a capnograph, pulse-oximeter and EKG. Respiratory rate was artificaly kept at 57 breaths/minute. End-tidal CO_2 varied between 35-45 mmHg and hemoglobin saturation in oxygen between 96-100%. Heart rate varied between 200-240 beats/hour.

Anesthesia lasted 3 hours, from intubation to extubation.

RESULTS AND DISCUSSIONS

There are also other studies regarding cataract surgery in rabbits [3]. Our study wants to encourage cataract surgeries in rabbits and to transform it into a routine procedure. General anesthesia with cisatracurium in our case study allowed a very good cataract procedure for the surgeon and for the technique. Lens problems solved well after this bilateral surgery.

Photo 2. Cisatracurium used in the study (original)

Cisatracurium is frequently administered in dogs and cats in cataract surgery at a dose of 150 µg/kg initialy, and 50 µg/kg the next boluses [4, 2]. Cisatracurium rate doses for rabbits are not recorded in literature. One study reported using a dose of 400 µg/kg cisatracurium in rabbits [1]. In this study we used the dose protocol recorded in dogs and cats, 50 µg/kg with very good results. Even if cisatracurium may produce severe tahicardia when administered fast intravenous, it did not appeare to be a problem in this study. All cardio-respiratory parameters were kept between physiological limits suggesting that cisatracurium do not interfere when is administered slowly intravenous.

Cataract surgeries without the use of neuromuscular blockade leads to hazard results because of the risk of eye movement during surgery. This is why we need to study the pharmacology of neuroblocking agents in different species were we will need to perform a cataract surgery.

CONCLUSIONS

Even if there are no studies regarding the use of cisatracurium in rabbits, our study used this neuromuscular blocking agent with great success at a normal dog and cat dose rate.
Following pharmacological researches are necessary to establish the exact dose of cisatracurium needed for a good neuromuscular blockade in rabbits.

Photo 3. Our case study

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DETERMINATION OF ZERDAVA DOG (KAPI KOPEGI) RAISED IN NORTHEAST OF TURKEY

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ABSTRACT
This is the first document on Turkish Zerdava dogs raised in northeast of Turkey. This study was carried out to define the morphologic traits of the Turkish Zerdava dogs raised in east of Turkey comparing with some other native dog breeds of Turkey. To this end, a total of 39 (19 male and 20 female) dogs was analyzed using the Minitab 15 statistical software program using ANOVA and Student's t-Test. Descriptive statistics and comparison results were for height at shoulders 51.2±0.35, height at rump 51.6±0.28, body length 56.3±0.35, heart girth circumference 50±1.43, chest width 25.6±0.22, cannon circumference 9.4±0.14, and head length 19.4±0.17 cm respectively. The overall results of the study demonstrated that Turkish Zerdava dogs had a very close resemblance to the Turkish Kangal (Karabash) and Akbash Shepherd dogs related with body measurements. The Zerdava dogs reach mature body weight and size at around 18 months of age.

Keywords: *Body measurement, genetic resource, hunting dog, live weight, phenotypic trait.*

INTRODUCTION

According to scientist the dog is the first domesticated animal in prehistoric times even though among scientist there is no full agreement on where and when dogs (Canis familiaris) originated [4]. Savolainen et al [8] reported that a genetic evidence for East Asian origin of domestic dog was found in China about 15,000 years ago. In Turkey Prof. Dr. Belli revealed that hunting seen with dog about 15.000 years ago rock carving in village of Calli, county of Kagizman, province of Kars, Turkey. Belli reported that the rock carving showed that dogs used to use to hunt deer and/orwild goats in ancient times [12]. Pang et al [6] reported that mtDNA data indicated a single origin for dogs South of Yangtze River, less than 16.300 years.

In the world there are more than 400 dog breeds [7]. In dog species (C. familiaris), guardian dogs are dogs bred to defend people and their possessions [10]. They are generally large, rugged and impressive in body. They possess great endurance and agility. These dogs are tall and powerful, yet not massive in build. This magnificent ancient working dog presents an impression of functional utility without exaggerated features. Large size is important, but correct breed type, soundness of movement, overall balance with correct temperament should be given precedence so as to preserve working ability. Flock guardian dogs show an alert, territorial and protective temperament of sheep and goats and their human family. Their possessive natural protective instinct is calm, noble, courageous, steady, intelligent, sensitive and affectionate with its own family and flock, loyal, proud, self-assured and independent. [9, 10].

In Turkey there are about dozen of native dog breeds, five of which are livestock guardian dogs listed in Table 1. In those breeds the Turkish Kangal (Karabash) Shepherd (TKnS) is the most common dog breed of Turkey. This breed can be seen almost all wide spread of country. Other breeds are generally local dog breeds. The Turkish Akbash Shepherd dog is located in triangle of Ankara, Afyon, and Eskişehir provinces. The Kars (Caucasian) Shepherd (TKrS) is mainly seen in east of Turkey. In province of Karaman and adjacent provinces there is a breed of Karaman Dog. The Turkish Tazi (Sighthound) (TT) is mainly raised especially in provinces of Konya and Sanliurfa. The

Tarsus Catalburun (Fork-nose) (TC) Dogs is a pointer type dog and can only be found in province of Icel. Dikkulak (Erect-ear) or Zagar (D/Z) dog is located in a place where TKrS dog lives. In northeast of Turkey, there are also three local dog breeds. One of them is Zerdava dog which is the subject of this study. This breed is a working type and medium size dog breed. The second breed is Fino of Tonya. This breed is a small size watch dog. The third breed is Rize Koyun dog (Bayburt Kelpi) which is another flock protection dog breed in north-east region of Turkey [10, 11, 12, 13, 14, 15, 16].

Table 1. Some morphologic traits on various Turkish Breeds of dogs

	LW (kg)	HS (cm)	HR (cm)	BL (cm)	HGC (cm)	CD (cm)	CC (cm)
Turkish Kangal (Shepherd)[11]	45.9	74.8	73.8	84.5	86.2	31.6	13.2
Turkish Akbas Shepherd[1]	44.9	75.3	74.2	81.8	86.5	32.6	13.3
Turkish Kars (Shepherd)[2]	44.6	72.4	71.1	87.3	84.7	31.3	12
Turkish Tazi[15]	18.4	62	62.2	60.3	63.3	22.8	10.2
Tarsus Catalburun[16]	21.7	48.5	48.5	49.1	64	20.8	10.5
Dikkulak (erect-ear)/Zagar[13]	10.6	27.8	29.1	46.3	50.9	21.8	9.5

The Zerdava dog is a hunting dog which is used to hunt boars, foxes, and jackals. Nowadays Zerdava dogs are used as a watch dog rather than hunting dog. Only one type of colour pattern can be seen. Main colour is dark brown or liver brown. On chest, legs, chest and point of tail there is white colour with small dark patches. This breed is potentially dangers to strangers. Zerdava Dogs are very brave, energetic and agile dogs. According to Zerdava owners, they chase a lure even for several days. They do not affair from wolves, so they are hunt by wolves in winter session. This is the main reason of decreasing number of Zerdava dogs during last several years (pers. com.).

The aim of this study is to present some morphologic traits of Zerdava Dogs by minding sex, region and age factors and by comparing with other dog breeds.

MATERIALS and METHODS

Experimental animals

The Zerdava dogs in this study were surveyed in November 2011 in the province of Trabzon (40°53'N; 39°17'E)[17]. A total of 39 dogs, 19 male and 20 female, were studied. The dogs were aged between 1 and 7 years, and divided into three age groups: 12-18 months, 24-30 months, and 36-84 months. In the first group there were 5 males and 11 females;in the second group there were 7 males and 6 females, and in the third group there were 7 males and 3 females. The ages of dogs were determined from the information given by their owners.

Measurements

The sampled dogs were measured for height at shoulders (HS), height at rump (HR), body length (BL), and chest depth (CD) by using a measuring stick calibrated in centimetres. Other linear measures such as hearth girth circumferences (HGC), cannon circumferences (CC) and head length (HL) were measured using a graduated plastic tape [5].

Statistical analysis

The data obtained were analyzed using the Minitab 15 statistical software program. Descriptive statistics for body dimensions were analyzed using ANOVA and Student's T-Test that also determined the impact of sex, country and age group on the response variables of HS, HR, BL, HGC, CD, CC and HL[1].

RESULTSANDDISCUSSION

The effects of sex, region and age on phenoltypic traits were given in Table 2. Between male and female dogs there were no significant differences for all morphological traits except the traits of HR and HL. For all results obtained male dogs yielded higher values than females except for the traits of BL and CC ($P<0.01$). For those traits male dogs yielded higher values than females.

Table 2. Descriptive statistics and comparison results of the phenotypic traits of Turkish Kars (Caucasian) dogs for different sexes, regions, ages and coat colours

	Traits		HS (cm)	HR (cm)	BL (cm)	HGC (cm)	CD (cm)	CC (cm)	HL (cm)
Sex	**Overall (n=39)**	$\bar{X} \pm S_{\bar{X}}$	51.2±0.35	51.4±0.28	56.3±0.35	58±1.43	25.6±0.22	9.4±0.14	19.4±0.17
	Male (n=19)	$\bar{X} \pm S_{\bar{X}}$	51.8±0.43	51.9±0.34	56.9±0.47	60.6±0.43	25.8±0.27	9.6±0.22	19.7±0.19
	Female (n=20)	$\bar{X} \pm S_{\bar{X}}$	50.7±0.53	50.8±0.40	55.7±0.50	55.6±2.69	25.5±0.35	9.2±0.18	19±0.26
Region	**Merkez (n=13)**	$\bar{X} \pm S_{\bar{X}}$	50.6±0.50	50.8±0.32	55.7±0.59	59.3±0.43	25.3±0.29	9±0.20	19±0.18
	Akçaabat (n=12)	$\bar{X} \pm S_{\bar{X}}$	51.3±0.76	51.5±0.65	56.3±0.68	54.3±4.52	25.3±0.40	9.1±0.26	19±0.32
	Maçka (n=11)	$\bar{X} \pm S_{\bar{X}}$	52.3±0.62	52.2±0.48	57.2±0.67	60.7±0.62	26.6±0.39	10±0.26	20.4±0.27
	Tonya (n=3)	$\bar{X} \pm S_{\bar{X}}$	50±0.58	50.3±0.33	55±0.58	57.7±0.33	24.3±0.88	9.3±0.44	19±0.29
Age (Month)	**12-18 (n=16)**	$\bar{X} \pm S_{\bar{X}}$	49.4±0.32	50±0.22	54.6±0.30	57.8±0.27	24.8±0.27	9±0.20	18.9±0.19
	24-30 (n=13)	$\bar{X} \pm S_{\bar{X}}$	52±0.54	52.4±0.47	56.8±0.51	56.3±4.30	26.5±0.35	9.5±0.24	19.7±0.33
	36-84 (n=10)	$\bar{X} \pm S_{\bar{X}}$	53.1±0.43	52.2±0.42	58.3±0.60	60.7±0.65	25.8±0.39	9.8±0.31	19.8±0.34

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

* There were no significant differences between means showed by the same letters of alphabet in the same line and factor group.

The impacts of region on live weight and body sizes are also given in Table 2. The TKrSdogs in provinces of Agri and Artvin were significantly different from others on measurements for BL (P<0.01). The dogs raised in Artvin yielded the lowest and the dogs in Agri the highest values.

With respect to ages, the descriptive statistics and comparison results are given in Table 2. Among the three age groups, 1-2 year-old TKrS dogs were significantly different to the other two groups for LW, HS, HR(P<0.01), BL, and HGC(P<0.05). After 3 years, there is minor difference for all traits. It can be concluded that the TKrS dogs grow up to 2-3 years of age, and after that there is only minor growth. In this study observed results (Table 2) were compared with other native dog breeds of Turkey (Table 1). According to results TKrS and TKnS dogs were almost similar for the traits of LW, HS, HR, BL, HGC, CD, and CC. The obtained value of TKrS dog was also in range of value of TAS dogs for live weight. TKrS dogs were two times heavier than TT, TC and four times heavier than D/Z dogs related with live weight. For other traits of HS, HR, BL, HGC, CD and CC results of TKrS were significantly higher than results of TT, TC, and D/Z dogs.

The phenotypic correlation coefficient values summarized in the Table 3 show that almost all observed traits are affected by selected factors. The highest value was found between HS and HR (r = 0.95) (P<0.01). Other high values were found between LW and HR (r = 0.81), LW and HS (r = 0.79), HR and HGC (r = 0.76), LW and HGC (r = 0.71), HS and HGC (r = 0.71) (P<0.01). The correlations of HS-BL, HR-BL, HS-CD, HR-CD, BL-CD, and HGC-CC also yielded higher values those than r = 0.50 (P<0.01). The lowest value (r = 0.30) was found between HS and CC(P<0.05). Other low correlation values were found between LW-CD (r = 0.49), LW-BL (r = 0.47), HGC-CD (r = 0.43), LW-CC (r = 0.43), BL-HGC (r = 0.42), and HR-CC (r = 0.36) (P<0.01). There were no negative correlations between the traits of BL-CC and CD-CC, as seen in Table 3.

Table 3.Phenotypic correlation coefficient values (r) between body measurements in Kars Shepherd dogs.

Traits	HS	HR	BL	HGC	CD	CC
HR	0.90**					
BL	0.76**	0.74**				
HGC	0.30	0.28	0.20			
CD	0.51**	0.47**	0.43**	0.04		
CC	0.68**	0.64**	0.36*	0.28	0.19	
HL	0.60**	0.66**	0.51**	0.34*	0.40*	0.65**

*P<0.05, **P<0.01

According to the results obtained in this research, Turkish Kars (Caucasian) Shepherd (TKrS) dogs are big-size livestock guardian dogs.

CONCLUSIONS

The overall results of this study demonstrate that TKrSdogs have a very close resemblance to the TKnSand TAS dogs for body dimensions.It can also be concluded that TKrS dogs are much bigger than the other Turkish dog breeds of TT, TC, and D/Z dogs. The TKrS dogs grow up to 2-3 years of age and that there is only minor growth. This suggests that TKrS dog reaches mature body weight and size at around 2-3 years of age. There were no significant differences among dogs From overall results of the current study revealed that the Turkish Kangal Dogs were bigger in size because of better life conditions.

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TECHNOLOGIES OF THE AGRO FOOD PRODUCTS PROCESSING

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EFFECTS OF COOKING METHODS ON THE HEAVY METAL CONCENTRATIONS IN THE FISH MEAT ORIGINATING FROM DIFFERENT AREAS OF DANUBE RIVER

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Abstract

Fish is a major source of mineral but it can also contain heavy metals. The present study has shown that the heavy metal concentration in fish meet can be affected by processing or cooking methods. Chromium (Cr), nickel (Ni), cadmium(Cd) and lead (Pb) were analyzed in cooked fish meat(4 sweet water fish species :bream, mackerel, carassius and perch, originated from the area of Sulina Arm and auxiliary canals). Various cooking methods were used such as grilling, frying, microwaving and baking. The Pb concentrations of the samples varied between 0.08 and 0.14 mg/kg . There were no significant differences ($P > 0.05$) in Pb concentrations between the raw, grilled, fried, baked and microwave-cooked fish. Ni contents decreased in grilled fish. Cr concentrations in grilled and microwave-cooked fish decreased significantly ($P < 0.05$).Cadmium was found only in fried meat fish. The results show that the grilling, microwave cooking and baking are suitable methods.

Key words: *heavy meta, fish meat, cooking methods*

INTRODUCTION

Fish meat has a special nutritive value due to its content of high quality proteins, fats rich in poli-unsaturated fat acids with a high efficiency in the human body, vitamins (especially A and D) and due to its high level of minerals (iron, phosphor, potassium, magnesium)[3]. Chromium (Cr) and nickel (Ni), which are present in fish meat, are essential for human life at low concentrations [4]. Heavy metals can be hazardous to consumers' health. Aquatic life environment makes it very easy to contaminate the fish meat with harmful heavy metals that reach the river waters through industrial residual waste, metals like: plumb, cadmium, mercury, copper, zinc. Unfortunately, some heavy metals and their compounds are considered cancerous for humans and animals [6]. The mineral content of fish can be affected by processing or cooking methods [2,5] and therefore, it is important to determine the concentrations of heavy metals in raw and cooked fish. In this context the present paper aims at determining the content of Cr, Ni, Cd and Pb from the meat of some 4 fish species : bream, carassius, perch and mackerel, originated from the area of Sulina Arm and auxiliary canals and highlighting how the concentration of these heavy metal can be affected by processing or cooking methods.

MATERIAL AND METHOD

Each of the fish species analyzed were collected during March-April 2012. We used fish weighing 200-250g on average and about 20-25 cm long. They were kept in an ice chest and transported to the laboratory. Fish were gutted, washed with tap water and filleted, and then fish fillets were divided into three groups (eight fish each). The first group was raw – not cooked (control). The other two groups (two replicates of each type of fish) were cooked in the microwave oven (2,450 MHz, 5 min.), baked in the oven (200^0C, 20 min.), in the grill oven (200^0C , 11 min) and fried (200^0C, 5 min) in sunflower oil. Raw and cooked samples were homogenized in a stainless-steel meat mince. Fish samples were digested according to the method using concentrated nitric acid (HNO_3), [1]. The digest was quantitatively transferred to a 50-mL volumetric flask and made up to volume with deionized water. A blank digest was carried out in the same way.

All metals were determined against aqueous and using a PERKIN ELMER-USA spectrophotometer by atomic absorption spectrophotometry.
The metal concentration was expressed as mg metal/kg dry weight (ppm).

RESULTS AND DISCUSSIONS

Table 1 gives the values of heavy metals content in cooked meat compared with raw fish.

