SCIENTIFIC PAPERS
SERIES D. ANIMAL SCIENCE
Volume LXI, No. 1, 2018

University of Agronomic Sciences
and Veterinary Medicine of Bucharest
Faculty of Animal Science

SCIENTIFIC PAPERS
SERIES D
ANIMAL SCIENCE

Volume LXI, No. 1

2018
BucharesT

PUBLISHERS:

University of Agronomic Sciences and Veterinary Medicine of Bucharest, Romania

Address: 59 Mărăști, District 1, 011464 Bucharest, Romania

Phone: + 40 213 182 564, Fax: +40 213 182 888**,** www.zootehnie.ro

CERES Publishing House

Address: 29 Oastei Street, District l, Bucharest, Romania

Phone: + 40 317 90 23, E-mail: edituraceres@yahoo.com, Webpage: www.editura-ceres.ro

To be cited: Scientific Papers. Series D. Animal Science, Volume LXI, No. 1, 2018

ISSN 2285-5750; ISSN CD-ROM 2285-5769; ISSN Online 2393-2260; ISSN-L 2285-5750

International Database Indexing: Web of Science Core Collection (Emerging Sources Citation Index), Index Copernicus, CABI, DOAJ, Ulrich's Periodicals Directory (ProQuest), PBN, Cite Factor (Academic Scientific Journals), Scipio, OCLC (WorldCat), Research Bible, Google Scholar.

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GENETICS AND BREEDING

Scientific Papers. Series D. Animal Science. Vol. LXI, Number 1, 2018
ISSN 2285-5750; ISSN CD-ROM 2285-5769; ISSN Online 2393-2260; ISSN-L 2285-5750

SOMATIC CELL COUNT OF MILK IN HOLSTEIN COWS RAISED IN TURKEY CONDITIONS: A COMPARATIVE EVALUATION

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Abstract

The objective of this paper was to discuss the level of somatic cell count (SCC) of milk in Holstein cows raised in Turkey. In total, 20 investigations conducted on Holstein breed Turkey (TR) and other countries (OC) were examined by SCC and effective non-genetic factors. The data were designed from the scientific journals on animal science published in the last decade. The means of SCC for TR and OC were calculated to be 486x10^3 cells/ml and 354x10^3 cells/ml, respectively. No statistical differences were found between two averages by log10 base. While parity, farm, month and stage of lactation were the significant factors affecting SCC for TR Holsteins, parity and calving season were the main factors for OC Holsteins. The findings revealed that reducing SCC have to be seen a priority target by the farm owners to achieve more productive herds.

Key words*: cow milk, environmental factor, Holstein, somatic cell count.*

INTRODUCTION

Milk industry has become one of the most important sector within today's animal farming. Not only breeder selection studies have been carried out, but also boosting raw milk quality has been intensified throughout the world. In this sense, detecting bacterial load is admitted as the prevalent procedure, many indirect techniques have been developed to determine milk quality degree. Such as, somatic cell count (SCC) is the most reliable indicator among the indirect parameters.

In normal, somatic cells are originated from body tissues and their amounts suddenly increase during intra-mammary infection or abnormality.

Thus, limit thresholds for SCC have been declared in many countries in respect to potableness of milk by human. While this level is 400x10^3 cells/ml in the EU countries, it has formally been affirmed to be 500x10^3 cells/ml in Turkey.

Actually, many studies investigating SCC of dairy cows have been conducted in different locations of the world and also in Turkey. However, comparative studies are still needed in this subject. Revealing the quality degree of raw milk of dairy cows in Turkey conditions will be gain an important information for milk industry of the country.

The aim of the study was to compare SCC of milk collected from Holstein cows raised in Turkey conditions and other regions of the world.

MATERIALS AND METHODS

To evaluate, 20 manuscripts those published in animal science journals and informed SCC results obtained from Holstein cows were investigated. All manuscripts had been published in the last decade and 10 papers of those were carried out in Turkey (TR). Before the evaluation, SCC data were transferred to logarithm 10 base to ensure homogeneity of variance. To compare SCC levels of Holstein cows belonging to Turkey with those noted in the other countries (OC; n=10), independent t-test were applied. The statistical processes were performed using SPSS 17 for Windows.

RESULTS AND DISCUSSIONS

In the present study, SCC means of Holstein raw milk in TR and OC are given in Table 1.

As seen, a wide variation among the SCC values is attractive. Also, the SCC average of TR was found to be 1.6 times higher than those obtained in OC.

However, the threshold for SCC of bovine raw milk in Turkey has been informed as $500x10^3$ cells/ml. Thus, calculated SCC mean might be assumed nearby to that limit. Besides, obtained SCC mean in OC might not be accepted as optimum.

While EU directives has been declared the highest SCC to be $400X10^3$ cells/ml, the worrisome average for OC was also observed here.

Table 1. Some study results on SCC ($x10^3$) of Holsteins

Researchers in TR	SCC	Researchers in OC	SCC
Erdem et al., 2007	572	Gaafar et al., 2010	313*
Atasever and Erdem, 2008	1071	Sefidmugzi and R. Baghal, 2014	250
Koc, 2008	456*	Sefidmugzi and Amer, 2015	88*
Atasever and Erdem, 2009	959	Ludovico et al., 2015	637
Koc and Kizilkaya, 2009	450	Sri Balaji et al., 2016	195
Kaygisiz and Karnak, 2012	506	Weglarz et al., 2008	968*
Alic Ural, 2013	879	Salamanczyk and Gulinski, 2013	427
Yilmaz and Koc, 2013	63	Stadnik and Atasever, 2015	302
Cinar et al., 2015	274*	Stadnik and Atasever, 2017	183
Yavuz and Kaygisiz, 2015	419	Jeretina et al., 2017	172*
Overall	~565	*Overall*	~353

*: estimated value

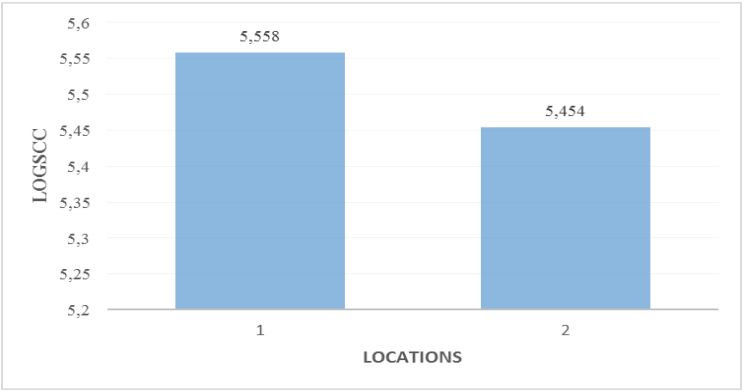

Figure 1. Change of logSCC means by locations (1=TR, 2= OC)

Distribution of environmental factors affecting SCC in both locations is presented in Table 2. While parity was the most important factor, farm, stage of lactation and month were other main factors causing high SCC for both locations.

Koc (2008) emphasized in a study that parity, herd, lactation month and milking time were significant ($P<0.05$) factors for SCC in Holsteins.

To compare SCC means by locations, all SCC data were transferred to logarithm 10 base (logSCC) before the analysis. According to final results, no significant difference was found between two locations of this study (Figure 1).

However, the findings clearly reflected that Holstein cows had high SCC not only in TR but also in OC.

In this context, giving more effort to reduce high SCC would be regarded in Holstein herds. Due to high association of management practices with milk SCC (Sefidmazgi and Amer, 2015), ensuring hygiene and applying precisely milking procedure should firstly be paid attention in the farms.

Table 2. Distribution of effective factors on SCC in TR (n=10) and OC (n=10) Holsteins (%)

Factors	TR	OC
Parity	23.07	27.77
Farm	15.38	11.11
Stage of lactation	15.38	11.11
Month	15.38	11.11
Milking time	11.38	-
Season	7.69	5.55
Calving season	7.69	11.11
Test day	3.84	5.55
Lactation length	-	5.55
Milk yield	-	5.55
Year	-	5.55

CONCLUSIONS

The SCC levels of Holstein cows raised in Turkey conditions were compared with those raised in the other countries. It was revealed that SCC means of Holstein herds in the both locations were relatively high according to EU directives.

In conclusion, taking substantial precautions to minimize non-genetic factors have to be seen as an imperative process by dairy farmers.

REFERENCES

Aliç Ural D., 2013. The Relationships Among Some Udder Traits and Somatic Cell Count in Holstein-Friesian Cows. Kafkas Univ. Vet. Fak. Derg., 19 (4), 601-606.

Atasever S., Erdem H., 2009. Association between subclinical mastitis markers and Body Condition Scores of Holstein cows in the Black Sea region, Turkey. J. Anim. Vet. Adv., 8(3), 476-480.

Atasever S., Erdem H., 2009. Estimation of milk yield and financial losses related to somatic cell count in Holstein cows raised in Turkey. J. Anim. Vet. Adv, 8(8), 1491-1494.

Cinar M., Serbester U., Ceyhan A., Gorgulu M., 2015. Effect of Somatic Cell Count on Milk Yield and Composition of First and Second Lactation Dairy Cows, Italian J. Anim. Sci., 14:1, 3646.

Erdem H., Atasever S., Kul E., 2007. Some environmental factors affecting somatic cell count of Holstein cows. J. Appl. Anim. Res., 32, 173-176.

Gaafar H.M.A., Basiuoni M.I., Ali M.F.E., Shitta A.A., Shamas A.Sh.E., 2010. Effect of zinc methionine supplementation on somatic cell count in milk and mastitis in Friesian cows. Arch. Zootech., 13, 36-46.

Jeretina J., Skorjanc D., Babnik D., 2017. A new somatic cell count index to more accurately predict milk yield losses. Arch. Anim. Breed., 60, 373–383.

Kaygisiz A., Karnak I., 2012. Evaluation of Somatic Cell Count in Raw Milk Samples Collected from Dairy Farms in Kahramanmaras Province for EU Norms and Subclinical Mastitis. KSU J. Nat. Sci., 15, 9-15.

Koc A., 2008. A Study of Somatic Cell Counts in the Milk of Holstein-Friesian Cows Managed in Mediterranean Climatic Conditions. Turk. Vet. J. Anim. Sci., 32 (1), 13-18.

Koc A., Kizilkaya K., 2009. Some factors influencing milk somatic cell count of Holstein Friesian and Brown Swiss cows under the Mediterranean climatic conditions. Arch. Tierz., 52 (2), 124-133

Ludovico A., Maion V.B., Bronkhorst D.E., de Santana E.H.W., 2015. Losses in milk production and quality due to milk somatic cell count and heat stress of Holsteins cows in temperate climate. Ciências Agrárias, 36 (5), 3455-3470.

Salamonczyk E., Gulinski P., 2013. Somatic cell level in dairy cows' milk during extended lactation. Ann. Anim. Sci., 13 (4), 859–868.

Sefidmazgi A.S., Amer P.R., 2015. Milk losses and quality payment associated with somatic cell counts under different management systems in an arid climate. Can. J. Anim. Sci., 95, 351-360.

Sefidmazgi A.S., Bagha F.R., 2014. Effects of herd management practices on somatic cell counts in an arid climate. R. Bras. Zootec., 43(9), 499-504.

Sri Balaji N., Saravanan R., Senthilkumar A., Srinivasan G., 2016. Effect of subclinical mastitis on somatic cell count and milk profile changes in dairy cows. Int. J. Sci. Envir., 5(6), 4427- 4431.

Stadnik L., Atasever S., 2015. Influence of some environmental factors on body condition score and somatic cell count in Czech Holstein cows. Indian J. Anim. Res., 49(6), 774-777.

Stadnik L., Atasever S., 2017. Influence of somatic cell count and body condition score on reproduction traits and milk composition of Czech Holstein cows. Indian J. Anim. Res., 51(4), 771-776.

Węglarz A., Gardzina-Mytar E., Zapletal P., Makulska J., Frelich J., 2008. Effect of the parity, lactation stage and season of feeding on the somatic cell count in cow milk. J. Agrobiol., 25(2), 209-214.

Yavuz S., Kaygisiz A., 2015. The Relationship Between Some Body and Udder Measurements with Somatic Cell Count in Holstein Cows. KSU J. Nat. Sci., 18(3), 9-18. (in Turkish).

Yilmaz H., Koc A., 2013. A research on milk yield, persistency, milk constituents and somatic cell count of Red Holstein cows raised under Mediterranean climatic conditions. Bulg. J. Agr. Sci., 19, 1401-1407.

Scientific Papers. Series D. Animal Science. Vol. LXI, Number 1, 2018
ISSN 2285-5750; ISSN CD-ROM 2285-5769; ISSN Online 2393-2260; ISSN-L 2285-5750

THE POLYMORPHISM OF *CAST* AND *GDF9* GENES IN THE TUVAN SHORT-FAT-TAILED SHEEP POPULATION

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Abstract

The Tuvan short-fat-tailed sheep is a local breed spread in the Russian Federation. This breed habites in Tuva. The aim of investigation was the identification of the genetic polymorphism of calpastatin (CAST) and the growth differentiation factor-9 (GDF9) genes in the Tuvan local sheep population. Calpastatin gene is known as a candidate gene of meat quality traits, and GDF9 gene is a potential genetic marker of prolificacy. Genomic DNA was isolated from samples of blood of 131 animals. Two primer pairs were used to obtain 622 b.p. fragment of CAST gene and 462 b.p. fragment of GDF9 gene. Calpastatin locus was digested with MspI restriction enzyme. Two genotypes (MM and MN) of CAST gene were observed. The polymorphism of GDF9 gene (CC and CD genotypes) was detected after amplicons digestion with AspLEI restriction enzyme. MM and MN genotypes were identified with 0.855 and 0.145 frequencies, M and N allele frequencies were 0.928 and 0.072, respectively. In this population CC and CD genotypes of GDF9 gene were identified with 0.878 and 0.122 frequencies, C and D allele frequencies were 0.939 and 0.061, respectively.

Key words*: sheep, genetic polymorphism, CAST, GDF9, PCR-RFLP.*

INTRODUCTION

The Tuva Republic is a one of the most important region of the Russian Federation characterized by conventional sheep breeding. That region is situated in southern Siberia, in the geographical center of Asia.

It's the region with alternation of mountain ranges and intermountain basins, characterized by steppe landscape. Mountains constitute over 80% of the region territory.

The climate of region is s sharply continental.