Table 1. The mean heavy metal concentration of the raw and cooked meat of bream,perch,mackerel and carassius

No.	Product type	Cooking methods	Chemical element(mg/kg dry weight)			
			Pb	Cr	Cd	Ni
1	Sample Bream	Raw	0,12±0,01	1,18±0,03	ND	0,40±0,03
		Grilled	0,10±0,05	0,58±0,10	ND	0,26±0,10
		Fried	0,11±0,05	1,28±0,02	ND	0,52±0,03
		Microway	0,07±0,02	0,09±0,03	ND	0,50±0,11
		Backed	0,09±0,01	1,19±0,04	ND	0,31±0,04
2	Sample Perch	Raw	0,13±0,01	1,22±0,03	ND	0,45±0,03
		Grilled	0,11±0,05	0,60±0,10	ND	0,28±0,10
		Fried	0,12±0,05	1,30±0,02	ND	0,55±0,03
		Microway	0,08±0,03	1,01±0,03	ND	0,53±0,11
		Baked	0,09±0,01	1,20±0,04	ND	0,40±0,04
3	Sample Mackerel	Raw	0,14±0,01	1,30±0,03	ND	0,58±0,04
		Grilled	0,12±0,05	0,80±0,10	ND	0,30±0,10
		Fried	0,13±0,05	1,41±0,02	0,03±0,01	0,63±0,03
		Microway	0,09±0,03	1,11±0,03	ND	0,59±0,11
		Backed	0,11±0,01	1,27±0,05	ND	0,35±0,04
4	Sample Carassius	Raw	0,13±0,01	1,28±0,03	ND	0,50±0,04
		Grilled	0,11±0,05	0,78±0,10	ND	0,35±0,10
		Fried	0,12±0,05	1,40±0,02	0,03±0,01	0,60±0,03
		Microway	0,08±0,03	1,08±0,03	ND	0,52±0,11
		Backed	0,10±0,0	1,20±0,04	ND	0,40±0,04

ND, not determined (below the limits of detection).

The Pb concentration in raw fish had values between 0.12-0,14 mg/kg in all types of fish investigated. There was no significant difference in Pb concentrations between the raw, grilled, fried, microwave-cooked and baked fish($P > 0.05$). A previous study on the effect of cooking methods on heavy metal concentrations of African catfish done by *Ersoy(2011*) correlates with our results. The determined Cr concentration in raw fish had values betwen 1.18-1,30 mg/kg in all types of fish investigated. The highest value was found in the fried meat as 1,41 mg/kg, samples of mackerel, while the lowest value was detected in the grilled meat as 0,58 mg/kg samples of bream. The decrease in Cr concentration was significant ($P < 0.05$) for grilling and microwave cooking methods when compared with the raw control.
Ersoy (2011) showed higher values of Cr levels in African catfish fried meat.

The Cd concentration of raw, baked, grilled, microwave-cooked and fried samples of bream and perch were not detectable (below limits of detection 0.02 mg/kg).
Cd was detected in samples of meat fried mackerel and carassius and in our opinion, the increase of metal concentrations may be related to evaporation that occurs during frying processing.
The Ni concentration of raw fish had values between 0.40-0.58 mg/kg in all types of fish investigated. The increase in Ni concentrations of microwave-cooked and fried samples when compared with the grilled fish was significant

($P < 0.05$) and are slightly higher than ones found by the African catfish *Ersoy(2011)* samples fried, baked, grilled and microwave-cooked

CONCLUSIONS

Of all the species investigated, mackerel raw meat recorded a higher content of heavy metals. To all types of fish investigated, by frying, a more pronounced concentration raising effect was observed on the heavy metal contents in fish than in the other cooking methods. Therefore, this method was found inappropriate for human consumption. Baked, microwave cooked and grilled samples lost a moderate amount of heavy metals during cooking. It is possible to reduce the heavy metal concentration in fish samples by choosing a suitable method of cooking. Further studies should be carried out on cooking methods at different conditions (time, temperature, medium of cooking) for reducing the dangerous effect of heavy metals.

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STUDY REGARDING THE SENSORIAL AND PHYSICO-CHEMICAL CHARACTERISTICS OF CERTAIN WINES PRODUCED IN ROMANIA

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Abstract

Romania is well recognized for the wine consumption, vineyards and for its specific important varieties of grapes. Accordingly to the statistical data (EUROSTAT), in Romania the gross grape annual consumption for 2008 was 5.532 kg / inhabitant and 25.379 l wine / inhabitant.
The objectives of this study were to analyze by comparison certain types of wines from Romania in order to underline the influence of the raw material and technology on the total quality of the final product. For this, there were selected home made wines, ordinary wines and also superior wines, red and white. The investigated parameters were: alcoholic grade, total acidity and pH, total and reduced sugars, sulphur dioxide, superior alcohols content and also sensorial analysis of the wine samples.

Key words**:** *wine, alcoholic grade, sugars, sulphur dioxide, sensorial analysis*

INTRODUCTION

There is a tradition in Romania regarding the wine consumption because of the fact that Romania detains important varieties of wines and vine.
Accordingly to the FAO dates, compared to other European countries, Romanian occupied five place for vine surface (after Spain, France, Italy and Portugal), six for the wine production (after France, Italy, Spain, Germany and Portugal) [6]
The National Institute for Statistics (INS) published in 2009 data that showed a decrease to 1.4% in vinery surface in 2005-2009. The decrease was also registered for the grapes production: 1.004.000 tons (2009) compared to 1.010.000 tones (2008). The Romanian wine exports in 2005-2009 for "wines from fresh grapes, including alcohol enriched wines" represented between 0.05% and 0.07% from total Romanian exports. [6].

The objectives of this work were to analyse by comparison different types of wines produced in Romania. We have chosen homemade wines, young wines and superior wines also.

MATERIAL AND METHOD

Alcoholic concentration was determined using the alcoholmeter after a simple distillation and the results were correlated with temperature [3].
Total and free SO_2 was determined by iodometric titration of the total reducing substances [5].
The *reducing sugars* were determined by standard methods (Schoorl) based on their propriety to reduce in alkaline medium at high temperature the Fehling solution. Results were expressed in mg glucose /l of wine [5].
Total acidity of wines was determined by titrimetric method using bromthymol blue as indicator [5].

Superior alcohols content was determined using ρ-dimetilaminobenzaldehyde using an etalon curve [3].

Sensorial analysis was conducted accordingly to descriptive analysis and affective testing using the hedonic scale with 5 points: (5) *like*; (4) *like moderately*; (3) *not like but not dislike*; (2) *dislike moderately*; (1) *dislike*. The evaluators were 120 students from faculty of *Animal Husbandry* [4].

Abbreviation of analysed samples of wines:

VAC – homemade white wine
VRC – homemade red wine
VAM – ordinary white wine
VRM – ordinary red wine
VAS – superior white wine
VRS – superior red wine
DOC-CMD A – controlled origin white wine harvested at full maturity
DOC-CMD R – controlled origin red wine harvested at full maturity

RESULTS AND DISCUSSIONS

Alcoholic concentration

The level of alcohol in wines is negative correlated with the residual level of sugars (the sweetness of wines). The alcohol results from sugars fermentation and the higher is the alcohol in wines, the higher is the consumption of sugars during fermentation, which determines a reduced level of residual sugars in wines (figure 1).

The highest level of sugars it was observed for ordinary white wine (VAM) up to 17.85g glucose/l wine. This is correlated to a low level of alcohol level (10.6% vol.).

It can be also observed from figure 1 that the lowest level of sugars was registered for DOC-CMD R – controlled origin red wine harvested at full maturity, at 2.5g glucose/l wine.

It is also interesting to observe that the home made wines (white and red) have a high level of alcohol (up to 11-11.5% vol.) and a low level of residual sugars. This could be due to the fact that the fermentation is intense and also confirm the local producer's declaration that they did not used added sugar for fermentation.

Acidity of wines

This parameter is reflected in Fig. 2, compared to the level of glucose content.

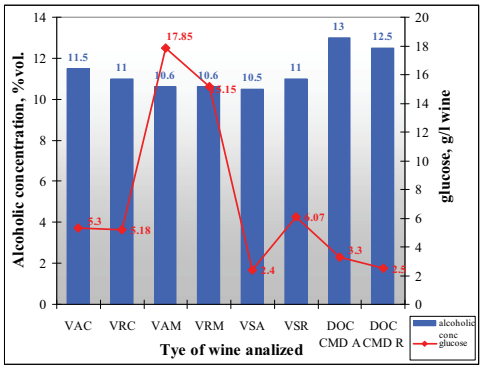

Fig.1. Alcoholic concentration of wines vs. reducing sugars content

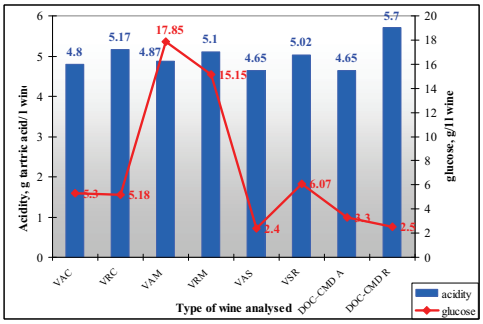

Fig. 2. Acidity of wines, compared to reducing sugars level

First of all, it can be observed from figure 2 that red wines had a higher level of acidity compared to white wines: 5.1g tartric acid/l wine for VRM sample compared to 4.87g tartric acid/l for VAM sample. For special wines with controlled origin, the red one (DOC-CMD R) has an acidity with 18.42% higher that the white one (DOC-CMD A). This can be explain by the fact that the technology of red wines includes the whole grape which determines the intense extraction of tannins, anthocyans and acids, responsible for the acidity in wines, but also for the specific astringent taste of red wines [2].

Comparing the white wines, it can be observed that the lowest level of acidity was registered for superior white wine (VAS) 2.4g tartric acid/l wine.

Level of SO_2 in wines

Sulphur anhydride (SO_2) can be considered as a natural component of wine because a lot of grapes because specific yeasts can metabolise the sulphur compound into SO_2. But the SO_2 resulted from this transformation is not enough to cover the antiseptic activity, the need to prevent enzymatic oxidation and wine clarification (this is the reason why in winery it is added SO_2).

Sulphur anhydride (SO_2) is found as free, active form (as gas) or bounded in covalent links. The total level of SO_2 is the result of addition of free and bounded SO_2 [1].

From figure 3 and 4 it can be observed that the red wines need less added SO_2 for conservation and stabilisation that white wines.

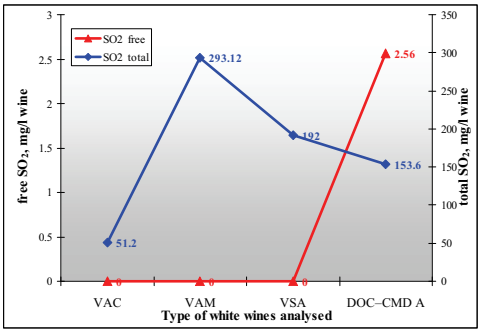

Fig. 3. Level of sulphur anhydride in white wines

It can be also concluded from figure 3 that the highest quantity of SO_2 in white wines was 293.12 mg SO_2/l ordinary white wine (VAM). This is correlated to the total level of residual sugars in wine; this wine is containing the highest level of sugars. The higher is the residual level of sugars, the bigger is the possibility of fermentation, so it is needed a higher level of SO2 for stabilization. The controlled origin white wine harvested at full maturity (DOC-CMD A) has a lower level of residual sugars, so, the level of SO2 is only 153.6mg/l wine.

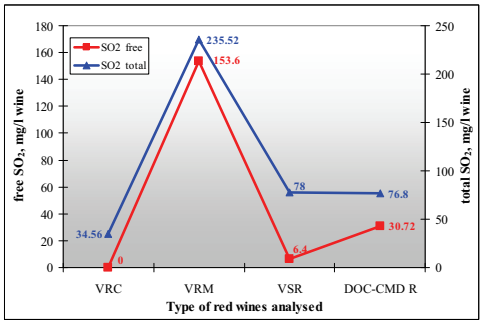

Fig. 4. Level of sulphur anhydride in red wines

The red ordinary wine (VRM) has the highest level of total SO_2 (235.52 mg SO_2/l wine), which is smaller than those in ordinary white wine (VAM).

Superior wines (DOC-CMD A and DOC-CMD R) registered similar level of total SO_2: 76.8 mg SO_2/l white wine respectively 78 mg SO_2/l red wine).

The level of SO_2 in homemade wines is low (total SO_2 is 51.2 and 34.56 mg SO_2 /l wine, for white and red respectively). This conduces to a smaller shelf life, maximum 6 months. But this is not a problem for producers because this type of wine is produced for self consumption of the family, not for commercial activities.

Superior alcohols

The main superior alcohols founded in wines are: isobutylic, izoamylic and amylic alcohols. Their content varies from 0.15-0.50g/l and represents 0.03-0.06% from total alcoholic grade of wine. They result from yeast fermentation of sugars and their quantity depends on the nitrogen compounds of wine, total sugars, yeast species and fermentation conditions.

So, their quantity is not so important, but their quality and role: they influence the palatability, texture and other sensorial characteristics of wine.

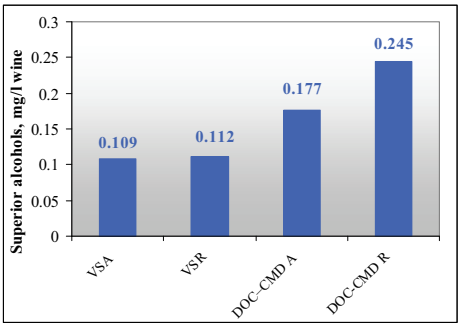

Fig. 5. Superior alcohol level in red and white wines

In figure 5 it can be observed that superior alcohols were founded only in superior wines (DOC-CMD red and white and VAS (VRS).
The higher level of superior alcohols was found in red DOC-CMD wine (0.245 g superior alcohol /l wine) and the smallest was in superior white wine – 0.109 g superior alcohol /l wine.

Sensorial analysis

After the sensorial evaluation of all samples, the panel expressed their preferences.

The sensorial profile for all types of wine regarding limpidity, colour, aroma and taste is shown in figure 6.

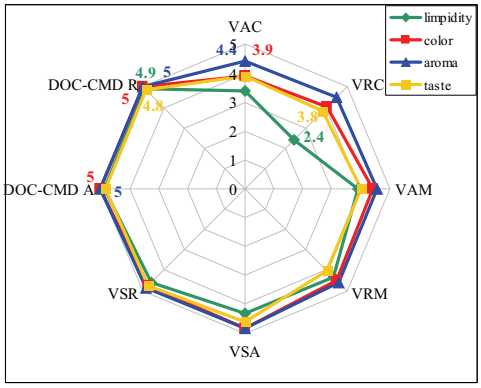

Fig. 6. Sensorial analysis for investigated white and red wines

For figure 6 it can be observed that the most appreciated *limpidity*, 4.9 points, was for DOC-CMD red wine and the less appreciated for homemade red wine (VRC), 2.4 points.

The best *colour and aroma* was found for superior wines, red and white equally (DOC-CMD) with 5 points.

Regarding the taste, the best score was registered for DOC-CMD red wine and the lowest for red homemade wine (VRC).

CONCLUSIONS

Experiments showed the correlation between the alcohol and residual sugars in wines (both white and red)
The highest level of SO_2 (293.12 mg SO_2/l wine) was registered in ordinary white wine (VAM) which is the sweetest wine of all samples, so it was needed additional SO_2 for conservation and stabilisation.
Only superior wines had superior alcohols, as a result of the yeast metabolism.
The most appreciated wines were superior controlled origin white wine harvested at full maturity, both red and white but the other were also well appreciated as aspect, aroma and colour, the less appreciated was a young homemade red/white wine which was not maturated and has no opportunity to develop these characteristics.

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THE CONTAMINATED MILK AND INFLUENCES ON HUMAN HEALTH

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Abstract

Milk product is the essential for new born babies and has an important influence in human daily food. It's important to know that quality milk directly influences the human health. This paper presents the effects of contamination milk on human health. Controllable factors that either positively or negatively influence the finally product are referred to the quality control. The use of good raw material is the primary importance for the achievement of the required finally product quality.

Key words: milk, pathogenic microorganisms, pesticides, antimicrobial.

INTRODUCTION

In industrialized countries, the percentage of the population suffering from food borne diseases each year has been reported to be up to 30%, while less well documented developing countries bear the brunt of the problem due to the presence of a wide range of food borne diseases, including those caused by parasites. The high prevalence of diarrhea diseases in many developing countries suggests major underlying food safety problems. The global incidence of food borne disease is difficult to estimate, but it has been reported that more 1.8 million people died from diarrhea diseases. A great proportion of these cases can be attributed to contamination of food and drinking water. Additionally, diarrhea is a major cause of malnutrition to infants and young children. Each year millions of people become ill and thousands die from a preventable food borne disease [16].

Quality control is the sum of all those controllable factors that ultimately influence positively or negatively the product quality e.g. selection of raw materials, processing methods, packaging, methods of storage distribution etc. Quality is defined as any of the features that make something what it is or the degree of excellence or superiority [7, 8].

MATERIALS AND METHODS

The milk is an alimentary product, of animal origin essential to the human alimentation. The milk quality is very important for the health of the new born babies and of humans. By milk, there may be brought many substances harmfully for human body, with negative effects upon human health.

This work paper presents the most important contaminating agents, which can be found in milk, the effects of these ones have upon animal and human organism, as well as the law foresights.

Proper food preparation can prevent many food borne diseases. As part of its global strategy to decrease the burden of food borne diseases, WHO identified the need to communicate a simple global health message, rooted in scientific evidence, to educate all types of food handlers, including ordinary consumers. In developed countries, surveillance of food borne disease is a fundamental component of food safety systems. Surveillance data are used for planning, implementing and evaluating public health policies. There is therefore a strong need to strengthen surveillance systems for food borne disease [12, 15].

RESULTS AND DISCUSSION

Selenium organic are found in the milk from the animal with have been feed with plants harvested from soils rich in selenium. The selenium is gathered in the plants and it replaces the sulphure from the molecule of the sulphure amino-acids. The selenium-methzonin, methyl-selenium-cistein. Within the digestive tube, the amino-acids liberate selenium which speeds in the organism and enters in the structure of muscle proteins and organs [3, 4].

The selenium content in milk and meat may reach the level of about 17,8 mg Se/kg product. The foods rich in selenium produce troubles in the human body: yellow colors of skin, nails defaults, cronical arthritis. The selenium passes easily in the placenta, negatively acting upon the body, it is secreted in the milk, send the lack of selenium leads to a sensitivity growth of the body against cancerigenic factors.

Toxic pollution and chemical contamination substances. Pesticides are toxic chemical substances used in agriculture. The pesticides may penetrate into the animal body on respiratory, oral or skin way. Feeding animals, with feeds which contain small doses of organically processed insecticides determine a significant gathering of residues during 1-2 months [4].

The organic processed insecticides are selectively accumulating in the animal body: in the fat mesenterical tissue, the fat deposited tissue, the fat perinneal tissue and the muscle tissue.

The concentrated feeds (grains, oil grist's) and the root plants are responsible with the accumulation of some pesticides residues in big amounts.