Sheep breeding is the main branch of agriculture of the Tuva Republic and the important part of traditional way of life of tuvans. The main target in the Republic is supplying of sheep meat.

The major indigenous sheep breed in that region is the Tuvan short-fat-tailed breed (Tuvan sheep breed).

Sheep of Tuvan breed are adapted to specific regional climate environment. Animals have a high immunity and stamina (Yuldashbaev et al., 2016).

The level of production traits of sheep depend on environment in each year and season.

The breed contains two breed types: steppe and mountain sheep, that are differ by level of productive traits. For example, the minimal live weight of adult rams of steppe type is 78 kg, of ewes - 56 kg. The natural wool is strong, 12-14 cm length. Adult rams of mountain type characterized by 55 kg of live weight, ewes - 42 kg. Length of wool is 10-12 cm.

The birth rate of the Tuvan sheep is 100-110 lambs per 100 ewes. The clean equivalent weight of wool is 50-60% (Yuldashbaev et al., 2016).

Sheep breeding in the Tuva Republic is extensive brunch of animal husbandry, expansion of production is achieved by increasing the total number of sheep and unlimited using of pasture.

Intensification of this brunch can be achieved by using methods which are increasing the level of production without extension of sheep and using maximum of resources from each sheep. The one of solution for intensification of sheep breeding is using DNA markers of productive traits for organization marker-assisted selection. Marker-assisted selection (MAS) is applying of DNA markers to improve

the response to selection in a population of animals. The markers should be closely linked to one or more target loci, which may often be quantitative trait loci.

One of the potential marker gene for growth traits and to improving meat tenderness after slaughter is ovine calpastatin gene (Deykin et al., 2016). Calpastatin gene (CAST) is of 100 kb length, includes four exons and is located on the fifth sheep chromosome (Palmer et al., 1998).

Growth differentiation factor-9 gene (GDF9), also situated on fifth sheep chromosome, knows as a potential genetic marker of prolificacy**.** This gene contain two exons and one introns (Bahrami et al., 2014).

Detection of polymorphic variants of genes is one of key moments of beginning of selection program**.** The polymorphic variant of gene, can be associated with different levels of productive traits.

The aim of this investigation is detection of polymorphism of CAST and GDF9 genes to estimate possibility of using these genes in selection programs.

MATERIALS AND METHODS

PCR-RFLP method was the basis for detecting genes polymorphism.

Blood samples for DNA analyses were collected from 131 purebred rams of Tuvan short-fat-tailed breed, were raising for herd replacements. Animals were presented from municipal unitary enterprise "Chalaaty". K3-EDTA tubes were used for good safety of samples. Approximately 9.0 mL blood samples were collected in sterile tubes. All volumes with blood were frozen in -20ºC.

Genomic DNA was isolated from blood samples using the commercial kits as per the manufacturer's instructions.

The DNA amplification of CAST gene was achieved by using following pair of primers: CAST - F: 5'-TGGGGCCCAATGACGCCAT CGATG-3' and CAST R: 5'-GGTGGA GCAGCACTTCTGATCACC-3' (Palmer et al., 1998). The PCR was implemented by following parameters: a preliminary denaturizing at 95ºC for 3 min, followed by 1 cycle of denaturing at 95ºC for 15 sec, annealing at 60ºC for 40 sec, and extension at 72ºC for 30 sec, followed by 35 cycles. A final extension by 5 min at 72ºC. The PCR products of CAST gene were digested at 37°C for 12-16 hours with *MspI* restriction enzyme (Alakilli, 2015).

To obtain GDF9 gene amplicons was used following pair of primers: GDF9-F: 5'-GAAGACTGGTATGGGGAAATG-3';

GDF9-R: 5'-CCAATCTGCTCCTACACACC T-3'. The amplification reaction conditions were carried out using 35 cycles at 94°C for 2 min., followed by 94°C for 30 sec, 63°C for 40 sec, 72°C for 30 sec, and final extension at 72°C for 4 min (Kolosov et al., 2015). The obtained GDF9 CAST gene PCR products were digested at 37°C for 12-16 h with *AspLEI* restriction enzyme.

All amplicons and digested PCR products of CAST and GDF9 loci were separated in 2.0 - 3.0% agarose gel and visualized by ethidium bromide staining under gel documentation system.

The data generated by electrophoresis of *MspI* and *AspLEI* digested product of samples was used for estimating the frequency of different restriction fragment patterns.

The genotypes and allelic frequency were estimated by standard procedure (Falconer, 1989).

Genotypes frequency was calculated in following formula:

$$P_i = \frac{n_i}{N},$$

where: P_i - ith genotype frequency;

n_i - number of samples of ith genotype;

N - total number of samples of individuals of all genotypes.

Allelic frequency was calculated by in the following way:

$$p_i = \frac{2n_{(homozygote)} + n_{(heterozygote)}}{2N},$$

where: p_i - ith allele frequency;

n - number of homozygotes of particular gene and heterozygotes, respectively;

N - total number of individuals.

RESULTS AND DISCUSSIONS

Amplified products of 622 b.p. fragment of CAST gene was obtained after amplification in all the analyzed samples (Figure 1).

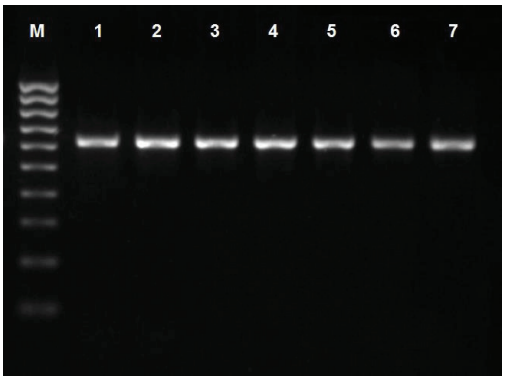

Figure 1. PCR products of CAST gene with size of 622 (lanes 1-7), lane M – 100 p.b. DNA ladder. Viewed on 2.0% agarose gel

N and M alleles of CAST gene were observed after the digestion of 622 b.p. PCR product with restriction enzyme. The *MspI* digestion of the amplicons produced fragments of 336 b.p. and 286 b.p. for allele M, and the allele N was not digested.

The homozygous genotypes MM were characterized by 336 b.p. and 286 b.p. bands. The heterozygous genotype MN was consist of 3 bands: 622 b.p., 336 b.p. and 286 b.p. NN genotype (622 b.p.) was absent in that population, but according the other literature sources It looks as a non-digested amplicon (Palmer et al., 1998; Tohidi et al., 2013).

PCR products of 462 b.p. fragment of GDF9 gene was obtained after amplification (Figure 2).

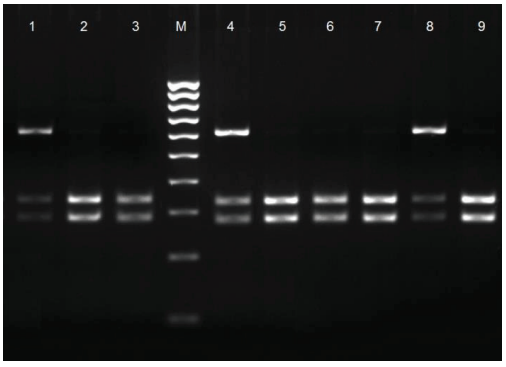

Figure 2. DNA electrophoretic pattern of CAST amplicons after digestion with *MspI* restriction enzyme: lane M - 100 p.b. DNA ladder, lanes 2, 3, 5, 6, 7, 9- MM genotype, lanes 1, 4, 8 - MN genotype. Viewed on 2.0% agarose gel

Alleles C and D of DGF9 gene were detect by digesting of PCR products by *AspLEI* restriction enzyme (Figure 3), then two genotypes presented by fragments of different size were established.