Inside the organism, these organically processed substances may be absorbed by tissues without undertaking significant changes in chemical or toxic structure. A part of them is eliminated by milk, this being the main way of detoxification for females. These substances concentrate themselves in the milk fat. The quality varies between 2-20 mg/kg in butter; we may reach quantities of 65 mg/kg. The skimmed milk and the whey comprise insignificant qualities. The pesticides in milk can be found in fresh cheese. The milk pasteurization has no effect upon the diminution of pesticides quantity, in exchange, the maturation process produce an important decrease of initial concentration.

The danger of being exposed to the pesticides, residues presents a close connection with the growth: the brain and nervous system development may be easily affected, especially during the new born babies uterus life, whose brains are incompletely developed and they may be also vulnerable to getting ill of cancer [4, 6].

According to the Order no. 147/2004 of ANSVSA for the approval of sanitary-veterinary norms and for the aliments safety concerning. The pesticides residues in animal and non-animal origin product and the veterinary use medicines, residues in the animal origin products, the highest toxic potential pesticides in milk are presented in table no. 1.

Table 1 Maximum pesticides residue limits for milk

Pesticide residue	MRL (mg/kg or ppm)
	Cow milk and cream
Aldrin, exprim in dieldrin (HEOD)	0.006
Chlordane	0.002
DDT	0.02
Endrin	0.0008
Heptachlor	0.004
Hexaclorbenzen	0.01
Hexaclorciclohexan (HCH)	Isomer alfa- 0.004 Isomer beta- 0.003 Isomer gama (Lindan)- 0.008
Deltametrin	-
Methidathion	0.02

In 2011 it was published a new Regulation (EU) No 1274/2011 that coordinated multi-year control programme of the Union for 2012, 2013 and 2014 to ensure compliance with maximum residue levels of pesticides and to assess the consumer exposure to pesticide residues in and on food of plant and animal origin and aims to reduce levels of pesticides in food.

Nitrites and nitrates are soil natural components coming from the mineralization of nitrous substances of vegetal and animal origin. Although, the animals may ingest nitrites quantities, from feed and water, the nitrites content is reduced. In milk, it can be excreted a small quantity of nitrates and their concentration doesn't surpass 40-50 mg/liter. The nitrites are practically absent in the milk.

In our country, it is allowed the nitrites addition to the milk meant for the cheese preparation, in order to prevent the early deterioration (produced by coliform bacteria's) and the swelling of maturate cheese (hard cheese) by the development of germens in the clostridium groups [2, 9].

In the powder milk, there were found nitrosamines in average of 1.9µg/kg in milk, yoghurt, kefir and fresh cheese, cream, butter and some cheese types were found nitrosamines.

The level of nitrates and nitrites present in the human body will depend upon the intake of

alimentary products which contain nitrates/nitrites.

The nitrates ingested once with the aliments reach the stomach and the intestines where they are is a less or bigger measure turned into nitrites. This nitrite formed in the stomach is a risk factor in the stomach cancer beginning. The nitrates are less toxically, having a local irritating action upon the digestive tube, causing congestions and blooding as well as kidney congestions.

The nitrites are a lot more toxically then nitrates. The nitrites inhibit the mitochondrial breathing and the oxidation phosphorus process, the effect being stronger in the case of a low pH. They diminish significantly the absorption of proteins and lipids. In case of toxic doses, the nitrites action taken place at the level of digestive system and of kidneys, leading to vomiting, colic's, diarrheas, poliuries and collapse. In combination with amines, the nitrites form nitrosamines with toxic mutagens, has a cancer action [9]. In some case, the nitrosamines content in cheese depends upon the nitrites content, the cheese kind and the preserved conditions. The forming of nitrosamines in cheese depends in great measure on the maturation process. The *Penicillium camemberti* and *Mucor ssp.* moulds present the capacity of favoring the nitrosamines formation. The nitrosamines content when cheese is maturated may increase from 5 to 20 μg/kg but it is estimated that it doesn't present any danger for the consumer's health.

Metals with toxic potential are the metals which present a toxically effect upon human body. The heavy metals belong to this category. These ones may get in the milk, but more ways: either by animal's intake of some aliments which contain maximal level of metals or by the milk transport or the equipments and devices corrosion.

Table 2 Maximum heavy metal residue limits

Aliments	MRL (mg/kg humid mass)						
	As	Cd	Pb	Zn	Cu	Sn	Hg
Cow milk	0.1	0.01	0.02	5	0.5	-	0.01
Dry milk	0.25	0.05	1.0	25	3.0	-	0.05
Cheese	0.15	0.05	0.5	25	2.5	-	0.05
Molten cheese	0.3	0.05	0.4	40	3.0	-	0.05

Radionuclide The aliments radioactivity represents an important reason of concern for consumers but also for the manufacturers of agro-food products. We find natural and artificial radionuclide in nature. The contamination sources are: the experimental nuclear explosions, exploded electral-nuclear centrals, radioactive mines exploitations [4].

Antimicrobial agents are administered in therapeutic treatment of cattle and constitute a common cause of the presence of chemotherapeutic drug residues in milk. Mastitis is the most prevalent disease of milk-producing cattle which requires antimicrobial treatment [5, 10].

The presence of certain antimicrobial agent residuals in milk constitutes a potential hazard for the consumer and may cause allergic reactions, interference in the intestinal flora, and resistant populations of bacteria in the general population, thereby rendering antibiotic treatment ineffective. Important losses are also provoked in the fermented products, by inhibiting the bacterial processes involved in the elaboration of cheese and cultured milk products [4, 11].

Biological pollution toxic substances: micotoxins. The molds may produce in a great measure, a series of toxin substances which are called micotoxins and they are very harmful to the human body [1, 3, 14].

Micotoxins are chemical substances, with a little analyzed chemical structure, having toxic effects upon the animal and humans, being synthesized by molds. The micotoxins may be comprised by the mold spores, by the whole fungus and they may also be excreted in the foods which represent a growing substratum for moulding. We can find the aflatoxins in milk.

Aflatoxins are secondary metabolites of mold *Aspergillus flavus*, contaminating diverse food and feed materials. In consideration of the carcinogenic properties of aflatoxin B_1, human exposure should be reduced to levels as low as reasonable achievable. Milk is contaminated with the aflatoxin M_1, following exposure of lacting animals to aflatoxin B_1, albeit its lower carcinogenic potency, maximum levels for aflatoxin M_1 have been st for consumable milk at 0.05 μg/kg, and 0.025 μg/kg for infant formulae, respectively, aiming to reduce human exposure to the lowest achievable level.

Table 3 Maximum aflatoxin M_1 limits

Cur. No.	Products	MRL (µg/kg)
1	Milk (raw milk, milk for the manufacturing of dairy products and the thermical treatment)	0.05
2	Infant formulae and the continuation formulae, including the milk for sucklings and the continuation of formula)	0.025

The toxic effects depend upon the dose, the administrating way, the exposure period, the feed quality, the breed and the animal age. The most frequent locations of tumor process are: the liver, the esophagus, the glandular stomach, the colon, the kidney, the duodenum and the skin. There is no specific treatment for the aflatoxins [13].

CONCLUSIONS

Presences of the residues in milk is very dangerous for human health and that's why there is a lot of norms and directives what try to impose some minimum residual level for all the substances, natural and synthesis substances, and in time to reduce this level at minimum. Each country establishes a MRL for every toxic substance for human body. That's way there is small different concerning this MRL. It is important to make a strategy to decrease the number of foodborne diseases, WHO identified the need to communicate a simple health message, rooted in scientific evidence, to educate all types of food handlers, including ordinary consumers. In developed countries, surveillance of foodborne disease is a fundamental component of food safety systems. Surveillance data are used for planning, implementing and evaluating public health policies. There is therefore a strong need to strengthen surveillance systems for foodborne disease.

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THE INFLUENCE OF USING DIFFERENT PROTEOLYTIC ENZYMATIC PRODUCES OVER THE CHARACTERISTICS OF FLOUR BREADING PRODUCTS

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Abstract

The utilization of enzymatic produces is a way of improving the process of breading products, moreover of the properties of the cereal products. Normal flour has a reduced proteolytic activity, while the flour made of sprouted wheat or attacked by the wheat bugs which has a special proteolytic activity, such that the resulted produces from the processing of such flour do not have the required quality. To improve this flour a supply of proteolytic enzymes will be used which gives the possibility of adjusting rheological indicators of the dough according to the technical requirements. After the analyzes being done, it has been noticed that for equal proteolytic activities, the enzymatic produces of various origins have a different effect on bread volume and porosity. The best results are obtained from the addition of fungal protease (Aspergillus oryzae, Aspergillus niger), bacterial protease (Bacillus subtilis) reducing the volume, even at minimal doses.

Key words**:** *bread, flour, protease, rheological indicators*

INTRODUCTION

During the last decades, due to the development of scientific research in bakery and cereal products appeared a diversification and modernization of processing technologies, a broadening of the product range, an increased use of additives for different purposes. In parallel with these, the risk of contamination with harmful substances of different foods increased, including bread, and the requirement of consumers to quality and food safety. All these factors have imposed a requirement to increase the control of products, in terms of their composition and safety [2].
The control strategies aim to know and use the most appropriate and accurate methods and analysis techniques, allowing the evidence of product quality indices. The aimed researches had as a highlight the influence of two proteolytic preparations from two different sources, namely a fungal and a bacterial enzyme preparation, over the quality of bread made from flour of type 680.

MATERIAL AND METHOD

In experiments, a wheat flour of type 680 has been used, for which the physic-chemical and rheological indicators have been determined. To achieve the technological tests compressed yeast from bakery has been used, according to the formulation.
For laboratory experiments the following proteolytic enzyme preparations have been used:
1. The Fungal Protease from the Mirpain company, Turkey, is an enzyme preparation containing peptihydrolase obtained by controlled fermentation with Aspergillus oryzae. The preparation is a mixture of acid proteases, neutral and alkaline, with both

exopeptidasic activity and endopeptidasic activity over the protein molecules.

2. The panazyme, from the Zytex company, India, is a bacterial enzyme preparation containing protease obtained by controlled fermentation of Bacillus subtilis.

During the research have been analyzed 7 recipes for the preparation of bread, respectively a control and 6 experimental samples, which were introduced in different amounts of protein and Panazyme Fungal enzyme preparations (Table 1).

In Table 2 are presented the recipes and technological parameters that used during the experimental period

Table 1. The experimental scheme

No. Sample	The used amount of enzyme preparation	The followed aims
Control sample	Without enzyme supplement	- indicators of quality of bread (nominal mass, volume, height, diameter, porosity, acidity, humidity) - physical indicators of bread (softening, elasticity)
Exper. sample 1	With addition of 3.0 g protein Fungal / 10 kg flour	
Exper. sample 2	With addition of 3.5 g protein Fungal / 10 kg flour	
Exper. sample 3	With addition of 4.0 g protein Fungal / 10 kg flour	
Exper. sample 4	With addition of 3.5 g Panazyme / 10 kg Veron GX meal preparation	
Exper. sample 5	With addition of 4.0 g Panazyme / 10 kg Veron GX meal preparation	
Exper. sample 6	With addition of 4.5 g Panazyme / 10 kg Veron GX meal preparation	

Table 2. The recipes and technological parameters used during the experimental period

Raw materials and technological regime	Indirect method																			
	Total							Yeast lees							Dough					
	M	P1	P2	P3	P4	P5	P6	M	P1	P2	P3	P4	P5	P6	M	P1	P2	P3	P4	P5	P6
Flour, kg	8	8	8	8	8	8	8	4	4	4	4	4	4	4	4	4	4	4	4	4	4
Water, l	4	4	4	4	4	4	4	2	2	2	2	2	2	2	2	2	2	2	2	2	2
Yeast, kg	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	-	-	-	-	-	-	-
Salt, kg	0.12	0.12	0.12	0.12	0.12	0.12	0.12	-	-	-	-	-	-	-	0.12	0.12	0.12	0.12	0.12	0.12	0.12
Fungal Protease, g	-	3	3.5	4	-	-	-	-	3	3.5	4	-	-	-	-	-	-	-	-	-	-
Panazyme, g	-	-	-	-	3.5	4	4.5	-	-	-	-	3.5	4	4.5	-	-	-	-	-	-	-
TFr, min.				-	-	-	-	2	2	2	2	2	2	2	-	-	-	-	-	-	-
TFe, min.				-	-	-	-	120	120	120	120	120	120	120	-	-	-	-	-	-	-
TFr, min.				-	-	-	-	-	-	-	-	-	-	-	4	4	4	4	4	4	4
Td, min.				-	-	-	-	-	-	-	-	-	-	-	40	40	40	40	40	40	40
Tb, min.				-	-	-	-	-	-	-	-	-	-	-	30	30	30	30	30	30	30
Toven, °C				-	-	-	-	-	-	-	-	-	-	-	-	220…230					

TFR = time kneading yeast, dough, yeast fermentation; TFE = time; Td = time leavening dough baking; Tb = time baking; Toven = temperature oven.

The used fungal protein and Panazyme doses had, in average, the following enzymatic activity: 484; 569; 644 SKB units / kg flour.

In the performed experimental tests have been used:

- 4 kg of flour for yeast and 4 kg of flour for the dough, therefore a total of 8 kg for each of the five samples and control sample;
- water at a rate of 2 liters and 2 liters of yeast dough, so 4 liters in total for each sample;
- 200 grams of yeast in the yeast lees for each sample;
- salt, 120 grams in the dough;
- no enzyme preparations have been used for the control sample; at the first test in the dough have been used 3.0 g of Fungal protease, for sample 2, 3.5 g of protease Fungal protease preparation, for sample 3, 4.0 g of protease Fungal protease preparation, for sample 4, 3.5 g of Panazyme protease preparation, for sample 5, 4.0 g of Panazyme protease preparation and for sample 6 were introduced 4.5 g of Panazyme protease preparation.

The time of leaven dough was two minutes. The leaven fermentation time was 120 minutes. The kneading of the dough was 4 minutes. Dough leavening took 40 minutes for the control sample and for the other samples, 30 minutes. Baking time was for each of samples, including the control one, 30 minutes. The temperature at which cooking was done for all the samples was $220\text{-}230^0$.

On each experimental group have been analyzed 16 loaves, which were obtained by the indirect procedure. The followed objectives were the determination of the physic-chemical indicators of quality, respectively nominal weight, volume, height, diameter, porosity, acidity, humidity, softening, elasticity.

RESULTS AND DISCUSSIONS

Quality indicators of the baking bread samples obtained after 3, 24, 48 and, respectively 72 hours out of the oven, are presented in Tables 3 and 4.

Table 3. Quality indicators of bread on their size

Quality indicator	*Time after baking (hours)*	*M*	*P1*	*P2*	*P3*	*P4*	*P5*	*P6*
Nominal weight. g	3	526.5	531.5	539.5	511.2	527.2	525.1	524.1
	24	523.1	528.4	536.3	508.4	525.1	522.3	520.6
	48	519.3	524.1	532.7	505.9	521.2	518.6	517.2
	72	511.7	518.2	525.4	500.1	518.5	512.0	509.7
Volume. cmc/100 g product	3	336	337	340	327	337	333	329
	24	328	334	337	323	330	325	325
	48	325	328	334	322	328	322	321
	72	323	328	332	316	326	320	318
Height (H). cm	3	9.9	10.3	10.8	10.1	10.4	9.9	9.7
	24	9.7	10.1	10.7	9.9	10.2	9.6	9.4
	48	9.5	10.1	10.5	9.8	10.0	9.5	9.2
	72	9.1	10.0	10.3	9.5	9.9	9.0	8.9
Diameter (D). cm	3	15.40	15.45	15.50	15.35	15.48	15.39	15.35
	24	15.21	15.32	15.35	15.24	15.25	15.21	15.20
	48	15.12	15.20	15.25	15.15	15.23	15.13	15.10
	72	14.92	15.15	15.17	15.05	15.16	15.04	15.00
H/D	3	0.64	0.67	0.70	0.73	0.67	0.64	0.63
	24	0.64	0.66	0.70	0.70	0.67	0.63	0.62
	48	0.63	0.66	0.69	0.70	0.66	0.63	0.61
	72	0.61	0.66	0.68	0.68	0.65	0.60	0.59

During the research 7 recipes of bread making have been analyzed, respectively a control and 6 experimental samples in which different amounts of Fungal Protease and Panazyme enzyme preparations have been introduced.

After the results being obtained for the three samples in which the Fungal Protease proteolytic enzyme preparation has been introduced, it can be observed that the nominal weight increased in the case of the sample for which 3.5 g Fungal Protease / 10kg flour were introduced, respectively the sample 2 to the control sample for which no enzyme preparation was used.

In case of using the Panazyme product, it can be observed that the nominal weight increased for sample 4 for which Panazyme 3.5 g / 10 kg flour was introduced to the nominal weight of the control sample for which no proteolytic preparation was used.

At 3 hours after baking, the lowest value of the bread's volume is that of the same sample 3 with 327 cmc/100g product. The sample 2 has the highest value of product, 340 cmc/100g, followed by sample 1 with 337 cmc/100g of product and the control sample with 336 cmc/100g of product.

At 24 hours the sample 3 has the lowest volume 323 cmc/100g of product and sample 2 has the highest volume of 337 cmc/100g. The control sample has a quite low value. At 48 hours the lowest volume is that of the sample 6, 321 cmc/100 g, followed by the value belonging to sample 5 and 3 with 322 cmc/100g of product.

At 72 hours after baking the sample 4 presents further the highest volume of 326 cmc/100g of product, remarking the fact that the volume of the bread has decreased as the amount of preparation of bacterial proteolytic introduced in bread increased.

After the analyzes being performed, it can be observed that still the amount of 3.5 g Fungal Protease/10kg flour introduced in the recipe determines the volume increase compared with the control sample.

Regarding the height on analyzed bread, it can be noticed that the better performance is still present in the Fungal Protease preparation which carries higher values of the height at the samples where 3.5 g were introduced, determining increases in height as opposed to

the control sample for which no preparation supply was used.

The loaves diameter recorded the highest values when using Fungal Protease preparation, introduced at a dose of 3.5 g, values which were higher than those recorded when using the Panazyme product.

Table 4 presents the physic-chemical indicators of quality of the produced bread obtained during the experimental period.