The *AspLEI* digestion of amplificated loci of gene produced fragments of 254, 156 and 52 b.p. for allele C, and the allele D was described like a 410 and 52 b.p. patterns.

The homozygous genotypes CC was characterized by 254, 156 and 52 b.p. bands. CD genotype was consist of 4 bands: 410, 254, 156 and 52 b.p. Fragments with size of 52 b.p. were bad visible (Figure 3).

DD genotype was not observed in the population. It contains 410 b.p. and 52 b.p fragments and has a low frequency, according the previous investigations (Bahrami et al., 2014; Kolosov et al., 2015).

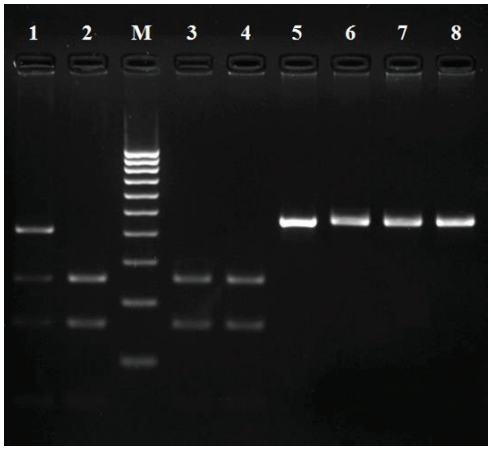

Figure. 3. PCR products of GDF9 gene and DNA electrophoretic pattern of amplicons after digestion with *AspLEI* restriction enzyme: lane M - 100 p.b. DNA ladder, lane 1 - CD genotype, lanes 2, 3, 4 - CC genotype, lane 5-8 - PCR products. Viewed on 3.0% agarose gel

The M allele of CAST gene and the homozygous MM genotype had the highest frequency in sheep of Tuvan breed (Table 1). Similarly the biggest value of frequency of C allele of GDF9 gene was in that population (Table 1). The largest part of population of sheep is homozygous of M and C alleles of CAST and GDF9 genes, respectively.

Table 1. The genotypes frequency for the CAST and GDF9 genes in Tuvan short-fat-tailed sheep breed in MUE "Chalaaty" (n=131)

Genes	Genotypes	The number of animals	Genotypes frequency
CAST	MM	112	0.850
	MN	19	0.145
	NN	0	0
GDF 9	CC	115	0.878
	CD	16	0.122
	DD	0	0

In the examined group of sheep the most frequent was the M allele (0.928) and C allele (0.939), whereas the frequency of the N allele and the D allele was 0.072 and 0.061, respectively (Table 2).

Table 2. The alleles frequency for the CAST and GDF9 genes in Tuvan short-fat-tailed sheep breed from MUE "Chalaaty" (n=131)

Genes	Alleles	Allele frequency
CAST	M	0.928
	N	0.072
GDF9	C	0.939
	D	0.061

Some researchers suppose this level of polymorphism is rather low and inconvenient for application in marker-assisted selection programs (Kolosov et al., 2015). But high total number of breed individuals can promote for search of sufficient number of animals with rare genotype for additional studying (Yuldashbaev et al., 2016).

CONCLUSIONS

The genotyping of the population of Tuvan sheep breed for the CAST and GDF9 genes is one of the steps in the implementation of the candidate gene approach in sheep breeding. The allelic variants of the CAST and GDF9 gene were discovered in the process of that research**.** It can be useful to analyze the other groups of Tuvan short-fat-tailed breed from other agriculture organisations of the Tuva Republic to get more information of genotypes frequency. In the future additionally needs to investigate meat quality, weight traits and prolificacy of Tuvan Sheep for each genotype groups for ascertainment the fact of associating genotypes with level of those traits.

ACKNOWLEDGEMENTS

Collecting of materials samples for this research was carried out with the support of faculty of agriculture of FSBEI HE "Tuvan State University" (The Tuva Republic, Kyzyl).

REFERENCES

Alakilli S.Y.M., 2015. Analysis of polymorphism of Caplstatin and Callipyge genes in saudi sheep breeds using PCR-RFLP technique. Int. J. Pharm. Sci. Rev. Res., 30(1), 340-344.

Bahrami Y., Bahrami S., Mohammadi H.R., Chekani-Azar V., Mousavizadeh S.A., 2014. The Polymorphism of GDF-9 Gene in Hisari Sheep. Biological Forum – An International Journal., 6(2), 46-52.

Deykin A.V., Selionova M.I., Krivoruchko A. Yu., Kovalenko D.V., Truhachev V.I., 2016. Genetic markers in sheep meat breeding. Vavilov Journal of Genetics and Breeding., 20(5), 576-583.

Falconer D.S., 1989. Introduction to Quantitative Genetics., Ed. 3. Longmans Green/John Wiley & Sons, Harlow, Essex, UK/New York, 438.

Kolosov Yu. A., Getmantseva L.V., Shirockova N.V.. Klimenko A., Bakoev S.Yu., Usatov A.V., Kolosov A.Yu., Bakoev N.F., Leonova M.A., 2015. Polymorphism of the GDF9 Gene in Russian Sheep Breeds. J Cytol Histol., 6, 305.

Palmer B.R., Roberts N., Hickford J.G., Bickerstaffe R., 1998. PCR-RFLP for MspI and NcoI in the ovine calpastatin gene. J. Anim. Sci., 76, 1499-1500.

Tohidi R., Elyasi G., Javanmard A., Shoja J., Rezaei R., Pirahary O., 2013. Molecular analysis of ovine Calpastatin gene in six iranian sheep breeds using PCR-RFLP. J. Anim. Prod. Adv., 3(9), 271-277.

Yuldashbaev Yu.A., Dongak M.I., Kulikova K.A., 2016. Perspective of studying gene polymorphism of useful traits in genome of Tuvan short-fat-tailed sheep. Izvestiya Saint-Petersburg State Agrarian University, 42, 141-148.

Scientific Papers. Series D. Animal Science. Vol. LXI, Number 1, 2018
ISSN 2285-5750; ISSN CD-ROM 2285-5769; ISSN Online 2393-2260; ISSN-L 2285-5750

THE EFFECT OF LAMENESS ON MILK PRODUCTION ON A HOLSTEIN-FRIESIAN FARM

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Abstract

The aim of this study was to determine the effect of lameness on daily milk yield, milk fat and protein. 3378 data of 978 animals were monitored for all conditions (locomotion, milk production and milk content) during two years, monthly. The prevalence of lameness was different in several parity and stage of lactation.
The effect of lameness on daily milk yield was complex and interacted with the stage of lactation. In the first stage of lactation the lame cows produced more milk than the not lame. The milk yield in the next period was almost equal, after the 120th day the milk production of not lame cows was higher than that of the lame cows. We conclude that lameness reduced milk production and milk protein content during the lactation. This same relation was not found in case of milk fat percentage.