Table 4. Physic-chemical indicators of quality of the produced bread obtained during the experimental period

Quality indicator	*Time after baking (hours)*	*M*	*P1*	*P2*	*P3*	*P4*	*P5*	*P6*
Porosity %	3	82.98	83.55	83.32	81.75	82.75	80.36	80.06
	24	81.06	82.52	82.12	80.36	81.26	79.65	78.85
	48	80.12	82.37	81.24	80.06	81.05	77.36	77.01
	72	79.07	81.81	80.19	80.01	80.97	77.14	76.14
Elasticity %	3	93	95	96	95	95	91	89
	24	91	92	93	92	90	88	85
	48	88	90	90	89	89	78	79
	72	79	87	86	77	81	76	69
Humidity %	3	43.69	42.79	42.95	42.30	43.38	42.33	41.89
	24	41.90	41.41	41.57	41.22	42.17	42.01	41.26
	48	40.41	40.24	40.36	41.01	41.13	41.69	40.25
	72	40.30	40.15	40.56	40.29	41.01	41.01	39.75
Acidity degrees	3	2.2	2.4	2.3	2.4	2.2	2.1	2.2
	24	2.1	2.4	2.2	2.3	2.1	2.1	2.0
	48	2.1	2.3	2.1	2.2	2.1	2.0	2.0
	72	2.0	2.1	2.0	2.2	2.0	1.9	1.8

After the results being obtained and the comparisons being made between the samples with supply of Fungal Protease and Panazyme and the control sample, it can be noticed that there are differences of porosity. Sample 1 with an addition of 3 g of Fungal Protease has the highest value in comparison with the one of the control sample.

Regarding the elasticity of bread belonging to the 7 lots, elasticity differences are found in case of addition of proteolytic preparation of fungal nature, respectively bacterial. The sample with the highest elasticity is found at sample 1, being of 89%, sample in which has been added the preparation of Fungal Protease of fungal nature and the highest value for the preparation of Panazyme of bacterial nature can be found at sample 4, being of 81% to the control sample which presents a low value in comparison with these, that of 72%. Elasticity increased in the upward direction of the dose of enzyme and it decreased over time. The smallest difference between the values at 3 hours and those at 72 hours was the P5 (sample with the highest dose of enzyme).

After the analyzes being performed to determine humidity, it has been shown that in comparison with the control sample, better results were obtained from samples in which the bacterial enzyme Panazyme preparation of 3.5 g and 4g was added.

The analyzed bread has recorded high values of acidity for the lots where an addition of 4 g of Fungal Protease preparation of fungal nature was used. In general, the bread has recorded close values at the experimental lots compared with the control sample without an addition of proteolytic enzymes, while there is a slight decrease in acidity.

Some authors think that for equal activities of proteases of different origins, the effect over the loaf volume is different. Better results are obtained by adding fungal proteases (Aspergillus oryzae, Aspergillus niger) [1, 4]. At low doses of 150-200 H/100 g flour units, the bread's volume increases, after which, increasing the dose to 500-600 H / g flour units, the volume does no longer modify and

at higher doses it decreases. The bacterial protease (B. Thermo-proteoliticus) visibly reduces the volume even for minimal doses. The addition of proteolytic enzymes also influences the bread porosity. The effect is also different for different proteases [3].

To adjust the dough plasticity, proteases are often used. The action of the proteases is more complex than the one of the amylases; the proteases causing the peptisation of the proteins from dough. The addition of proteolytic enzyme adjustes the rheological qualities of dough, according to technological needs. The proteolytic action starts during the kneading process and it continues throughout the fermentation until the enzymes are inactivated by heat [5].

CONCLUSIONS

After the research being done on the influence of a proteolytic enzyme preparation in order to improve the baking properties of flour. the following conclusions have been taken:

♦ the addition of proteolytic enzymes followed an increase in volume. The most visible increase was observed at the sample with a supply of 3.5 g of enzymatic Fungal Protease preparation/10 kg flour (sample 2) and at the one with an addition of 3.5 g of enzymatic Panazyme preparation /10 kg flour (sample 4).

♦ the bread height recorded a slight decrease in time of all samples. A higher value of bread height was observed for sample 2 (with an addition of 3.0 g enzymatic Fungal protease preparation/10 kg flour) and sample 4 (with an addition of 3.5 g enzymatic Panazyme preparation/ 10 kg flour).

♦ the diameter and the ratio H / D of the bread presented insignificant differences from the control sample during the baking period until 72 hours.

♦ the bread porosity decreased over time. The highest value recorded for the bread made from flour to which were added 3.0 g Fungal Protease/10 kg product (sample 1) and 3.5 g enzymatic Panazyme preparation/10 kg flour (sample 4).

♦ the elasticity of bread was not significantly influenced by the introduction of proteolytic preparations, the elasticity of the loaves obtained for the experimental samples being similar to that observed at the control sample without addition of proteolytic enzymes.

♦ the bread humidity showed a slight decrease tendency in humidity over time, the supply of enzymatic preparation not influencing this indicator of bread quality.

♦ The bread acidity registered close values at the experimental lots than the control sample without addition of proteolytic enzymes. In time there is a slight decrease in these values of the bread's acidity.

Summarizing the results of research on the influence of addition of proteolytic enzymes on flour quality, it is noticed that they can be used to improve the baking qualities of wheat flour of type 680, the recommended dose being of 3.5 g enzymatic Fungal Protease preparation/10 kg flour, which ensures obtaining high quality bread.

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SCIENTIFIC EVIDENCES THAT PIG MEAT (PORK) IS PROHIBITED FOR HUMAN HEALTH

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Abstract

Among all animal meats pork is the filthiest diet to consume by human beings. Pig is the cradle of harmful germs. Scientific evidences prove that pig meat is least healthy having different harmful agents like Cholesterol and Fatty Acids, Bacteria and Toxins and a number of parasites. The pig meat is high in fat and cholesterol that causes the cardiovascular diseases, obesity, the incidence of large intestine cancer. Bacteria and Toxins associated with pigs spread many diseases like salmonellosis, which leads to the acute gastroenteritis and diarrhea. Many other diseases like, Tuberculosis, Yersiniosis, Listeriosis, Leptospirosis, Brucellosis, Small Pox, Influenza, Anthrax, Balantidial dysentery, Foot rot, Cholera and Erysipeloid are attributed to pork consumption. Parasitic Diseases Ascaris, Ancylostomiasis, Toxoplasmosis, Trichinellosis, Cysticercosis showing signs of mental disorders, pneumonia, bleeding of the lungs (haemoptysis), which may lead to death or madness. The patient may become blind and deaf. Nitrates used in pork and pork products as additives are converted into nitrosamines which cause hepatic cell tumors. Flesh of the pork is hard to digest and may lead to chronic digestive disturbances. Pimples, boils, cysts are common in pork eaters. The pig excretory system secretes 2 percent of its uric acid that is injurious for the human health. Pork consumption seriously affects human health and adversely injurious one's moral values. A person gets pig like characteristics by eating pork, Indecency, obscenity and vanished honour of women.

Key words**:** *Pig meat (pork), Prohibition, Human Health*

INTRODUCTION

The health and socioeconomic impacts of pig meat (Pork) are growing continuously and are increasingly felt by meat consumers. Pig production is an important part of the economy in many countries. Domestic and wild pigs (Sus scrofa) are susceptible to a wide range of infectious and parasitic diseases. Some of these diseases are specifically limited to pigs while some of the other diseases are shared with other species of wildlife and domestic livestock [1]. As the number and geographic distribution of wild and domestic swine continues to increase, it is certain that the number of contacts between these swine and domestic livestock will also increase, as will the probability of human exposure to the parasites of swine directly or indirectly [2]. In this review article we discuss diseases of medical importance that swine may transmit to humans.

Salmonella typhimurium (S. Typhimurium) is a common zoonotic pathogen in pigs and the pork industry is considered to be an important food vehicle in its transmission to humans [3]. Once contamination with S. typhimurium takes place there is every possibility of contamination through the food chain to contaminate pork and pork products [4]. The importance of fat intake in the human diet has been emphasized by many researchers. The composition of pork has higher levels of essential and non-essential fatty acids, polyunsaturated fatty acids and mono-unsaturated fatty acids. When humans ingest undercooked contaminated pork meat, the adult worm develops in the small intestine. After two months of asymptomatic infection, this tapeworm starts producing thousands of eggs that, once released with the stools, can contaminate the environment, infecting pigs (rapidly differentiating into cysticerci mainly in the muscle) and humans (where most severe symptoms are observed due to the presence of cysticerci in the brain) [5]. God had prohibited all His PROPHETS to eat pork. They include Prophet Abraham, Prophet Moses, Prophet Jesus, Prophet MUHAMMAD and others

(peace upon them all). Scriptures clearly guide us on this subject. This prohibition applies naturally to the Followers of the PROPHETS as well.

THE QUR'AN REFERENCE:

In Qur'an Allah has been forbidden pork for muslims at four places (2: 173, 5: 3, 6: 145, 16:115), for the health and wellbeing of man as:

1. "He has only forbidden to you dead animals, blood, the flesh of swine, and that which has been dedicated to other than Allah. But whoever is forced [by necessity], neither desiring [it] nor transgressing [its limit], there is no sin upon him. Indeed, Allah is Forgiving and Merciful. (Verse 2:173) - sūrat l-baqarah}".
2. "Say (O Muhammad): I find not in that which has been revealed to me anything forbidden to be eaten by one who wishes to eat it, unless it be Maytah (a dead animal) or blood poured forth (by slaughtering or the like), or the flesh of swine (pork); for that surely, is impure or impious (unlawful) meat (of an animal) which is slaughtered as a sacrifice for others than Allaah (or has been slaughtered for idols, or on which Allaah's Name has not been mentioned while slaughtering)…" [Al-An'aam:145]
3. "Prohibited to you are dead animals, blood, the flesh of swine, and that which has been dedicated to other than Allah , and [those animals] killed by strangling or by a violent blow or by a head-long fall or by the goring of horns, and those from which a wild animal has eaten, except what you [are able to] slaughter [before its death], and those which are sacrificed on stone altars, and [prohibited is] that you seek decision through divining arrows. That is grave disobedience. This day those who disbelieve have despaired of [defeating] your religion; so fear them not, but fear Me. This day I have perfected for you your religion and completed My favor upon you and have approved for you Islam as religion. But whoever is forced by severe hunger with no inclination to sin - then indeed, Allah is Forgiving and Merciful. (Verse (5:3). - sūrat l-māidah")
4. "He (God) has only forbidden you what dies of itself , and blood, and flesh of swine, and that over which any other (name) than (of) God has been invoked …"2: 173"… Or (eating) flesh of swine-for that surely is unclean …"6: 145

Prohibition of pork eating have been explained in definite terms by the ALLAH REHMAN

ALLAH loves to his believers so He asked to get away from all the injurious food items:

ALLAH Almighty, for his lovely creature sent prophet (PBUH) with a complete code of life "religion Islam" and a complete eternal book "Quran". Quran gives detailed teaching of health not just spiritual but also gives sound scientific reasoning on each and every aspect. These facts are being accepted by science and scientists in every genera of life. There lies wisdom behind each word of Quran for the betterment of mankind. Subject of Quran is man and humanity. Allah had made something's exorbitant for the benefit of man. Man is subordinate of almighty ALLAH. When he tried to navigate certain norms, faith he always had defeat "what GOD will, no frost kill". Every tide has its ebb; ALLAH has prohibited for eating pig meat as numerous injurious aspects lies behind it. In the upcoming section we describe available scientific evidences related to human health associated with the consumption of pork and its products.

THE BIBLE REFERENCE:

As quoted in the Qur'an, the Bible too has declared the meat of pig as forbidden and impure:

1. "You may not eat their meat or even touch their dead bodies; they are forbidden foods for you." [6].
2. "And the swine … it is unclean unto you: You shall not eat their flesh, nor touch their dead carcase." [6].
3. "And swine, because it has divided hoofs but it is not a ruminant (does not chew the cud), it is unclean to you. You shall not eat any of their flesh nor touch their carcasses." [6].
4. A people that provoketh Me to anger continually to My face; … which eat swine's flesh, and broth of abominable thing is in their vessels. Isaiah. 65:3/4;
5. Condemning Pagan Mysteries: "who eat the flesh of pigs, reptiles, rats: Their deeds and their thoughts shall end all at once- it is

Yahweh who speaks." Isaiah 66: 17 See also Deut. 19: 19/21

6. "The pig must be held unclean, though it has a cloven foot, it is not a ruminant. You must not eat the meat of such animals, nor touch their dead bodies; you must hold them unclean. Leviticus 11:8

7. "Now some distance away there was a large herd of pigs feeding, and the devils pleaded with Jesus, "If you cast us out, send us into the herds of pigs." And he said to them, "Go then," and they came out and made for the pigs; and at that, the whole herd charged down the cliff into the lake and perished in the water. The swine herds ran off and made for the town, where they told the whole story." Mathew 8: 28-33

The Jews too did not consume the pork because there is clear commandment in the old Testament of the Bible.

THE HARMFUL NATURE OF PIG MEAT:

It is sufficient for a Muslim to abhor pig meat because ALLAH (swt) has simply ordered us to do so and the Prophet (peace and blessings of Allah be upon him) declared it as haram. But Muslims also believe that Allah (SWT) is aware of all the ingredients and effect of everything. The food and drinks which have been declared forbidden by Islam are ultimately harmful for humans, regardless of whether we are actually aware of this or not. As a doctor prevents his patient from a particular food, he does so to benefit the patient, though the patient may not realize this at the time. In the past, simply accepting knowledge of the unseen was sufficient, but today we can witness the grave harm of pig meat, its effect on those who consume it and its link to diseases, through the means of medical knowledge and research. One particular disease associated with pig meat is Trichinosis.

"Trichinosis or Trichiniasis: A disorder resulting from infestation with the small roundworm Trichinella Spiralis, commonly acquired by humans by the eating of undercooked pork containing encapsulated larvae of the parasite. Trichinosis is more common in pork consuming countries than in other parts of the world. In the United States the incidence of infection may be as high as fifteen to twenty percent." (The New Encyclopaedia Britannica, 15th Edition, 1995).

THE MORAL DAMAGE OF PIG:

Not only does the domestication of the pig cause illness in the human body, but also the consumption of its meat affects the person's inner soul, and causes severe moral damage. This is because bad company and impure food greatly affect and influence a person's physical and spiritual wellbeing. Consider this quote from the author of The English Pig:

Domestication alters the nature and behaviour of the controlled animal. (Similarly) Domestication also generates a familiarity with the animal that affects human consciousness. (The English Pig, London: The Hambledon Press, 1998, p. 129)

THE NATURE OF PIGS:

Pigs are extremely dirty, idle, disliked and indecent animals. Here are a few references to highlight this fact. The irony is that these quotes come from exactly those people who raise and consume pigs:

1. "The pig is a fat, sleepy, stupid, dirty animal, wallowing constantly in the mire." (Ibid, p. 1)

2. "They will eat small quantities of many materials from a very early age, including feed,

3. Earth and the faeces of the dam." (Fream's Agriculture, London: Butler & Tanner Ltd, 16th Edition, 1983, p. 684)

4. "A man from St Helens, Lancashire, born in 1893, recalled of the houses: 'Not only was there the open lavatory in the back yard, many of the people when I was young, kept a pig in the yard'." (The English Pig, p. 42)

5. "The pig is the Husbandman's best Scavenger, and the Huswives most wholesome sink; for his food and living is by that which will else rot in yard …; for from the Husbandman he taketh pulse, chaff, barn dust, man's ordure, garbage, and the weeds of his yard: and from the huswife her draff, swillings, whey, washing of tubs, and such like, with which he will live and keep a good state of body, very sufficiently." (Ibid, p. 34)

6. "The hog during life does not render the least service to mankind, except in removing

that filth which other animals reject." (Ibid, p. 30)

7. "It would be accurate to say that the pig was generally acknowledged to have a character, but that this character was not considered in any way attractive or admirable." (Ibid, p. 1)

THE WORD 'PIG' AS A FORM OF INSULT:

Muslims and Jews abhor pigs and consider the word as a grave insult. In fact, the word is used as an insult in the English language: Indeed the pig was usually thought to be brutish, insensitive and filthy – so much so, in fact, that it became a common place metaphor for human greed, grossness and intemperance. (Ibid, p. 1)

Pig, swine and hog all have a similar usage in English. When these words are used in reference to a human, then it means the person is greedy, dirty, ill mannered, selfish, unpleasant, obnoxious, foul smelling and disliked. (The Oxford Advanced Learner'sDictionary, 4th Edition, 1989).

WHY IS PIG MEAT HARAM?

It is clear from this analysis that the pig – physically and literally – has no appeal, and nor its behaviour or food is worthy of any praise. Its meat opens the door to several illnesses. Such is the extent of its filth and gross impurity that if it was left in a clean place, it would excrete and then eat this to feed its sickening appetite. For Muslims, even to think about consuming its meat is extremely vile; even looking at it is nauseating.

PARASITIC AND MICROBIAL TRANSMISSION THROUGH THE PORK:

Trichinella spp:

T. spiralis a nematode affects humans and animals. The encysted larvae of this parasite cause the disease trichinellosis acquired by consuming muscle tissue of the infected animal. After eggs are hatched in the intestine of the host, trichinae becomes encysted in the muscle where no further development occurs until eaten by other animal, they become sexually mature when they are set free in the intestines. Worldwide consumption of the undercooked pig meat has been traditionally most common source of trichinellosis in humans. It is more common in the United States and the Europe as compared to other parts of the world. Incidence of infection in United States is high as fifteen to twenty per cent, [7].

TAENIA SOLIUM:

Raw and undercooked meat contains the cysticerci of this pork tapeworm, [8]. This is subclinical infection and clinically it is observed by the signs nausea, abdominal pain and diarrhea. In humans it cause severe health burdens and is most prevalent in the South America. Their cysticercus not only persists in the muscle but it migrates towards the brain meninges, cerebral cortex, spinal cord and eye which is most of the time treated only by the surgical operation, [9].

STRONGYLOIDES RANSOMI:

The threadworm of pig has a life cycle comprises males and females free living generations, parthogenetic females in the intestine. It is highly prevalent source of diseases in the humans.

ENTAMOEBA POLECKI:

Entamoeba polecki is a cosmopolitan intestinal parasite of pigs, wild boars and humans. This protozoan parasite occasionally affects humans but morphologically resemblance with a pathogenic spp. Entamoeba histolytica makes it difficult to distinguish, [10].

BLASTOCYSTIS SPP:

Blastocystis sp has worldwide variety of hosts. B. hominis like organisms have wide spread over birds, domestic pigs, wild boars and reptiles. Although its pathogenic potential is yet controversial but the association of this parasite is with symptomatic gastrointestinal diseases, [11].