Key words*: dairy cow, lameness, milk production, milk component.*

INTRODUCTION

The genetic progress of the dairy cattle population is significant, however selection for increased milk, fat and protein yield has led to unfavourable correlated changes in reproductive performance (Chagas et al., 2007; Mokhtari et al., 2015; Pryce et al., 2004), and also to some diseases like ketosis (Raboisson et al., 2014), milk fever, lameness (Dechow et al., 2004) mastitis, and others (Alawneh et al., 2014).
Lameness in dairy cows is a multifactorial and progressive disease with complex interactions between risk factors contributing to its occurrence (Randall et al., 2015). Extensive effects on herd performance are published, including milk yield loss (Alawneh, Stevenson, Williamson, Lopez-Villalobos and Otley, 2014) and impaired reproductive performance (Mokhtari, Moradi Shahrbabak, Nejati Javaremi and Rosa, 2015). The significance of these effects extends beyond the financial implications.
The rate of lameness depends on milk production, body condition and parity, and according to (Archer et al., 2010) it was more likely associated with high milk yield in multiparous cows.
In addition, lameness is a major problem for the dairy industry in terms of animal well-being (Alsaaod et al., 2012; Bicalho and Oikonomou, 2013; Solano et al., 2015). Lame animals show behavioural signs of being in pain (Vieira et al., 2015) such as reduction in mobility and alterations in behaviour (Miguel-Pacheco et al., 2014; Navarro et al., 2013). Due to discomfort and changes in behaviour lameness has been associated with a reduction in milk production. The signs of changed behaviour included impaired locomotory ability and reduced feed intake which can be associated with weight loss and milk yield reduction (Charlton et al., 2016). Beside milk yield losses, the correlation between lameness and BCS is significant.
Results of Lim et al. (2015) suggested that both a decrease and an increase in BCS influence the risk of becoming lame and regular monitoring and maintenance of BCS on farms could be a key tool for managing the risk of lameness.
In summary, lameness is one of the most significant endemic disease-problems facing the dairy industry (Thomas et al., 2015).
Lameness has an economic impact (Ettema and Østergaard, 2006) on the herd, involving

decreased milk production, loss of value of production, change in live weight, treatment cost, replacement costs, early culling, extra labour costs and prolonged calving interval (Enting et al., 1997).
The importance of prevention is unambiguous and the early recognition and treatment of lameness is fundamental to mitigate its negative effects (Solano et al., 2016).
The early treatment of lame dairy cows resulted in the development of less severe lesions, increasing the chance of full recovery and decreased the amount of time an animal was lame (Groenevelt et al., 2014).
According to Defrain et al. (2013) the collected foot health records are useful in monitoring the degree of lameness within dairy herds and, perhaps more importantly, providing insight into the underlying factors causing lameness. In addition, locomotion scoring has been globally adopted to determine the prevalence and severity of lameness.
The aim of this study was to investigate the impact of clinical lameness in Hungarian dairy cows on milk yield and milk composition in different parities and stage of lactation.

MATERIALS AND METHODS

The study was carried out on a dairy cattle farm in Hungary. The dataset included 3378 monthly test day milk yield, from 976 cows in first to five lactations during a two years period.
Once a month after the milk-test-day the cows were examined for lameness according to their locomotion (lame, non-lame).
Individual cow milk yields were estimated on a monthly test throughout the lactation.
The milk fat and protein-content was collected from evening and morning milking (alternate samples), the samples weighed, and a subset of the combined evening and morning milking taken for the determination of SCC, milk fat and protein concentration (Hungarian Dairy Herd Recording).
Statistical analyses were performed using SPSS 18. Data were analysed using two-ways ANOVA model. The prevalence of lameness in the different lactations and the different stages of lactation were compared using a Chi-squared.

RESULTS AND DISCUSSIONS

The prevalence of lameness (Figures 1 and 2) is varied widely between parity (13-41%) and the different stages of lactation (16-32%). During the examination of DIM (Figure 1) number of lame cows were the highest between 231-300 days of lactation. Lameness prevalence increased with increasing days of lactation and it differed statistically (Chi^2=39.93, df=5, P<1%). The lowest lameness prevalence occurred in the first stage of lactation (1-60 days). A 16%-point increase was present in lameness cases from 1-60 days to 300 days whereas prevalence was the highest (38%, n=176). This tendency was observed in other authors' studies. According to Solano et al. (2015) lameness increased with increasing DIM. Main et al. (2010) and Espejo and Endres (2007) found that longer time spent in milking was significantly associated with increased prevalence of lameness.

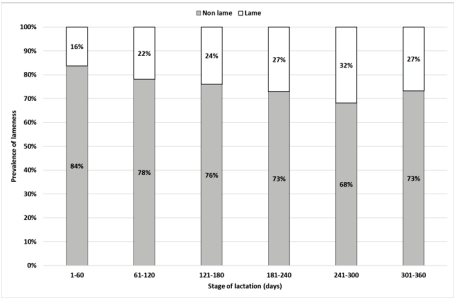

Figure 1. The prevalence of lameness in different stage of lactation

The prevalence of lameness was also different in several parities. Figure 2 shows that the lowest prevalence occurred in first lactation (13%), while it was highest in 5th lactation (41%). The difference between the first and 5th parity was 287% (Chi^2=144.55, df=4, P<1%). Summarizing the results, lameness was associated with increased DIM and parity.
The effect of lameness on daily milk yield was complex and interacted with the stage of lactation (Table 1). In the first stage of lactation the lame cows produced more milk than the not lame. The difference between groups was 1.25 kg (P>5%). The milk yield in the next period was almost equal, after the 120th day the milk production of not lame cows was higher than that of the lame cows.

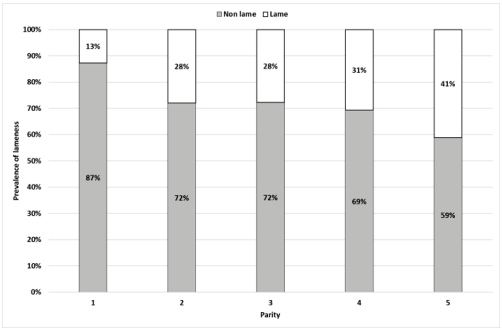

Figure 2. The prevalence of lameness in different parities

The milk production difference of lame and not lame cows changed from 1.15 kg to 1.8 kg (241-300 days).

During the lactation the decline in milk production is a natural process, however the decrease of the milk yield of lame cows was higher (16.28 kg) than in case of not lame ones (13.1 kg).

According to the two ways anova method the effect of the stage of lactation and lameness on daily milk yield is significant, similarly to the interaction of two effects (P=0.016).