SALMONELLA TYPHIMURIUM:

Salmonella typhimurium is most frequently involved in the human salmonellosis, which leads to the acute gastroenteritis and diarrhea. S. typhimurium present in the pigs and pig meat is the most common vehicle in its transmission. Pigs may also be involved in the diseases like, yersiniosis (Y. enterocolitica) , Listeriosis (L. monocytogenes), leptospirosis (swineherds

disease), brucellosis (Brucella suis), anthrax, balantidial dysentery (Balantidium coli) and erysipeloid (Erysipelothrix rhusiopathiae), [12, 13].

SHAMELESS AND FILTHIEST ANIMAL

Its consumption not only affects the human health seriously but is also injurious for the inner, soul and moral values of human beings. A person is physically and spiritually greatly influenced by what he eats. It is very well explained by the English author in the following quote:

"Domestication alters the nature and behavior of the controlled animal. (Similarly) Domestication also generates a familiarity with the animal that affects human consciousness." (The English Pig, London: The Hambledon Press, 1998, p. 129)

HIGH CHOLESTEROL AND FAT

The pig meat is high in fat and cholesterol and causes the cardiovascular diseases. Animal fat is rich source of energy and majorly causes obesity. However, obesity is also correlated with heredity and exercise. Coronary heart diseases is frequently prevalent by the combustion of the pork which is rich in fat and cholesterol; as it is well studied by the WHO that " CHD is mass characterized by the diets having high cholesterol level ,high intake of saturated fat, low carbohydrates combustion. The experimental and epidemiological revealed that the incidence of large intestine cancer in humans is observed with the intake of diet high in fat, [14].

HARMFUL EFFECTS OF ADDITIVES USED IN PORK INDUSTRY

To increase the weight and feed efficiency various growth promoting substance and antibacterial are used in the intensive farming .these agents may cause the harmful effects to man as many of them may persist in the meat. Nitrates are used in pork and pork products as additives in the curing process frequently as mostly pork is consumed after curing in the form of different types of sausages .nitrates are converted into nitrosamines which causes hepatic cell tumors.

From above commandments in the scriptures, it appears that God Almighty attaches such importance to the prohibition of human consumption of pork that He uses strong language to show His extreme displeasure on the violators.

The wording of the prohibition is clear, precise, powerful and coming from God himself. It also clearly tells us the consequences of breaking the Law. If it were to be abrogated, the language must be equally specific and emphatic and from God (in first person) and should categorically nullify the Law, remove the displeasure and wrath of the Lord, making pork pure and wholesome. Detailed insights of scriptures unravel the fact that pork is prohibited. The health hazards associated with eating pork and pork products are well documented. Besides pork being high in salt, fat, and cholesterol, it also carries parasitic disease like pork tapeworm, trichinosis, Balantidium coli organism. These cause disease in human beings. Cysts have been reported to lodge in brain and act as brain mass, giving rise to seizures, needing brain surgery. The diseases that may result from eating pork include; dysentery, trichinosis, tape worm, round worm, hookworm, jaundice, pneumonia, intestinal obstruction, high fever, and death, [15]. This hazard still exists in advanced countries, including America, but more so in developing countries both in western hemisphere and eastern hemisphere.

One of the triglyceride molecules in pig fat cannot be hydrolyzed, so it is deposited in humans as "pig fat." In animals like cattle, sheep, goat, etc. the animal fat is completely hydrolyzed and reconstituted as human fat. Pig intestine makes the food move very fast, so that there is not enough time to fully digest all that it eats. Thus, many toxins are absorbed and deposited in its flesh, which is then consumed by some humans. He strongly dissuades his congregation of eating pork and pork products.

CONCLUSION

From what we have read from the Scriptures of the Christians, scientific findings, Last Testaments (The Holy Qur'an) and the saying of the Last Prophet Muhammad (SAW) about health and the flesh of the swine, we now come

now to the conclusion that eating of swine flesh or pork is sin and sickness. Therefore, we must protect ourselves for it is a fact that health is a trust and best blessing bestowed upon us by ALLAH.

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THE QUALITY OF MEAT IN SOME HYBRIDES PIG

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Abstract

Aspects commonly found in the literature on meat quality of swine, shows the fact that, in a large number of qualitative peculiarities of muscle tissue, it can select the Napole Technological Yield as some of the most important genetic elements. These parameters are closely interdependent with pH at 24 hours in the meat after slaughter. In this work, the research has been conducted on 40 samples, taken from the semimembranos muscles from Perhib, F1 hybrids (Marele AlbxLandrace) xPietrain and F1 (Marele AlbxLandrace) xSeghers for the determination of Napole Technological Yield. Methods used to analyze the parameters listed are those in the literature. Age of the animals on slaughter was 183-185 days. The results put in evidence that the F1xPietrain hybrid have RN- in their genotype.

Key words: *Genetic type, pig meat, quality of meat*

INTRODUCTION

Quality of pork is conditioned by many factors. Some of them act in the animal's life, others after killing it. Thus, race, sex, age, state of maintenance, etc., are decisive factors on meat quality, and nature, direction and speed of development of biochemical processes in muscle tissue after suppression of animal life, have an important role. Factors influencing meat quality can be grouped into: farmed factors, genetic factors, factors related to preparing pigs for slaughter (transport, fasting, etc.) and factors in the slaughterhouse. Pig meat consumption presents a significant growth and at the same time establishes a growth of consumer preferences for a good quality, to satisfy the tastes of the refined and more of them and to ensure a healthy and balanced nutrition.

Technological and sensory quality of pork meat is influenced by various factors including genetic.

Thus, the pH of meat recorded after slaughter, which has a strong effect on indices of meat quality is mainly influenced by genetic factors, major genes N/n and RN^-/rn^+ [6] . Since 1985, RTN is, due to correlation with the index of meat quality, selection criteria for certain populations of swine [1,2].

RN^- gene has been reported since 1985 by using an original method for measuring the efficiency of boiling, a method called "Napole Technological yield" (RTN).

Based on this method were considered to gene RN-free animals, animals which attained a value of 91% higher RTN and animals-the carrier of the RN gene whose RTN was of less than 91%.

RN-gene effect is to abnormal increase the amount of glycogen after slaughtered by decomposition to the level of lactic acid, causing a decrease of pH in meat at 24 hours, thus reducing the efficiency of meat technology [5].

Knowing the influence of genetic factors on meat quality in pigs, we have proposed in this paper to study the effect of major genes on meat quality from some populations of swine and commercial hybrid in Romania.

In this work, the research has been conducted on 40 samples, taken from the semimembranos muscles from breeds Marele Alb, Landrace, Syntetic Line 345 Periş, Duroc, Hampshire, Perhib, F1(Marele AlbxLandrace)xPietrain and

F1 (Marele AlbxLandrace)xSeghers for the determination of RTN. Methods used to analyze the parameters listed are those in the literature (Georgescu, Banu, Oţel, 2000, 1986). Age of the animals on slaughter was 183-185 days. Also, it was determinate pH of meat at 24 hours after slaughter (pH_{24}) and the phenotipical correlation between pH_{24} and RTN (using classical statistic methods).

MATERIAL AND METHOD

In this work, the research has been conducted on 40 samples, taken from the semimembranos muscles from hybreeds Perhib, F1(Marele AlbxLandrace) x Pietrain and F1 (Marele AlbxLandrace)xSeghers for the determination of RTN. Methods used to analyze the parameters listed are those in the literature [3,4]. Age of the animals on slaughter was 183-185 days. Also, it was determinate pH of meat at 24 hours after slaughter (pH_{24}) and the phenotipical correlation between pH_{24} and RTN (using classical statistic methods).

RESULTS AND DISCUSSIONS

In Table 1 are presented RTN values for samples of semimembranosus muscle from pig genetic types studied.

Table 1. RTN values from studied genetic types

Genetic type	n	RTN $\bar{X} \pm S_{\bar{X}}$
Perhib	40	95.85 ± 1.023
F1xPietrain	40	89.07 ± 1.112
F1xSeghers	40	94.90 ± 0.879

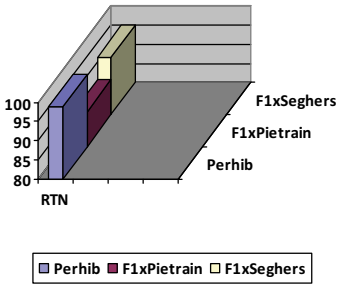

Fig.1. RTN values from genetic studied hybreeds

From results presented in Table 1 and Fig. 1 we can see that most analyzed pig genetic types presents higher RTN values of 91%, which advocates for the absence of RN^- gene. The commercial hybrid F1xPietrain (89.07 ± 1.112) based on RTN values can be suspected the presence of RN^- gene, with all the consequences of technological properties of meat.

RTN values confirm the results of other studies that have concluded that this breed is genetically predisposed to exudative myopathy and settled by other methods that is the bearer of the RN gene.

According to previous research reports that between different genotypes for susceptibility to stress the important difference in the quality indices of the meat registers in the pH, making it, in many breeding companies, selection criteria (in slaughterhouse) to identify animals susceptible to stress.

Thus, we present in Table 2 the values of pH at 24 hours for samples of semimembranosus muscle from pig genetic types studied.

Very important to finalize the selection criteria is the degree of interdependence between RTN and pH value at 24 hours [7]. Thus, we present in Table 3 phenotypic correlations between the two parameters.

Analyzing correlations between RTN and pH_{24}, their values advocates RN gene absence from most types of analyzed genetic type, except hybrid F1xSeghers.

Table 2. pH at 24 hours values from studied genetic types

Genetic type	n	pH_{24}
Perhib	40	5.700
F1xPietrain	40	5.380
F1xSeghers	40	5.216

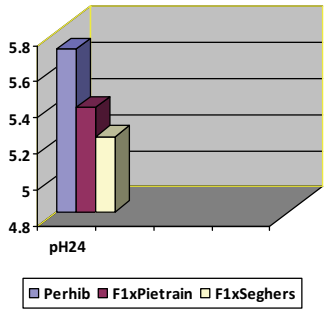

Fig.2. pH_{24} values from genetic studied hybreeds

Table 3. The interdependence between RTN and pH_{24}

Genetic type	n	r_F RTN/pH_{24}
Perhib	40	0.67
F1xPietrain	40	0.57
F1xSeghers	40	0.55

All breeds has a high quality of meat that allows using of those carcasses to achieve various preparations, such as ham of "Paris" type. Regarding hybrids, the RTN good values were obtained to Perhib and F1xSeghers. To combination F1xPietrain, RTN has a low value, due to race Pietrain, which is known to be stress gene bearer.

CONCLUSIONS

RTN values, pH_{24} and correlations between the two parameters of studied breeds and hybrids, places these animals in category "animals that produce quality carcasses," except F1xSeghers hybrid.

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EFFECT OF THAWING METHODS ON PHYSICAL CHARACTERISTIC AND CHEMICAL COMPOSITION OF RIB EYE MEAT ONGOLE CROSSED

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Abstract

The research was aim to know effect of thawing methods on physical characteristic and chemical composition of rib eye meat Ongole Cross, and to know method best thawing based physical characteristic of and chemical composition. Research is conducted experimentally used Completely Randomized Design (CRD) with four treatments thawing, that is: refrigeration temperature (5-7^{0}C), room temperature (27-30^{0}C), running water at room temperature (22-24^{0}C), and warm water temperature (50-55^{0}C) each treatment was repeated five times. Analysis of variance conducted to determine the effect thawing methods on the phyisical and chemical composition of rib eye meat Ongole Crosses, while to know difference between the treatment used Duncan's test. The result of statistic analysis and discussion indicated that the method of thawing significant effect (P<0,05) of physical characteristic and chemical composition of rib eye meat Ongole Crosses. The method of thawing with refrigeration temperature has the best effect on chemical composition and physical characteristic of rib eye meat Ongole Cross.

Key words*: Thawing, physical, chemical, meat, Ongole*

INTRODUCTION

Ongole Cross is a type of cow that is have dual function as a workers and beef cattle. Ongole cross is the result of crossbreeding of local cows with Ongole cattle of India and has long been maintained by farmers in Indonesia Rib eye meat is a beef carcasses are taken from the back of the sixth to the twelfth rib (Crown and Damon Jr., 1960). Rib eye steaks consists of Longissimus dorsi muscle around the rib cage area and Thoracic vertebrae (Aberle *et al*., 2001). The chemical composition of rib eye meat is 17,4% protein, 59% water, 23% fat, 0,8% ash, and calories by 280 kkal/100 gram (AMIF, 1960 in Tien. R. Muchtadi, and Sugiyono, 1992). The chemical composition is a good medium for the growth of microorganisms (Eneji *et al*, 2007), to slow the process of microbiological it is required preservation. At a temperature of -18^{o}C beef can be stored up to 12 months, whereas at a temperature of -25^{o}C beef can last up to 18 months, and at a temperature of -30^{o}C maximum storage time was 24 months (FAO, 1990 in Chalid Thedjokusuma, 2002).
Freezing can inhibit the activity of microbial growth, enzymatic and chemical reactions that cause damage and decay (Gokalp *et al*, 1999). The resulting ice crystals will inhibit the growth of more than 99% of bacterial decay (Ragab, 2010). Freezing has the disadvantage that the drip at the time of refreshment (thawing) followed by the loss of nutrient meat. Thawing with wrong technique will lead to lower quality, and otherwise the proper thawing will ensure the quality of frozen meat is more stable and consistent. Thawing method commonly used according to Ragab (2010), that is: thawing refrigeration temperature (5-7^{o}C) with a length about 12-24 hours of thawing; running water thawing at room temperature (18-20^{o}C) with a length about 2-4 hours of thawing; thawing in the microwave; thawing at room temperature (26-30^{o}C); thawing in warm water temperature (45-50^{o}C) (Ragab, 2010).
Differences in temperature and time in various methods of thawing will affect the physical quality of the texture, firmness, and juiciness of meat and meat chemical composition. Thawing at refrigeration temperatures require slow heat transfer and takes a long time, but did not make a lot of drip (His, 2000), in addition to the water content and physical and chemical quality of the meat can be sustained (Xiong and Blanchard, 1993), while thawing using a high

temperature can cause meat loses more fluid (Lametsch and Ballin, 2008), which is 15-25% more than the thawing using a low temperature, so the meat becomes dry, because a lot of water loss (Escundern *et al*, 2010). Proteins, peptides, amino acids, lactic acids, purines and various salts are substances that compose a drip (Ballin, 2008), too much drip loss effecting meat becomes dry and pale due to loss of fluids and nutritional value (Lawrie, 2003), so need to do research on the effect of various thawing methods on the physical characteristic and chemical composition of PO Beef Rib Eye cuts.

MATERIAL AND METHOD

Research is conducted experimentally used Complete Randomized Design (CRD) with four treatment methods of thawing, that is : refrigeration temperature (5-7^{0}C), room temperature (27-30^{0}C), running water at room temperature (22-24^{0}C), and warm water temperature (50-55^{0}C), each treatment was repeated five times in order to obtain 20 combinations of treatments. To find the difference between the treatments used Duncan multiple range test.

Research Procedure: Preparation of meat samples, Aging, Freezing, Thawing and measure of variables (physical characteristic and chemical composition)

1. Preparation of meat samples

Rib eye meat of the 20 head of cattle carcasses Ongole Cross between the ages of 4 to 4.5 years.

2. Aging

Samples are packed in plastic stored in a chiller (5-6 °C) for 2 days in the withering process.

3. Freezing

Performed on the freezer (slow freezing method, temperature of -20 °C for 4 days).

4. Thawing

Meat melting process of solid phase into the liquid phase again. Post rigor of fresh meat Ongole Cross cattle were used as controls.

Variables measured:

1. Shrinkage of meat (Modified Xiong and Blanchard, 1993)

$$S\ (\%) = \frac{\text{Length of fresh meat (cm)} - \text{length of thawed meat (cm)}}{\text{Length of fresh meat (cm)}} \times 100\ \%$$

2. Cooking Lost

$$CL\ (\%) = \frac{\text{Weight before cooking} - \text{Weight after cooking}}{\text{Weight before cooking}} \times 100\ \%$$

3. Water Holding Capacity (Honikel and Hamm, 1994)

$$Mg\ H_20 = \frac{\text{Wet area (cm}^2\text{)}}{0,0948} - 8,0$$

$$\text{Water Holding Capacity} = \%\ \text{Water Content} - \frac{(\text{mg } H_2O)}{300\ \text{mg}} \times 100\%$$

4. Tenderness (penetrometer)

$$\text{Tenderness (mm/gram/10 seconds)} = \frac{\text{Average Measure}}{\text{10 Seconds}} \times 100\ \%$$

5. Kjeldahl Method Protein Content (AOAC, 1984)

6. Gravimetric Method Water Content (AOAC, 1984)

RESULTS AND DISCUSSIONS

The results in Table 1 indicate that the highest water content and protein meat on thawing refrigeration temperature treatment significantly different (P <0.05) from the thawing using higher temperature.

Table 1. Effect of Treatment on Chemical Composition of Beef Rib Eye Cuts

Item	Treatment			
	TRf	TAm	TAw	Tww
Water Content (%)	71,75 a	70,76 ab	70,38 b	57,32 c
Protein Content (%)	22,20 a	20,89 b	20,28 b	16,49 c

Description: TRf = Refrigeration Temperature, TAm= Room Temperature,
TAw = Running Water Temperature ,Tww = warm water Temperature.
Values followed by different letters towards the line significantly different (P <0.05)

This is due to the high thawing temperatures cause protein denaturation, which will be followed by the opening of the muscle fibers that inflict water released from the protein of meat. As stated Wismer-Pedersen (1971) that at the time the meat protein has denaturation would have released a number of free water in between the protein molecules. The results of this study strengthen the research Escundern *et al*., (2010), that the amount of liquid coming out of the meat more on thawing temperatures above 40 °C compared with lower temperatures.

The highest water content of beef rib eye with the refrigeration temperature thawing treatment, not significantly different from thawing at room temperature, because refrigeration and thawing at room temperature was slow so it can retain a liquid meat, this is in accordance with the opinion Varnam and Sutherland (1995) that the slow thawing produce a minimal drip. Similarly, thawing at room temperature due slow heat transfer will not generate a lot of meat fluid. According to the statement of Lawrie (2003) and Phillips (1995) that the heat transfer at refrigeration temperature and room temperature is slow so that the meat does not cause excessive fluid loss.

Running water is a rapid conductor of heat transfer, thus leading to flesh out a lot more fluid, is shown by the high water content compared with the thawing meat refrigeration temperature (P <0.05). This is supported by Philips (1995) which states that the running water dissipates heat faster than heat transfer in water is not running.

Lowest levels of meat protein in the treatment of thawing warm water temperature 50^{o}C significantly different (P <0.05) compared with other treatments thawing. This is due to the treatment of warm water temperature 50^{o}C thawing cause damage to the muscle fibers due to denaturation of proteins, so the meat will increase the loss of nutrients dissolved in water and disappeared along with the drip. This is in accordance with the opinion of Ragab (2010), that the use of high temperature thawing results in damage to the muscle fibers and sarcolema resulting in a number of muscle protein denaturation. Supported by the opinions of Varnam and Sutherland (1995) which states that protein denaturation occurs at a temperature of 45^{o}C will lead to protein loss with drip.

The lower temperature used for thawing, it will minimize the resulting drip so that the nutrient content of meat can be maintained, from this study demonstrated that the protein of meat can be kept at refrigeration temperature thawing which is supported by the opinions of Xiong and Blanchard (1993) that the thawing using refrigeration temperatures 5 ± 1^{o}C will not cause too much fluid loss of meat because of the heat transfer was slow and takes a long time so that the meat quality of both physical and chemical properties can be maintained.

The results showed that the water content of meat by the use of thawing methods at

refrigeration temperature (5^{o}C) can better maintain the water content of beef rib eye cuts, according to the research Ballin and Lametsch (2008) that the thawing method at refrigeration temperature can maintain the water content of meat, so that thawed meat still has a water content that is not different from fresh meat.

Effect of thawing methods on the physical characteristic of meat (Table 2), indicating that the pH of meat from various treatments were not significantly different, namely in the range of 5,66 to 5,88 means that the meat has reached the ultimate pH, thus obtained are not significantly different pH. Soeparno (2005), states that the final meat pH in the range of 5,1 to 6,1. This is due prior to frozen storage, the flesh withered at refrigeration temperature for 48 hours. Overhaul of glycogen into lactic acid persists at low temperature storage through anaerobic glycolysis, so that the pH of the meat reach to 5,5-5,6 (Lawrie, 2003).

Table 2. Effect of Treatment on Physical Characteristics PO Beef Rib Eye Cuts

Items	Treatment			
	P1	P2	P3	P4
Daya Ikat Air (%)	71.24 a	70.25 ab	69.58 b	56.71 c
Susut Masak (%)	16.00 a	32.00 bc	26.00 ab	44.00 c
Pengerutan (%)	2.13 a	2.27 a	2.27 a	3.07 b
Keempukan (mm/g/10 dtk)	106.06 a	95.58 b	90.12 bc	64.36 c

Description: TRF = Refrigeration Temperature, TAM = Room Temperature, Taw = Running Water Temperature, Tww = Warm Water Temperature.
Values followed by different letters towards the line significantly different (P <0.05)

Meat with the treatment of refrigeration temperature thawing method has a water holding capacity value were not significantly different by thawing at room temperature, however both significantly (P <0.05) higher than other treatments. The difference in results may be explained by the slow of heat transfer and a long time, this provide an opportunity for meat to reabsorb the water to minimize the amount of fluid that comes out from the meat. As noted Hendrickson (1978) that thawing at a not too high temperature will cause the re-absorption of water, so the loss of fluid from the meat is reduced. Otherwise to the method of thawing with warm water temperatures, due to thawing at high temperatures lead to changes in structure tissue of the meat that it will reduce the ability of tissue cells in maintaining the extracellular fluid. Ophart (2003), states that the heating will make the meat protein denatured and cause the water binding capacity decreased. In the opinion Aberle, et al (2001) that the decrease in water holding capacity began to be seen on heating above 40^{o}C that cause alteration in water holding capacity of meat significantly.

The low percentage of meat cooking lost at refrigeration temperature thawing method (P1) indicate that still many water bound within and between muscle fibers. This is due to the low thawing temperature affected on slightly meat protein which may denatured cause lower drip losses. Supported by the opinion Chandirasekaran and Thulasi (2010), meat stored at temperatures below 35 ˚C, makes fluid exudation on the inside meat work slowly.

The results showed that the lowest cooking lost at refrigeration temperature thawing method. Meat with the lowest cooking lost having a better quality rather than meat with higher cooking lost. This is supported by the opinion Hermanianto Joko (2008) that the use of thawing temperature which not reach 40 °C not caused muscles denaturation so the structure of the meat muscle fibers has not been damaged and was not caused a lot of shrinkage in the meat. Unlike the case at high thawing temperature caused denaturation of

proteins, thus decreasing the ability to bind water. In line with the opinion of Ranken (2000), that the heating process with a higher temperature of 50-60 ˚C can lead to loss of water up to 80%.

High meat shrinkage on warm water thawing treatment 50^{o}C compared to other treatments (P <0.05) due to protein denaturation of myofibrils by the heat that would cause the number of muscle fibers in the meat will shrink. This opinion is supported Xiong and Blanchard (1993) which states that meat protein denaturation by heat resulted in shortening the length of the muscle fibers because the muscle fibers was shrink. This is supported by the opinion of Buckle et al., (1987) that during the thawing process of muscle fibers tend to shrink at a pace that is affected by the temperature level, at high temperatures there is rapid reduction of ATP reserves and muscle fibers can shrink quite a lot. Unlike the case with thawing at lower temperatures (refrigeration, room temperature, and running water at room temperature) with one another are not significantly different. This happens because at temperatures between 5-30^{o}C myofibrils protein denaturation has not occurred so that the use of thawing method using the temperature reaches 30^{o}C insignificant. According to the research Chandirasekaran and Thulasi (2010) that the use of the thawing method at temperature 5^{o}C or until the temperature reaches 30^{o}C will not cause a lot of meat shrinkage due to thermal effects which are used by the thawing temperature will not much affect the length of the muscle fibers.

The highest value of meat tenderness thawing method with refrigeration temperatures significantly different (P <0.05) compared with other treatment methods of thawing, because the ability of meat to retain water better. Kramlich (1973) claimed that the tenderness is influenced by moisture content, fat and protein. In addition, Aberle, *et al*., (2001) stated that tenderness is affected by the water holding capacity. Increased water holding capacity due to the extraction of protein-protein interacting and resulting in the space between the filaments becomes larger, so the water can be retained and increased water holding capacity. In contrast the use of thawing method with warm water temperatures, resulting in more drip out and the lower water content, due to the low ability of meat to bind water so the meat becomes tougher and low juicy.

CONCLUSIONS

1. Thawing method significantly (P <0.05) affected the physical characteristic and chemical composition of rib eye meat Onggole Crossed.
2. The best chemical composition and physical characteristic of rib eye meat Onggole Crossed on the treartment thawing method at refrigeration temperature (5 ^{o}C).

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ROBUST REGRESSION MODELS FOR PREDICTING THE LEAN MEAT PROPORTION OF LAMBS CARCASSES

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Abstract

The aim of this study was to develop and evaluate robust regression models for predicting the carcass composition of lambs. One hundred and twenty lambs (34 females and 86 males) were slaughtered and their carcasses were cooled for 24 hours. The subcutaneous fat thickness (C12) was measured between the 12th and 13th rib, and the left side of carcasses was dissected and the proportions of lean meat (LMP) was calculated. A multiple regression model was fitted using robust regression (RR) methods, and the results were compared to ordinary least squares (OLS) estimates. For RR methods, the Bisquare and Welsch weighting functions were used, and model fitting quality was evaluated by the following statistics: the root mean square error (RMSE), the median absolute deviation (MAD), the mean absolute error (MAE), and the coefficient of determination (R^2). The parameters obtained by RR presented lower standard error for C12 measurement (decreases by 12% when compared with OLS estimates). The RR methods or weighted least squares methods represents a good alternative to OLS approach for modelling the LMP of lambs carcass. In this study, the Bisquare weighting method presented the best results, however other weighting functions are available and should be tested and compared in the near future.

Key words*: carcass, quality, ordinary least squares, robust regression*

INTRODUCTION

An accurate objective system for the classification of carcasses at the slaughter-line is of great importance for meat industry, and research results attained by [4,1,2] indicate that the lean meat proportion (LMP) of lamb carcasses can be predicted by simple models using the hot carcass weight (HCW) and one fat depth measurement as explanatory variables.

Multiple regression models has been the most important statistical tool applied to develop models to predict carcass composition. The ordinary least square (OLS) method has been generally used considering this purpose, however data sets, usually, contain outliers which presents higher residuals. Thus, OLS estimates can be biased when the distribution of the residuals is not normal, specially when the residuals distribution is heavy-tailed [3]. When this occurs the OLS estimates are unreliable. The removal from the data set of influential observations can be a solution to this problem, however is observations are real this procedure leads to looses of information.

Robust regression (RR) methods are resistant to unusual data, and the most common general method of RR is M-estimation developed by [5]. When data are linear and residuals are normally distributed, the OLS and RR estimates are similar. The results will be quite different when the residuals are not normally distributed or when data contains a considerable number of outliers [8]. Thus, RR can be used, as a complement to OLS estimates, to handle unusual data records that are not following the general data trend, since RR is resistant to outliers and often performs better in the presence of heteroscedastic residuals.

In the case of models for predicting carcass composition, RR methods can be used to estimate equations parameters, whilst the unusual values are taken into account.Thus, the aim of this study was to compare OLS and RR methods for modelling the LMP of lamb carcasses.

MATERIAL AND METHOD

Data. One hundred and thirty five (45 females and 90 males) of Churra Galega Bragançana breed were randomly selected from the experimental flock of the Escola Superior Agrária de Bragança. The lambs were slaughtered, and their carcasses were weighed approximately 30 min after slaughter in order to obtain the hot carcass weight (HCW). After chilling at 4ºC for 24 hours, the carcasses were halved through the centre of the vertebral column, and the kidney knob and channel fat were removed and weighed. During quartering of the carcasses, the subcutaneous fat thickness (C12, mm) between the 12th and 13th rib was measured with a calliper. The left side of each carcass was dissected into muscle, subcutaneous fat, inter-muscular fat, and bone plus remainder (major blood vessels, ligaments, tendons, and thick connective tissue sheets associated with muscles). The carcasses' lean meat proportion (LMP) was calculated as a proportion of the total tissues in the carcasses.

Statistical analysis

In linear regression, the ordinary least squares (OLS) estimator is sensible to extreme observations, to overcome this problem [5] developed the a general method of robust regression (RR), the M-estimation. This class of estimators represents a generalization of maximum-likelihood estimation. Thus, in this study we used the following multiple regression model used was:

$$LMP = \alpha_0 + \alpha_1 \times LMP + \alpha_2 \times C12 + \varepsilon_i$$

where α_0, α_1, and α_2 are regression coefficients and ε_i is the error term.

All statistical analyses were undertaken using the software "R" [6], and the add-on package "robustbase" [7] was used to fit model using RR estimators.

The model fitting quality was evaluate using the following statistics: the root mean square error (RMSE), the median absolute deviation (MAD), the mean absolute error (MAE), and the coefficient of determination (R^2). The RR methods assign a weight to each observation of the dataset, and in this study we compared two weighting functions Bisquare and Welsch, with the OLS estimates (Table 1). The RR methods were fitted using the default tuning constant (Table 1), which leads to coefficient estimates that are approximately 95% as statistically efficient as the OLS estimates, if the response variable has a normal distribution without outliers. In the weight functions, the value of r is $r = resid / \left(tune \times s \times \sqrt{(1-h)}\right)$, where $resid$ is the vector of residuals from the previous iteration, h is the vector of leverage values from a least-squares fit, and s is an estimate of the standard deviation of the residuals given by $s = MAD / 0.6745$.The MAD is the median absolute deviation of the residuals from their median.

Table 1. Weighting function equations for RR methods

Weight function	Equation	Default tuning constant
OLS	$w=1$	none
Bisquare	$w = \left(\left((r)\right) < 1\right) \times \left(1 - r^2\right)^2$	4.685
Welsh	$w = \exp\left(-\left(r^2\right)\right)$	2.985

RESULTS AND DISCUSSIONS

The estimated parameters and summary statistics for the three estimation methods "OLS", "Bisquare", and "Welsch" are presented in Table 2. The OLS estimates ignores the presence of outliers in the data set, these estimates differ from RR estimates. The efficiency of the RR estimates compared to the OLS estimates is expected to increase in the presence of outliers [3], which is expectable for carcass composition traits. The parameters obtained by RR are characterized by lower standard errors, the reduction of the standard errors was especially visible for C12 measurement (decreases by 12%). Thus, modelling the LMP of lambs carcasses ignoring the presence of outliers results in inefficient estimates.

Table 2. Estimation results of the three methods

Parameters	**OLS**	**Bisquare**	**Welsh**
Intercept	63.0	62.9	62.9
SE	0.788	0.806	0.804
Pr(>|t|)	<2e-16	<2e-16	<2e-16
HCW	0.201	0.198	0.198
SE	0.075	0.079	0.079
Pr(>|t|)	0.008	0.014	0.013
C12	-2.61	-2.58	-2.58
SE	0.210	0.185	0.185
Pr(>|t|)	<2e-16	<2e-16	<2e-16

Table 3 shows model fitting quality statistics. The RR methods presented better overall fit, indicated by the highest (0.591 for OLS, 0.651 for Bisquare, and 0.611 for Welsch).

The standard errors of the regression coefficients and the standard errors of the estimates (SEE) were larger in the OLS estimates, specially for the C12 measurement. The RMSE, the R2, and MAD statistics show that Bisquare is the best weighting function for LMP of lamb carcasses (RMSE = 2.55, R^2 = 0.651, MAD = 2.43). The RR methods presented better fitting quality statistics because they have the ability to minimize the effect of influential observations on the estimation of model parameters.

Table 3. Model fitting quality statistics for the three methods

Weighting function	MAE	RMSE	MAD	R^2
OLS	2.15	2.80	2.46	0.591
Bisquare	2.14	2.55	2.43	0.651
Welsh	2.15	2.55	2.49	0.611

CONCLUSIONS

The RR methods or weighted least squares methods represents a good alternative to OLS approach for modelling the LMP of lambs carcass. These methods can be used to minimize the effects of influence of observations (outliers) in data sets. However, if the data set do not have outliers, the results of OLS and RR will be similar. Thus, we can recommend the use of RR methods to modelling the lambs carcass quality traits. The Bisquare presented the best results in this study, however other weighting functions are available and should be tested in the near future.

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PRELIMINARY RESULTS ON THE LOWER DANUBE STURGEON MIGRATION MONITORING

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Abstract

Amongst the many severely endangered species range the sturgeons. The diminishing stocks are considered to have been the result of several factors such as energetically hydro technical work, hydro technical work to improve conditions of navigation, excessive fishing, poaching and the increasing pollution. Typical migratory, the Black Sea sturgeons reproduce in rivers flowing here and hence the Danube. Deepening knowledge of migration and breeding conditions in this stage are a must. During the 2011 autumn migration there were monitored 50 copies of sturgeons from 4 species. Initial results showed that some marked specimens haven't remained on the Lower Danube to winter but returned to the mouth of the sea, using the Old Danube route between km 197 and km 186.

Key words**:** *sturgeons, migration, acoustic telemetry, transmitter, reception stations*

INTRODUCTION

Sturgeons are among the oldest fish that inhabited the waters of the earth; their dissemination areas are the northern waters. The sturgeons belong to Acipenseriformes order, Acipenseridae family and include 4 genus: Huso, Acipenser, Scaphyrhyncus and Pseudoscaphyrhyncus. On the Romanian territory it can be found 6 species of sturgeon, according to the literature: Huso huso, Acipenser gueldenstaedti, Acipenser stellatus, Acipenser sturio, and Acipenser Acipenser nudiventris ruthenus [1].
The typical migratory route from the Black Sea to the Danube is more or less similar; it differs the places of quartering, time of penetration and migration of juveniles [2]. Currently their number decreased dramatically and a large number of species are endangered due to pollution, poaching or hydro technical works for the Iron Gates I and II, which impede migration to natural spawning areas [3].

The estimation of the environmental impact of hydro technical works began to be realized with the United Nations Conference on the environment - Stockholm Declaration, in June 1972. This proclaimed that environmental protection and the improvement of the environment are major problems affecting the welfare and economic development worldwide. Thus our research will focus on the hydro technical work which can improve navigation on the Danube but without having a negative impact on the sturgeon population.

MATERIAL AND METHOD

In the project "Monitoring the environmental impact of improvement works to navigation conditions on Danube between Calarasi and Braila, km 375 and km 175" for sturgeon migration monitoring on the Lower Danube started in 2011, acoustic telemetry was chosen as a method of tracking.

Acoustic telemetry is used to obtain data of relatively continuous record of fish movements and is ideal for asking fine scale questions. There are two methods of monitoring: actively, in real-time, or passively, using a listening station (Photo 1). For both the first step is to secure an acoustic transmitter or "pinger" to a fish. This pinger emits an acoustic pulse at a frequency from 32 to 300 kHz. This sound pulse is then recorded by a hydrophone.

Photo 1. Reception station

With passive tracking the hydrophone is mounted on a secured listening station. The listening station picks up any tags within its range and logs the occurrence of the tagged fish. The range is generally 100 to 1000 meters and depends on the frequency of the transducer and the power output of the tag. (Photo 2). Thus, depending of the ultrasonic sensors embedded in the transmitter is received information on the date and time of the detection, the depth or temperature shift [4]. By fitting stations in strategic points specific information can be revealed on the migration routes used by sturgeons upstream or downstream, when they go back into the sea.

For sturgeon monitoring were used submersible reception stations VR2W Vemco. Their position in water was carried by the following method: the principle supposed the use of a design composed of concrete anchor placed on the fairway to bind to a steel cable attached to the shore with a metal stake.

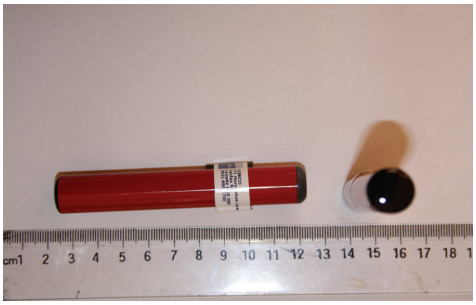

Photo 2. Acoustic transmitter

By this cable has been fixed a metal plate bound to a relon rope length of 0.7 m, onto which was attached a VR2W station, vertically supported by a raft (Photo 3). The entire assembly was positioned so to capture signals from the marked fish transiting that area.