Table 1. The effect of lameness on daily milk-, fat- and protein yields and SCC

Stage of lactation (days)	Lameness	n	Daily Milk Yield (kg)	P %	Fat (%)	P %	Protein(%)	P %
1-60	non lame	349	31.61±7.09	0.064	3.46±0.75	0.262	3.04±0.29	0.021
	lame	68	33.36±6.99		3.35±0.63		2.95±0.33	
61-120	non lame	459	30.37±6.71	0.966	3.64±0.73	0.834	3.27±0.31	0.000
	lame	129	30.34±7.48		3.65±0.68		3.11±0.33	
121-180	non lame	425	28.28±6.60	0.012	3.81±0.71	0.719	3.36±0.28	0.002
	lame	134	26.62±6.73		3.83±0.78		3.27±0.32	
181-240	non lame	440	25.11±6.64	0.053	3.86±0.82	0.998	3.45±0.32	0.001
	lame	164	23.96±6.08		3.86±0.62		3.35±0.30	
241-300	non lame	377	21.14±7.15	0.005	4.02±0.80	0.598	3.60±0.33	0.003
	lame	176	19.34±6.67		3.98±0.85		3.50±0.36	
301-360	non lame	255	18.51±6.60	0.136	4.11±0.90	0.958	3.74±0.38	0.035
	lame	93	17.33±6.40		4.12±0.78		3.64±0.36	
Effects and interactions			Stage of lactation P<1%, lameness P<1%, Lameness x Stage of lactation P<5%		Stage of lactation P<1%, lameness P>5%, Lameness x Stage of lactation P>5%		Stage of lactation P<1%, lameness P<1%, Lameness x Stage of lactation P>5%	

Several authors reported relationship between milk solids (fat and protein) and lameness. Penev and Stankov (2015) reported that the milk fat percentage of lame cows reduced by 0.16%, and milk protein - by 0.04% compared to healthy cows.

According to Olechnowicz and Jaskowski (2010) the cows, which were never lame in early lactation and the cows, which were mildly lame (score 2), produced more milk, fat, protein, and lactose per month as compared with cows, which were clinically lame for one month and compared with the cows, which were clinically lame longer than one month.

According to Enting et al. (1997), cows, which were culled for lameness, had lower milk fat, and protein production, by 14.1%, and 16.4%, respectively. In our study, the milk fat % was greater with higher days in milking, however the value did not differ significantly by lameness. Not lame cows had greater milk protein content compared with lame cows (P<5%).

CONCLUSIONS

In this study, we investigated the effect of lameness on milk production, milk fat and protein.

Lameness prevalence increased with increasing days of lactation and it differed statistically (Chi^2=39.93, df=5, P<1%). Prevalence of lameness also differed significantly in terms of the number lactation (Chi^2=144.55, df=4, P<1%). Lame cattle in all lactation periods (except for the first period) had lower milk production than that of the not lame cows. We conclude that lameness reduced milk production and milk protein content. during the lactation. This same relation was not found in case of milk fat percentage.

REFERENCES

Alawneh J.I., Stevenson M.A., Williamson N.B., Lopez-Villalobos N., Otley T., 2014: The effects of liveweight loss and milk production on the risk of lameness in a seasonally calving, pasture fed dairy herd in New Zealand. Preventive Veterinary Medicine. 1/1/, 113, 72-79T.

Alsaaod M., Römer C., Kleinmanns J., Hendriksen K., Rose-Meierhöfer S., Plümer L., Büscher W., 2012: Electronic detection of lameness in dairy cows through measuring pedometric activity and lying behavior. Applied Animal Behaviour Science. 12/31/;142:134-141.

Archer S.C., Green M.J., Huxley J.N., 2010: Association between milk yield and serial locomotion score assessments in UK dairy cows. Journal of Dairy Science. 9//;93:4045-4053.

Bicalho R.C., Oikonomou G., 2013: Control and prevention of lameness associated with claw lesions in dairy cows. Livestock Science. 9//;156:96-105.

Chagas L.M., Bass J.J., Blache D., Burke C.R., Kay J.K., Lindsay D.R., Lucy M.C., Martin G.B., Meier S., Rhodes F.M. et al., 2007: Invited Review: New Perspectives on the Roles of Nutrition and Metabolic Priorities in the Subfertility of High-Producing Dairy Cows. Journal of dairy science. 9;90:4022.

C harlton G.L., Bouffard V., Gibbons J., Vasseur E., Haley D.B., Pellerin D., Rushen J., de Passillé A.M., 2016: Can automated measures of lying time help assess lameness and leg lesions on tie-stall dairy farms? Applied Animal Behaviour Science. 2//;175:14-22.

Dechow C.D., Rogers G.W., Sander-Nielsen U., Klei L., Lawlor T.J., Clay J.S., Freeman A.E., Abdel-Azim G., Kuck A., Schnell S., 2004: Correlations Among Body Condition Scores from Various Sources, Dairy Form, and Cow Health from the United States and Denmark. Journal of Dairy Science. 10//;87:3526-3533.

DeFrain J.M., Socha M.T., Tomlinson D.J., 2013: Analysis of foot health records from 17 confinement dairies. Journal of Dairy Science. 11//;96:7329-7339.

Enting H., Kooij D., Dijkhuizen A.A., Huirne R.B.M., Noordhuizen-Stassen E.N., 1997: Economic losses due to clinical lameness in dairy cattle. Livestock Production Science. 9/15/;49:259-267.

Espejo L.A., Endres M.I., 2007: Herd-level risk factors for lameness in high-producing holstein cows housed in freestall barns. Journal of dairy science. 01/01;90:306.

Ettema J.F., Østergaard S., 2006: Economic decision making on prevention and control of clinical lameness in Danish dairy herds. Livestock Science. 6//;102:92-106.

Groenevelt M., Main D.C.J., Tisdall D., Knowles T.G., Bell N.J., 2014: Measuring the response to therapeutic foot trimming in dairy cows with fortnightly lameness scoring. The Veterinary Journal. 9//;201:283-288.

Lim P.Y., Huxley J.N., Willshire J.A., Green M.J., Othman A.R., Kaler J., 2015: Unravelling the temporal association between lameness and body condition score in dairy cattle using a multistate modelling approach. Preventive Veterinary Medicine. 3/1/;118:370-377.

Main D.C.J., Barker Z.E., Leach K.A., Bell N.J., Whay H.R., Browne W.J., 2010: Sampling strategies for monitoring lameness in dairy cattle. Journal of Dairy Science.93:1970-1978.

Miguel-Pacheco G.G., Kaler J., Remnant J., Cheyne L., Abbott C., French A.P., Pridmore T.P., Huxley J.N., 2014: Behavioural changes in dairy cows with lameness in an automatic milking system. Applied Animal Behaviour Science. 1//;150:1-8.

Mokhtari M.S., Moradi Shahrbabak M., Nejati Javaremi A., Rosa G.J.M., 2015: Genetic relationship between heifers and cows fertility and milk yield traits in first-parity Iranian Holstein dairy cows. Livestock Science. 12//;182:76-82.

Navarro G., Green L.E., Tadich N., 2013: Effect of lameness and lesion specific causes of lameness on time budgets of dairy cows at pasture and when housed. The Veterinary Journal. 9//;197:788-793.

Olechnowicz J., Jaskowski J., 2010: Impact of clinical lameness, calving season, parity, and month of lactation of milk, fat, protein, and lactose yields during early lactation of dairy cows. Bulletin of the Veterinary Institute in Puławy.54.

Pryce J.E., Royal M.D., Garnsworthy P.C., Mao I.L., 2004: Fertility in the high-producing dairy cow. Livestock Production Science. 3;86:125.

Raboisson D., Mounié M., Maigné E., 2014: Diseases, reproductive performance, and changes in milk production associated with subclinical ketosis in dairy cows: A meta-analysis and review. Journal of Dairy Science. 12//;97:7547-7563.

Randall L.V., Green M.J., Chagunda M.G.G., Mason C., Archer S.C., Green L.E., Huxley J.N., 2015: Low body condition predisposes cattle to lameness: An 8-year study of one dairy herd. Journal of Dairy Science. 6//;98:3766-3777.

Solano L., Barkema H.W., Pajor E.A., Mason S., LeBlanc S.J., Nash C.G.R., Haley D.B., Pellerin D., Rushen J., de Passillé A.M. et al., 2016: Associations between lying behavior and lameness in Canadian Holstein-Friesian cows housed in free stall barns. Journal of Dairy Science. 3//;99:2086-2101.

Solano L., Barkema H.W., Pajor E.A., Mason S., LeBlanc S.J., Zaffino Heyerhoff J.C., Nash C.G.R.,

Haley D.B., Vasseur E., Pellerin D. et al., 2015: Prevalence of lameness and associated risk factors in Canadian Holstein-Friesian cows housed in free stall barns. Journal of Dairy Science. 10//;98:6978-6991.

Thomas H.J., Miguel-Pacheco G.G., Bollard N.J., Archer S.C., Bell N.J., Mason C., Maxwell O.J.R., Remnant J.G., Sleeman P., Whay H.R. et al., 2015: Evaluation of treatments for claw horn lesions in dairy cows in a randomized controlled trial. Journal of Dairy Science. 7//;98:4477-4486.

Vieira A., Oliveira M.D., Nunes T., Stilwell G., 2015: Making the case for developing alternative lameness scoring systems for dairy goats. Applied Animal Behaviour Science. 10//;171:94-100.

Scientific Papers. Series D. Animal Science. Vol. LXI, Number 1, 2018
ISSN 2285-5750; ISSN CD-ROM 2285-5769; ISSN Online 2393-2260; ISSN-L 2285-5750

ESTIMATES OF THE TRENDS COMPONENTS IN THE MILK YIELD OF HOLSTEIN FRIESIAN COWS

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Abstract

This study was carried out to estimate the trend components (the phenotypic, genetic and environmental trends) for 305-day milk yield in Holstein Friesian Cattle raised between the years 1989-2012 at the Ceylanpınar State Dairy Farm. In order to estimate the trend components 6165 lactation records of 2055 Holstein cows was analysed. It was found that the lactation period average was 313.23 ± 28.47 days, the annual average lactation milk yield was 6197.88 ± 1681.35 kg and adjusted 305-day lactation milk yield was found to be 6164.41 ± 1713.90 kg. In order to estimate of genetic parameters, standardized milk yield according to 305-day lactation period (lactation 1, 2 and 3) were analysed primarily with repeated-measured animal models by using MTDFREML programs. According to the data obtained in the study, the lactation length, average lactation milk yield and average 305-days milk yield adjusted were calculated as 313.23 ± 28.47 days, 6197.88 ± 1681.35 kg and 6164.41 ± 1713.90 kg, respectively. The phenotypic, environmental and genetic trends for 305-days milk yield were found to be -70.72 kg/year, -70.53 kg/year and -0.19 kg/year, respectively.

Key words*: Ceylanpınar, Holstein, genetic trends, phenotypic trends.*

INTRODUCTION

In order to increase yield per animal can be grouped into two groups: one is to regulate environmental factors and the other is to improve the genotypic level. While the impact of the rehabilitation of the environment arises shortly, it takes longer to heal the genotype. However, the positive effect of the created environment and the increase in productivity are limited by the genotype of the animal. The genotypic correction to be carried out in parallel with the improvement of environmental conditions is carried out by separating the individuals identified as having high genotypic value as parents and contributing to their next generations (Özyurt and Akman, 2009).

Genetic trends are the best parameter for an efficient selection prediction (Falconer and Mackay, 1996). Selection of genotypes suitable for the environment is possible by evaluating genotype performances based on scientific researches (Gönül, 1974).

While the impact of the rehabilitation of the environment arises shortly, it takes longer to heal the genotype. However, the increase in the yield due to the positive environment effect is limited by the genotype of the animal (Akman, 1993).

Main problem in animal breeding research is determining genetic gain that resulted from performing animal breeding programs during several years. In a population, which selection has carried out and mating between animal designed based on genetic characteristics, deal of changes that obtained in several years from animal breeding programs must investigated, thus genetic trend of selected traits in population estimated. Estimation of genetic trend may be providing investigation of animal breeding methods (Wilson and Willham, 1986; Kovac and Groeneveld, 1990).

The change in production per unit of time due to change in mean breeding value is called the genetic trend (Harville and Hendeason, 1966).

For the estimation of efficient selection, the best parameter is genetic trend (Falconer and Mackay, 1996). Genetic changes in a population should be checked in the case of selection on more traits at the same time

because that is the most powerful analysis to evaluate the selection work in a population. Dairy cattle have a long generation interval end low reproductive rate. In addition, it is costly and time-consuming to carry out dairy cattle selection on a large experimental scale. Methods to determine variance component have been greatly improved over the last three decades (Mashhadi et al., 2008). The estimation of genetic trend is the best tool to follow genetic changes in a population (Potocnik et al., 2007).

Several researchers (Burnside and Legates, 1967; Lee et al., 1985; Meinert and Pearson, 1992; Powell et al., 1977; VanVleck et al., 1986) have studied genetic trends in dairy cattle. Most of these researchers estimated genetic trends over periods of less than 20 years. The precision of genetic trend estimates is enhanced greatly as the number of years studied increases (Burnside and Legates, 1967). In the State-owned Agricultural Enterprises, although records of milk yields have been recorded for many years, it can be seen that these records are not utilized sufficiently for cattle breeding (Tuna et al., 2007).

As in other countries, also in Turkey as a result of work done in terms of dairy cattle, with effect share of the yield increases achieved in milk production genotype and environmental factors, these factors need to be discussed will be focused on what level of culture has been a major issue. There are many studies on how cattle breeding studies, which have been carried out for many years in especially livestock advanced countries, have been influenced by genetic and environmental sources. Various researchers estimated the annual genetic change by evaluating the yield records obtained under different conditions with appropriate statistical methods for their own trial materials (Alpan and Arpacık, 1998).

MATERIALS AND METHODS

In this study, 6165 records of milk production of Holstein Friesian Cattle raised between the years 1989-2012 at the Ceylanpınar State Dairy Farm in Turkey were used. In this study, the records of 305-days the first three lactation and twice milking per day were used.

Prediction of Lactation Milk Yield

The lactation milk yields were calculated using the Test Interval Method that is the reference method by ICAR (ICAR, 2003).

$$LMY = I_0M_1 + I_1 \times M_1 + M_2/2 + I_2 \times M_2 + M_3/2 + \ldots + I_{n-1} \times M_{n-1} + M_n + I_nM_n$$

In which:

M_1, M_2, …, M_n is milk yielded in 24 hours of the recording day, kg;

I_1, I_2, …, I_{n-1} is the intervals between recording dates, days;

I_0 is the interval between the lactation period start date and the first recording date, days;

I_n is the interval between the last recording date and the 305th lactation day, days.

Estimation of Genetic Parameters

Repeated-measure animal models was used to estimate genetic parameters by using the MTDFREML program (Van Vleck and Boldman, 1995). In the analysis, the additive genetic effect, the maternal additive genetic effect, the mother effect and permanent environmental effect included to the model as genetic effects and calving year-season, calving year-lactation order, calving year-age and service period as the environmental factors. In addition, the first calving age was included as a co-variable to the model.