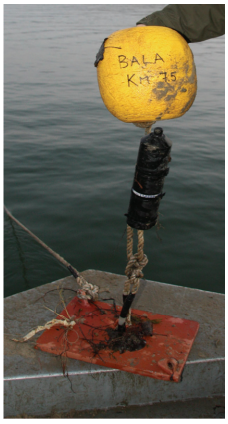

Photo 3. System fixing for station

After the monitoring system was positioned, the fish were captured and marked with ultrasonic transmitters. Each sturgeon was introduced inside a tube cell (Photo. 4) that was specially made to allow maintenance of vital signs during transmitter's implantation (Photo. 5), without having to be removed from the water, thus eliminating the stress. As tranquillizer it has been used an electroshock therapy system (DC 28-30 V) and lidocaine was used as a local anesthetic, injected into 3 points. The suture of the incision has been made with resorbable wire and the disinfecting was made with terramycin spray.

Photo 4.Mobile tube used for implantation

Photo 5.Implantation of the transmitter

Besides the marking with acoustic transmitter it has been used a second type of mark called "Floy tag ". This is made from plastic materials and has the shape of a spaghetti engraved with a unique identification code and contact date of the National Institute of Research and Development for Environmental Protection in Bucharest, necessary when a fish is recaptured by fishermen. The mark is attached to the fish dorsal fin implantation with the help of a special gun without harming the specimens (Fig. 6). This method offers limited information. The information which is gathered will transmit the area where the fish was released and the area where it was captured without being able to draw its specific route. In this case the method is used for both the above mentioned goal and to easier identify specimens marked with ultrasound mark, captured and then released by fishermen.

Photo 6.Implantation Floy tag type

For each marked sturgeon were made the following biometric measurements: total length (TL), standard length (SL) and weight (Fig. 7); the data obtained were recorded on observations sheets and then introduced in a centralized database. The identification of the sex of the sturgeons, which were captured and marked with ultrasonic transmitters, was done using a nondestructive method, by using a rigid Welch Allyn endoscope (Fig. 8). Its probe was inserted into the abdominal cavity through the genital pore and guided by the fiber optic lighting system, which displayed the evidence of milk, in the case of males or of caviar, in the case of females.

Photo 7.Biometric measurements

Photo 8.Determination of sex

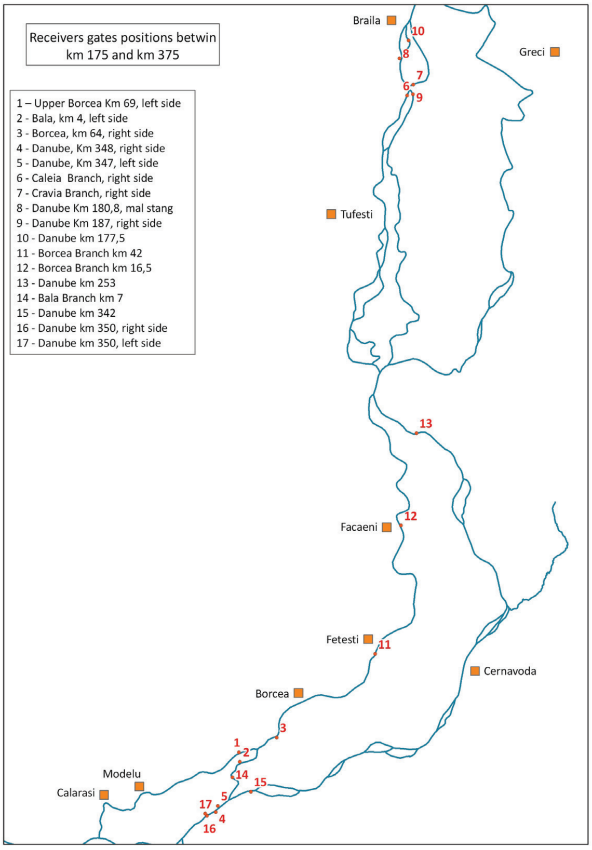

Fig 1. Automatic positioning stations

After the marking operation and the biometric measurements have been taken, sturgeons have been released into the wild. Reading data has been periodically conducted from the VR2W Vemco reception stations using the Vemco User Enviroment (VUE) software for data extraction. After this, the stations were relocated to their initial location.

RESULTS AND DISCUSSIONS

On the Calarasi – Braila monitoring sector, from km 175 to 375, there were located a total of 17 VR2W submersible Vemco automatic stations (Fig. 1). There was also been fixed an additional station outside this area, on the Danube, at km 100, to record fish that return from the Danube Delta to the Black Sea. The storage capacity is 1,000,000 records and the average battery life is 15 months. The Operating Temperature is between -5^{0}C and + 60^{0}C. Their installation was made in places where there weren't interfering with the fishing and outside the mooring areas marked on the sides.

Between October and December 2011 there have been marked a total of 50 copies of sturgeon species.

Amongst them, 30 were marked both with ultrasonic transmitter and a conventional mark (Floy tag), and 20 using only a conventional mark (Floy tag). The weights of the fish were between 8 kg (*Acipenser gueldenstaedtii*) and 180 kg (*Huso huso*). The highest percentage of captures was in the Borcea branch km 30-47 with 75% captures, followed by the Borcea branch km 50-57 with 17.86% of the catch (Fig. 2). On the Caleia branch and the Danube, between km 195 and 197, have been marked only 3.5% of the total catches of the autumn campaign.

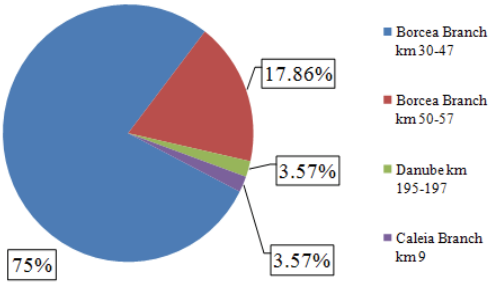

Fig 2. Distribution of catches by fishing areas

Regarding the allocation of catches by species it can be seen that the highest number of catches is sturgeon morun, followed by sturgeon cega. The latter is a specific Danube species migrating on the river. A single female specimen was marked from the sturgeon nisetru and two from the sturgeon pastruga. It is known that sturgeon pastruga migrate in smaller numbers in the fall migration than in the spring when they make clutch. The project provides for the capture and marking of males species than females, which are more sensitive. This can be seen from the diagram below (Fig. 3).

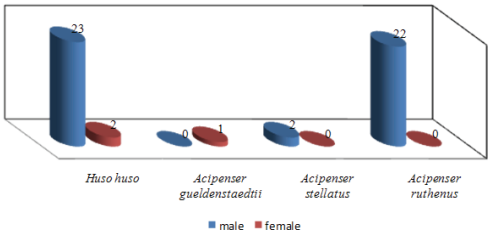

Fig 3. Distribution of sturgeons captured during October-December 2011 by species and gender

After the processing of the collected data stored on the VR2W automatic reception stations it has been noted that 16 copies of sturgeon have crossed 8 perimeters out of the 18 stations located on the Danube. Most of the specimens have had the tendency to move downstream. A total of 7 fish were recorded in the Isaccea station, on the Danube km 100, and heading to the mouth of the Danube into the sea. In the downstream at km 197, the Danube is divided in two branches, namely: Caleia arm and the Old Danube arm stretching for 10 km. At km 186 the two arms meet for a short distance then a new fork is following. In their descent migration 4 moruni sturgeons used the Old Danube as their route, not Caleia, where there will be built a bottom threshold for improving the navigation. On Caleia arm there were no records available. There is only one catch of morun sturgeon weighing 100 kg and being 228 cm long dated 11/04/2011, marked and released by the INCDPM team. This capture was made at km 7 by authorized INCDPM fishermen for scientific purposes.

CONCLUSIONS

The high percentage of catches on the Borcea arm highlights its importance regarding the sturgeon migration route. The VR2W recordings on the Danube km 100 show that not all specimens of sturgeon which climb the river in the fall migration to wintering remain in deep holes over 15 to 20m. Some of them prefer to go down to the mouth of the sea, following that next spring to return to the deposit tip. I noticed that in their downstream route 4 sturgeons used the Old Danube arm between km 197 and km 186. This is very important in the context in which on the other side arm, Caleia, it will be build a bottom threshold that would prevent upstream fish migration. Research in the area will continue in coming years both during the development works and post construction to determine the exact extent the threshold may affect the reproduction of sturgeons.

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RESEARCH ON SPERM QUALITY OF NORTH AMERICAN STURGEON SPECIES *POLYODON SPATHULA*

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Abstract
The paper presents research on sperm quality and affecting factors in male of North American sturgeon species Polyodon spathula recently acclimated in Romania. Quality assessment was performed by microscopic and macroscopic analysis of sperm from a total of 5 males used in artificial spawning experiments in 2012. Microscopic analysis was to determine the concentration of sperm by haemocytometer method, sperm shaped and size determination using electronic microscopy and identification of specific staining, motility determination using dark field microscopy, testing the viability of sperm after sperm activation. Sperm quality parameters were interpreted by correlation with following factors: hormonal stimulation, the spawning season, temperature, extenders properties, short or long term storage of sperm, the biology of spawning (age, weight, length), health and wellness condition.

Key words*: Polyodon spathula, spawning, sperm quality*

INTRODUCTION

Quantity and quality of sperm affect decisively the success of artificial spawning.
Milt quality is a measure of the ability of sperm to successfully fertilise an egg which such ability mostly depends on qualitative parameters of milt i.e. composition of seminal fluid, milt volume, sperm density and sperm motility.[6]
Making artificial spawning without the control of sperm quality can have a negative effect on its biotechnological indicators. [1]
It is necessary to understand the importance of theoretical and especially the dissimilar practical significance of quality control as it is accepted the quality control need of agricultural seeds.
On this context, inside of S.C.D.P Nucet were initiated research work to determine the sperm quality of *Polyodon spathula* sturgeon species by making some current spermiogram and reveal the correlations between environmental factors and fecundate capacity of sperm.

MATERIAL AND METHOD

The spawning experiments accomplished during the years 2002-2010, revealed that paddlefish males can yield sperm a period of 5-6 days consecutively, due to hormonal stimulation of spermiation.
In the first part of breeding season, paddlefish males give a small amount of sperm and therefore require hormonal stimulation of spermiation.
Hormonal stimulation of breeders was accomplish by receiving Nerestin 5A synthetic hormone in a single dose of 0,1 mg/kg of body weight, on 4 of the 5 males used (M1 – M4). A male – wasn't stimulated being used as control in our experiments.
The semen was collected successively for 5 days, starting from the moment of hormonal induction.
Males were characterized biometrically, and the quality of sperm from each male was assessed by macroscopic and microscopic analysis.

Each male was contention, beat out from tank and well drain off. Sperm sampling, (Photo 1) was accomplished with 20 ml plastic siring, dried, provided to end with a cannula made by a 10 cm infusion tube. By this sampling technique can be obtain a sensibly amount of semen, without the risk of being contaminated by faeces or water.

Photo 1. Sperm sampling on *P. spathula*

Macroscopic analysis was to make the following determinations:
-Volume (measuring cylinder, expressed in ml);
-Color;
-Density or consistency;
-Impurities;
-Smell.
Microscopic analyses consisted of:
-Determination of spermatozoa concentration – spermatozoa number/ml (haemocytometer method);
It is based on the principle similar to the appreciation of figurative elements from blood, using Goreaev, Thoma, Burker etc. like counting chambers, and Potain dropper.
The sperm is aspirated in the Potain up to 0.5 division and is filled with a solution of 3 % sodium chloride up to 101 division.
Concentration of spermatozoa on sperm/mm^3 is calculated as:

$$C = \frac{N.I.S.D.}{n}$$

where:
N – number of spermatozoa counted in 5 big quadrates;
I – height or depth of chamber (1/10 mm);
S – surface of a square (1/400 mm^2);
D – amount of sperm dilution;
n – number of squares in which the sperm were counted.
-Determination of shape and size of *P. spathula* spermatozoa by electronic microscopy (Optika microscope) and determination of specific staining to measure the main components – head and tail – Photo 2)

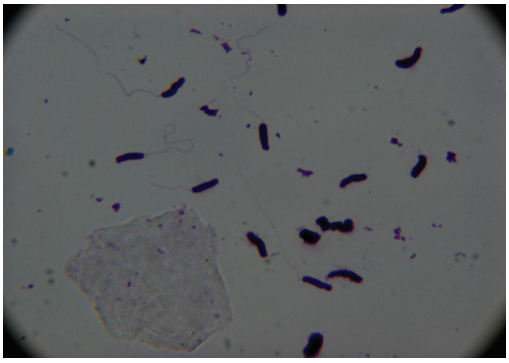

Photo 2. Shape and size of *P. spathula* spermatozoa – May-Grumwald-Giemsa dyeing

- Determination of sperm motility was performed using a simple microscope (ML-4 IOR tip). Optical combination suitable for this control is ocular 10X with lens 20X or 40 X. Was used a grid blade (Burker). The smear was read extemporaneously, according to conventional method known in the practice of artificial spawning after sperm activation in technological water and normal saline solution.

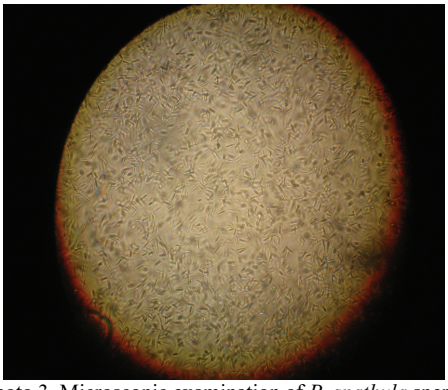

Photo 3. Microscopic examination of *P. spathula* sperm

- The way of spermatozoa movement: straight, cuff, riding, etc.
- Testing spermatozoa viability after sperm activation – examining the preparation

found between blade and slide;
Semen has been collected for five days, every 24 hours.
Sperm concentration was evaluated as billions / ml of semen.
Semen was collected in weighing bottles with lid. By the macro and microscopic sperm analysis was made the usual semen analysis with qualitative and quantitative indicators that can give us important information about the status of breeders used in the process of spawn.
The main biometric characteristics of males are presented in Table 1. After hormonal induction, the male breeders were stoked in canvas tanks specially made for this species and located into the hatchery.
Tank capacity is 4000 l of water; water flow is 15 l / min., dissolved oxygen - 9,0 mg O_2/l, water temperature 13 – 14 ^{0}C.
During the experimental period breeders were monitoring continuously, and water temperature was measured and registered hourly.

RESULTS AND DISCUSSIONS

Males used in artificial spawning experiments, in 2012, were aged 9 and 10 years, average weight between 9325 and 13200 g and standard length between 120.0 and 146.0 cm.

Table 1. Biometric characteristics of *P. spathula* males

Male	Age (years)	Weight (g)	Length (cm)
M1	10	13200	146
M2	10	13180	143
M3	9	10260	136
M4	9	10710	134
M5	9	9325	120

The literature mentions that the volume of sperm released by sturgeon males is sometimes large (e.g. 25–200 ml for sevruga, *Acipenser stellatus*, 25–500 ml for Russian sturgeon,*Acipenser guldenstaedti,* and 800–1000 ml for kaluga, *Huso dauricus*) [2] .
In sturgeons the concentration of spermatozoa was usually reported as low compared to teleost fish. [5] ; [2]
In artificial propagation, the amount of sperm obtained per male is limited in paddlefish and in white sturgeon (*Acipenser transmontanus*), especially in the first period of the reproductive season, and therefore spermiation must be stimulated. [4]
The total amount of sperm per male during of the 5 days of sperm sampling was significantly bigger after injection with Nerestin 5A 0.1 mg/kg towards the uninjected male (Table 2).

Table 2. Evolution of paddlefish sperm after hormonal induction with Nerestin 5A

Male	Volume of sperm harvested after n days (ml)					
	1	2	3	4	5	Total
M1	8.0	96.7	117	105.8	111.8	432.2
M2	8.4	96.0	110.0	98.8	95.0	408.2
M3	8.5	103.8	138.1	108.2	121.0	479,6
M4	10.1	96.0	107.2	102.2	91,1	396,5
M5	3.5	15.5	31.0	16.5	4.1	67.1

The sperm was obtained at 00: 35 h (19 April 2012). (Table 3).
The amount of sperm harvested from one hormonally induced male was between 91.1 ml and 138.1 ml.
Sperm volume increased from dai 2 to day 5. It was found that the amount of sperm harvested from the 9 years old males was higher than that collected from the 10 years old males.
Data from literature shows that the volume of sperm collected from paddlefish during the experiment was similar to that of the sturgeon and Russian sturgeon. Also the concentration of paddlefish spermatozoa was similar to that of the sterlet or Persian sturgeon, but lower than that of the Russian sturgeon, sevruga and beluga. [2;5].
Semen color varies from milky white to dirty white with gray tint (Photo 3, Table 3).

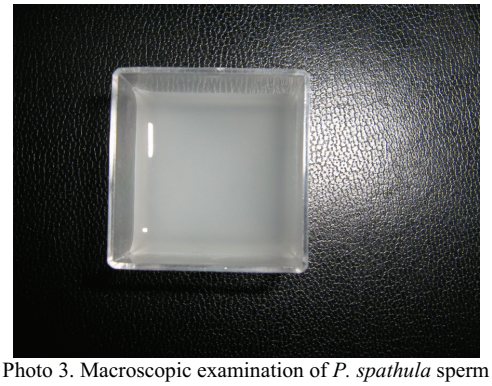

Photo 3. Macroscopic examination of *P. spathula* sperm

Consistency is aqueous (Table 3), and the smell is distinctive.

Table 3. Evolution of paddlefish sperm number hormonal induction with Nerestin 5A

Male	Number of sperm harvested after n days ($\times 10^9$)					
	1	2	3	4	5	Total
M1	14.1	46.3	49.4	25.2	9.1	144.1
M2	9.3	44.7	36.3	22.3	5.6	118.2
M3	9.6	42.8	43.9	29.0	9.6	134.9
M4	11.2	13.4	12.3	5.6	4.5	47.0
M5	10.3	2.0	9.3	2.0	1.0	14.3

The microscopic analysis shows that the total number of sperm per male ranged from 4.5 and 49.4 x 10^9.

Total number of sperm per male was higher on day 2 (46.3 x 10^9 per male), the highest in day 3 (49.4 x 10^9 per male), and then easily lower in day 4 (29.0 x 10^9 per male) and day 5 (9.6 x 10^9 per male).

It was fond that although semen volume remains high on the day 4 and 5, sperm concentration decreases.

Fish sperm concentration, which is an important parameter of artificial reproductive management is very variable and depends on the species, individual fish size and season. [3]

Sperm motility was regularly observed during the 5 days in each experimental variant (Table 3). The percentage of motile sperm was not significantly different between males during the harvesting of sperm this ranging from 80 to 95 %.

Survival times of fresh spermatozoa after activation with water and saline solution varied in large limits (Table 4, Table 5).

Regarding the viability percentage, found that it was higher after sperm activation with saline solution than activation with technological water.

Studying sperm movement was observed that they have clear forward movement in the first 5 minutes from activation. After 8 minutes, only 20 – 30 % still has forward movement, others have weaker movements, of oscillation. It was observed that sperm are still motile al approx. 20 minutes of activation.

From studies on semen stored at room temperature, it was found that sperm are mobile even after there activation with saline solution at 4 hours after harvesting.

Table 4. Usual semen analysis on *P. spathula*

Date/ hour Male	Temperature on harvest ^{0}C		Survival time after activation (minutes, seconds)		Volume ml/fish	Concentration nr. of sperm billion/ml	Motility %	Consistency	Impurities	Color
	Air	Water	Water	Saline solution						
Male sperm sampling on 19.04.2012, starting at 00^{35}										
Mascul 1	10	13	1'42"	6' -8 '	96.7	46,3	95	Aqueous	No	Dirty white-gray tint
Mascul 2	10	13	1'40"	2'30"	96.0	44,7	85	Aqueous	No	Milky white
Mascul 3	10	13	1'10"	1'30"	103.8	42,8	85	Aqueous	Urine	Milky white
Mascul 4	10	13	45"	3'30"	46.0	13,4	90	Aqueous	No	Milky white
Mascul 5	10	13	25"	1'10"	15.5	2	80	Aqueous	Urine	White-gray
Sperm analyses of male 1, date 21.04.2012										
Male 1	11.5	13.5	1'48"	8'56"	111.8	9.1		Aqueous	No	

Table 5. Data on *P. spathula* sperm viability after activation

Data	Temperature at harvest °C		Storage temperature ^{0}C	No. hours from harvest	Motility %	Survival time after activation – minutes, seconds		Observations
	Air	Water		-ore-		water	saline solution	
19.04.2012, from 00^{35}								
Male 1	10	13	20,0	2	90-100	1'42" -2 '	6' -8 '	Sperm from one male
	10	13	20,0	24	-	-		Agglutinated sperm has not been activated
	10	13	4,0-8,0	24	80- 50	1'20" - 1'45"	6'	
	10	13	4,0-8,0	48	2-3	-	10"	
Male 2	10		20,0	2,5	-	-	-	Semen has not been activated
Male 3	10	13	20,0	2,5	70-25	1'35"	5'	Sperm has not been activated, agglutination
Male 4	10	13	20,0	2,0	10-13	35"	35"	
Male 5	10	13	20,0	1	100	1'25"	6'45"	
	10	13	20,0	4	70 - 90	55"	3'35"	
	10	13	20,0	24	-	-	-	Sperm has been agglutinated
	10	13	4,0-8,0	24	50	50" -55"	2'35"	
	10	13	4,0-8,0	48	-	-	-	Sperm has not been activated
Analyses of male 1 sperm, date 21.04.2012								
Male 1			2.0 -4.0	-	90-100	1'30" - 1'48"	6'56" -7'	
			2.0-4.0	24	90	1'30"	5'	
			2.0-4.0	48	70- 40	1'10"	5'	
			2.0-4.0	72	50- 30	45"	2'30"	
			2.0-4.0	96	5-10	-	1'20"	

CONCLUSIONS

To increase the volume of semen in the spawning season the male hormonal stimulation is required. Although sperm volume is high even at four, five days after stimulation, the number of sperm decreases.

Qualitative parameters of milt (i.e. seminal fluid composition, spermatozoa motility and sperm production) could be influence by

several factors including biological characteristics of brooders (age, weight and length), rearing conditions of brooders (temperature, photoperiod, nourishment, and animal welfare and health), artificial induction of spawning, spawning season (repeated milt collection and spermiation time) and post stripping factors (chemical properties of diluents and short-term and longterm storage of milt).

The analyses results were materialized in achievement of usual semen analysis with quality indicators of semen. Understanding of the factors that affect sperm quality could be useful for adjustment and efficient management of them.

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STUDY CONCERNING THE EVALUATION OF THE GENETIC DETERMINISM OF SOME BIOECONOMIC AND ECOECONOMIC CHARACTERS IN INEU CARP BREED

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Abstract

Increased competitiveness in the biological fish material market required the improving the quantitative characters of exploited species. One of the essential problems of heredity study quantitative characters is knowing the degree of hereditary transmission of characters from parents to offspring. Assessment of genetic determinism was based on heritability. This procedure requires knowledge of causal components of variance.
The biological material studied consisted of 50 descendants of Ineu carp breed, who came from five mothers and ten fathers. Each descendant was measured for three characters: body weight, maximum body height and body length at the end of the first three summers of growth. For variance components analysis was used BLUP (Best Linear Unbiased Prediction) methodology, applied to an individual animal model. The results showed that the characteristics considered their genetic determinism is low to medium and medium to high. Thus, it was found that, after the first summer of growth, morphological characters measured have a low genetic determinism (0.1615; 0.1894; 0.1708). After the second summer of growth, body weight and maximum body height have medium genetic determinism and body length high genetic determinism (0.4426). After the third summer, weight and body length have high genetic determinism and maximum body height has medium genetic determinism (0.3270).
In conclusion, genetic determinism of considered characters is low to medium, an aspect which leads to the conclusion that to maximize the effect of selection is necessary to consider family selection.

Key words**:** *carp, genetic variance, morphological characters*

INTRODUCTION

The methods of intensive animal growth to obtain high productions of meat, has located in the forefront the research and study of the genetic capacity for growth as the main factor that interferes with the development of high bioeconomic and ecoeconomic productions.
Making the animal production is the result of simultaneous or separate action of the three factors: the genetic potential of the individual, the number of individuals and the operating conditions. Short-term increase of animal production can be achieved by increasing the workforce, but on long term, this approach is unfeasible, because the vegetal production grows as an arithmetic progression, while the animal one, as a geometric progression, leading to an excess of the capacity support [1].
In the context of sustainable animal production, the way which should be followed is to increase the animal production based on improving the genetic potential, together with the improvement of the operating conditions [1].
The followed goals in the growth of valuable species of fish, carp in our case, consist of transforming some bioeconomic and ecoeconomice features , in order to be useful for humans [4].
The aim is to obtain species with:
- high growth rate;

- a certain external morphological aspect, according to biological and economic considerations;
- increased precocity and prolificity;
- resistance to diseases and unfavorable environment factors.

In practice, these objectives are reflected in the transformation of some characters of individuals belonging to the population with which the work is done.

Also, at fish, they can be considered domestic animals, whose genetic evolution is under long human intervention, and that any negligence or mistake in the management of the genetic fund, may have most serious consequences, until the disappearance of some races or local populations [2].

MATERIAL AND METHOD

In our country, the carp, which as a wild species was, decades ago, the basic fishery production in the lower Danube basin, was, is and it will remain, at least as goal, the main fish species in Romania, regardless the economic circumstances.

The studied biological material consisted of 50 offspring of Ineu carp (Photo 1), which belonged from five mothers and ten fathers.

Each descendant has been measured for three characters: body weight (W), maximum body height (H) and body length (l), at the end of the first three summers of growth.

The body weight (W) has been determined by weighing with a scale for small weights.

The maximum body height (H) has been measured in the highest region of the body, at the level of the first radiating from the dorsal fin, with the help of a graduated ruler.

The body length (l) has been measured on the midline of the body, from the top scaly snout to the end cover to the caudal fin, with the help of a graduated ruler.

Photo 1. Ineu carp breed (Source: S.C.P. Nucet)

For the analysis of the variance components the BLUP (Best Linear Unbiased Prediction) methodology has been used, being applied to an individual animal model.

RESULTS AND DISCUSSIONS

Knowing the level of quantitative hereditary transmission of characters from parents to offspring is one of the important problems of their heredity study [3].

To highlight the genetic determinism of quantitative characters, subject to genetic improvement programs to breed the Ineu carp using heritability.

Heritability expresses the proportion of phenotypic manifestation of a character that can be attributed to environmental effects of genes involved in that genotype [1].

Table 1. Genetic determinism of the weight, maximum body height and body length, on a first summer carp of Ineu breed

Breed/age	Characters	Error variation	Additive genetic variation	Phenotypic variation	Heritability
Ineu 0+	W (g)	19.0851	3.6757	22.7609	0.1615
	H (mm)	81.3038	18.9949	100.2987	0.1894
	l (mm)	450.3851	92.7415	543.1267	0.1708

After the first summer of the Ineu carp individuals growth, there is weak genetic determinism of the measured morphological characters (Table 1). This means that the average effect of genes from the genotype is responsible for more than 20% of the phenotypic manifestation of the analyzed characters.

The value of heritability is also influenced by the environmental conditions in which the analyzed individuals of the population evolve. Any change in the action of the environmental factors influences their share in the total phenotypic variance, changing by default, and the share of the other components of the variance, including the additive variance [1].

Table 2. Genetic determinism of the weight, maximum body height and body length, on a second summer carp of Ineu breed

Breed/age	Characteristics	Error variation	Additive genetic variation	Phenotypic variation	Heritability
Ineu 1+	W (g)	11026.1	4360.9	15387.0	0.2834
	H (mm)	68.2523	25.9540	94.2063	0.2755
	l (mm)	232.5263	184.6654	417.1917	0.4426

At the end of the second summer of growth, an intense genetic determinism is found in the length of the body characteristic. The other two characters, the body weight and maximum body height are intermediate heritability (Table 2).
After the second summer of growth, in the phenotypic expression of genes character average effect from the genotype provides over 40% of the phenotypic expression of the body length and between 20-40% for the weight and for the maximum body height.
The results can be the result of uniformity of the environmental conditions and lower sensitivity of the breed towards them.

Table 3. Genetic determinism of the weight, maximum body height and body length, on a third summer carp of Ineu breed

Breed/age	Characters	Error variation	Additive genetic variation	Phenotypic variation	Heritability
Ineu 2+	W (g)	78896	55637	134533	0.4136
	H (mm)	119.4897	57.9617	177.4513	0.3270
	l (mm)	427.0243	296.9444	723.9687	0.4102

At the end of the experimental period, respectively the third summer of growth, the weight and body length characters have hard heritability determinism, having similar values. The maximum body height is, as in the end of the second summer of growth, an average heritability characteristic (Table 3).
Except for the body length, the other analysed morphological characters, the body weight and the maximum body height, there is an increasing tendency of heritability. In the same tendency is the body length characteristic, making an exception after the second summer of growth.

CONCLUSIONS

As a consequence of the study on the population of Ineu carp, in what regards the evaluation of the genetic determinism of some useful bioeconomic and ecoeconomic characters, the following has been observed:
1. After the first summer of growth, the measured morphologic characters have a low genetic determinism (0.1615; 0.1894; 0.1708).
2. At the end of the second summer of growth, the body weight and the maximum body height have an average genetic determinism (0.2834, 0.2755) and body length has a high determinism (0.4426).
3. At the end of the third summer of growth, the weight and the body length have a high determinism (0.4136; 0.4102), and the maximum body height has an average determinism (0.3270).
4. The genetic determinism of the characters is considered low to intermediate, which means that to maximize the effect of the selection, the family selection is required.

5. The heritability values of the analyzed characters refer only to the study of the analyzed population and to the environmental conditions in which it has developed.

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STUDY CONCERNING THE HERITABILITY ESTIMATION FOR SOME BIOECONOMIC AND ECOECONOMIC CHARACTERS IN ROPSA CARP BREED

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Abstract

Increased competitiveness in the biological fish material market required the improving the quantitative characters of exploited species. One of the essential problems of heredity study quantitative characters is knowing the degree of hereditary transmission of characters from parents to offspring, issue that can be quantified by heritability.
The biological material studied consisted of 50 descendants of Ropsa carp breed, who came from five mothers and ten fathers. Each descendant was measured for three characters: body weight, maximum body height and body length at the end of the first three summers of growth. For variance components analysis was used BLUP (Best Linear Unbiased Prediction) methodology, applied to an individual animal model. Thus, it was found after the first three summers of growth, morphological characters measured have a medium genetic determinism, to the lower limit (0.20). The exception was the maximum body height that had a genetic determinism 0.2757 after the first summer of growth and 0.1910, after the second summer.
The results in the investigated effective showed that the determinism of the considered characters is low to intermediate. This thing shows that the main performance is not a sufficiently precise indicator for additive genotype; for this reason is necessary to supplement this source with information provided by family candidate selection. As a result we can expect increased accuracy of selection and consequently the selection effect.

Key words**:** *carp, genetic variance, morphological characters*

INTRODUCTION

The intensive animal growth in order to obtain high productions of meat, has determined the research orientation towards the study of the genetic capacity for growth. It is considered to be one of the main factors which interfere in obtaining high bioeconomic and ecoeconomic productions.
Highlighting the animal production is the result of simultaneous or separate action of the genetic potential of the individual, the number of individuals and the operating conditions.
During a short term, the increase of animal production can be achieved by increasing the workforce. On the other hand, on a long term, the solution is not useful, because the animal production grows as a geometric progression, which leads to the overcome of the support capacity [1].
In the context of sustainable animal production, the way which should be followed is to increase the animal production based on improving the genetic potential, together with the improvement of the operating conditions [1].
The genetic improvement of fish can be defined as a process of change, by specific methods and ways, of the genetic structure of the populations, in the wanted direction given by human, keeping in mind the characteristics of the species and of the environment [5].
Therefore, the aim of the genetic improvement is to identify and quantify the elements which get involved in the creation of useful bioeconomic and ecoeconomic characters,

moreover, to determine whether a data value is transmitted to the offspring.
In the growth of carp, species which is considered valuable in our country, the followed goals are similar to the ones of the fishes of bioeconomic and ecoeconomic interest [4].
They are represented by: high growth rate; a certain external morphological aspect, according to biological and economic considerations; increased precocity and prolificity; resistance to diseases and unfavourable environment factors.
In practice, fulfilling these objectives is done in the transformation of some characters of individuals belonging to the population with which the work is done.
Any negligence or mistake in the management of the genofund may have most serious consequences, until the disappearance of some races or local populations [2].

MATERIAL AND METHOD

In Romania, the carp, which as a wild species was, decades ago, the basic fishery production in the lower Danube basin, was, is and it will remain, at least as goal, the main fish species in Romania, regardless the economic circumstances.
The studied biological material consisted of 50 offspring of Ropsa carp (Photo 1), which belonged from five mothers and ten fathers.
Each descendant has been measured for three characters: body weight (W), maximum body height (H) and body length (l), at the end of the first three summers of growth.
The body weight (W) has been determined by weighing with a scale for small weights.
The maximum body height (H) has been measured in the highest region of the body, at the level of the first radiating from the dorsal fin, with the help of a graduated ruler.
The body length (l) has been measured on the midline of the body, from the top scaly snout to the end cover to the caudal fin, with the help of a graduated ruler.

Photo 1. Ropsa carp breed (Source: S.C.P. Nucet)

For the analysis of the variance components the BLUP (Best Linear Unbiased Prediction) methodology has been used, being applied to an individual animal model.

RESULTS AND DISCUSSIONS

Knowing the level of quantitative hereditary transmission of characters from parents to offspring is one of the important problems of their heredity study [3]. To highlight the genetic determinism of quantitative characters, subject to genetic improvement programs to breed the Ropsa carp using heritability.
Heritability expresses the proportion of phenotypic manifestation of a character that can be attributed to environmental effects of genes involved in that genotype [1].

Table 1. Genetic determinism of the weight, maximum body height and body length, on a first summer carp of Ropsa breed

Breed/age	Characters	Error variation	Additive genetic variation	Phenotypic variation	Heritability
Ropsa 0+	W (g)	41.4315	11.8120	53.2435	0.2218
	H (mm)	32.0216	12.1912	44.2128	0.2757
	l (mm)	333.5126	85.1395	418.6521	0.2034

After the first summer of the Ropsa carp individuals growth, there is weak genetic determinism of the measured morphological characters (Table 1). This means that the average effect of genes from the genotype is responsible for more than 20% of the phenotypic manifestation of the analyzed characters.

The results may be the consequence of an increased resistance of crap individuals of the Ropsa breed towards the environment conditions.

Table 2. . Genetic determinism of the weight, maximum body height and body length, on a second summer carp of Ropsa breed

Breed/age	Characters	Error variation	Additive genetic variation	Phenotypic variation	Heritability
Ropsa 1+	W (g)	8225.2	2330.1	10555.3	0.2207
	H (mm)	29.7920	7.0351	36.8271	0.1910
	l (mm)	229.6105	63.6016	293.2121	0.2169

At the end of the second summer of growth, gn intense genetic determinism is found in the length of the body characteristic. The other two characters, the body weight and maximum body height are intermediate heritability (Table 2).

After the second summer of growth, in the phenotypic expression of genes character average effect from the genotype provides over 40% of the phenotypic expression of the body length and between 20-40% for the weight and for the maximum body height.

Table 3. Genetic determinism of the weight, maximum body height and body length, on a third summer carp of Ropsa breed11

Breed/age	Characters	Error variation	Additive genetic variation	Phenotypic variation	Heritability
Ropsa 2+	W (g)	40397	10241	50638	0.2022
	H (mm)	185.6248	47.5118	233.1366	0.2038
	l (mm)	1602.0406	424.1594	2026.2000	0.2093

At the end of experimental period, respectively the third summer of growth, the morphological analysis tended to maintain intermediate heritability, at the lower limit (Table 3).

For all the three characters, the average effect of genes from the genotype assures more than 20% of their phenotypic expression.

The heritability is influenced by the environmental conditions in which the analyzed individuals of the population develop. Any change in the environmental factors influences their share in the total phenotypic variance, changing by default the share of other components of variance, including the ones of the additive variance [1].

The Ropsa carp breed is recognized as a breed resistant to the environmental conditions and it is possible that they may not interfere too much in modifying the phenotypic expression of the analyzed characters.

CONCLUSIONS

As a consequence of the study on the population of Ropsa carp, in what regards the evaluation of the genetic determinism of some useful bioeconomic and ecoeconomic characters, the following has been observed:

1. After the first summer of growth, the measured morphologic characters have an average genetic determinism, at the lower limit (0.20).
2. The maximum body height had a slightly modified genetically determinism from the other characters, being the largest after the first summer of growth (0.2757) and the lowest after the second summer (0.1910).
3. In the conducted study, it is possible that the own performance is not a sufficiently precise indicator for the additive genotype, reason why the supplement of this source with the provided information is imposed by the family of the

candidate at selection. As a result we can expect an increased accuracy of selection and thus of the effect of the selection.

4. The heritability values of the analyzed characters refer only to the study over the population and environmental conditions in which it has evolved.

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