The analysis model used to predict the genetic parameter are as follows:

$$Y_{ijklm} = \mu + CYS_i + CYLO_j + CYA_k + IP_l + A_{ijklm} + MA_m + PE_m + bX_{ijklm} + e_{ijklm}$$

In which:

Y_{ijklm} is 305-days total lactation milk yield corrected (lactation 1, 2 and 3)

μ is 305-days average lactation milk yield corrected

$CYS_{i.}$ is calving year-seasonal effect;

$CYLO_{j.}$ is calving year-lactation order effect;

$CYA_{k.}$ is calving year-age effect;

IP_l is Involution period effect (1: heifer, 2: 0-60 days, 3: 61-90 days, 4: 91-120, 5: 121-150, 6: ≥151days);
A_{ijklmn} is additive genetic effect;
MA_m is maternal additive genetic effect;
PE_m is uncorrelated random effect of cow;
b is regression coefficients;
X_{ijklm} is co-variable (first calving age: 20, 21, 22, 23, … ,50 and more);
e_{ijklm} is random environmental effect.

The Phenotypic trends as regressions of the corrected milk yield averages on years for 305-days milk yield. The environmental effect on the phenotypic trend was estimated by using corrected milk yield and lactation length records of cows for 2 consecutive years. The difference between the first and second year milk records of a cow was assumed to be a result of environmental fluctuations. The genetic trend was calculated as the regression of cow's breeding values to cow birth years.

RESULTS AND DISCUSSIONS

Lactation Milk Yield

Test Interval Method was used to estimate Lactation Milk Yield from control day yields. According to the results of the research, average annual lactation milk yield, 305-days corrected lactation milk yield and average lactation length was calculated as 6197.88 ± 1681 kg, 6164.4 ± 1713 kg and 313.23 ± 28.4 days, respectively (Table 1).

Table1. Average annual milk yield, corrected 305 days milk yield and lactation length

	N	Mean	SE	Min	Max.	Cv
Lactation milk yield	6165	6197.88	1681.35	715.2	13684.8	27.13
Lactation length	6165	313.23	28.47	198.6	355.2	9.09
Corrected 305-day lactation yields	6165	6164.41	1713.90	1014.8	13561.1	27.80

Lactation milk yield has been observed to be much higher than average lactation milk yield due to the purchase of high-yielding dairy cows from other enterprises between 2009 and 2012. The changes in lactation milk yield during the years is shown in Figure 1.

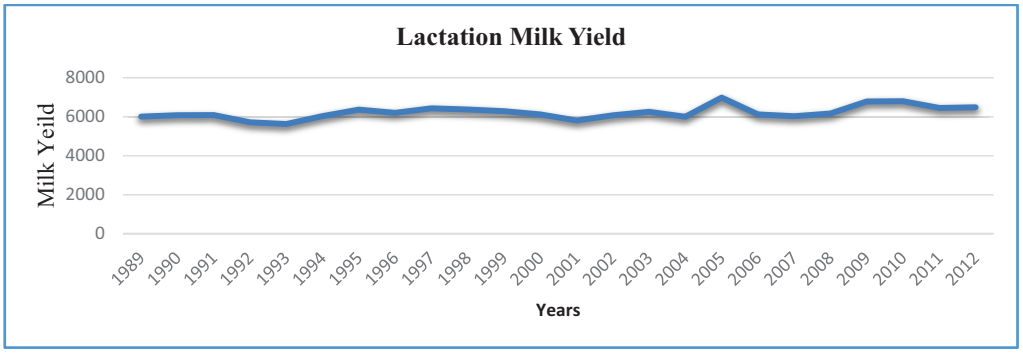

Figure 1. 305- day milk yields during the years

Between 1989 and 2012, the average lactation milk yield was calculated as 6197.88 ± 1681.35 kg. The highest milk yield was calculated as 6979.90 ± 1480.59 kg in 2005 and the lowest milk yield was 5625.08 ± 1600.63 kg in 1993. Milk yield has shown fluctuation over the years (Table 2).

Table 2. Average Lactation milk yields by the years 1989-2012

Years	N	Mean	SE	Min.	Max.	CV
1989	7	6004.69 ^{cdef}	1860.44	4565.8	9957.4	30.98
1990	155	6074.72 ^{cdef}	1700.96	2945.8	11758.4	28.00
1991	267	6087.83 ^{cdef}	1758.45	2260.8	12821.2	28.88
1992	358	5714.83^{ef}	1738.31	1684.0	11865.0	30.42
1993	316	5625.08^{f}	1600.63	715.2	11394.4	28.46
1994	258	6033.61 ^{cdef}	1534.17	1768.8	9881.6	25.43
1995	252	6365.91^{bcd}	1481.84	2611.0	11145.0	23.28
1996	245	6198.22 ^{cdef}	1581.60	1900.8	11605.0	25.52
1997	268	6432.30^{bc}	1652.29	2517.4	11864.4	25.69
1998	273	6367.88^{bcd}	1705.40	825.6	11992.0	26.78
1999	248	6284.30^{bcde}	1599.05	2243.6	11725.8	25.45
2000	297	6117.98 ^{cdef}	1698.04	1336.8	10835.4	27.75
2001	348	5806.65^{def}	1635.30	855.4	9892.0	28.16
2002	311	6073.81 ^{cdef}	1674.15	829.4	10889.6	27.56
2003	317	6253.17^{bcde}	1365.19	2198.0	12399.4	21.83
2004	274	6000.11 ^{cdef}	1330.21	1943.4	11197.2	22.17
2005	332	6979.90^{a}	1480.59	2669.2	12258.0	21.21
2006	313	6119.31 ^{cdef}	1463.56	2061.6	9917.6	23.92
2007	354	6025.48 ^{cdef}	1668.63	1241.2	11044.0	27.69
2008	307	6166.96 ^{cdef}	1769.92	893.6	13402.4	28.70
2009	314	6783.72^{ab}	2132.68	1727.0	13520.8	31.44
2010	205	6794.63^{ab}	1935.36	2575.4	13684.8	28.48
2011	120	6447.06^{abc}	1646.95	2371.8	10290.6	25.55
2012	26	6484.67^{abc}	1486.10	3672.0	8830.0	22.92

P<0.05

305-day milk yield (6164 kg) calculated in this study shown similarity to the results obtained by the other researchers (Özçakır and Bakır, 2003; Bakır and Çetin, 2003; Şahin, 2012; Arslan and Cak, 2013).

305-day milk yield in this study was lower than the reports of (Soysal and Özder, 1989; Yener et al., 1994; Yaylak, 2003; Uğur, 2000; Özkök and Uğur, 2007; Erdem et al., 2007; Yılmaz and Bayrıl, 2010; Şahin and Ulutaş, 2010).

It has been calculated higher than the reports of Şekerden et al. (1987), Kumlu et al. (1989), Gürdoğan and Alpan (1990), Soysal and Özder (1990), Ulutaş et al. (2002), Bilgiç and Alıç (2005), Bilgiç and Yener (1999), Duru and Tuncel (2002), Koç (2006), Tapkı et al. (2007), Akkaş and Şahin (2008), Çilek (2009).

Lactation length

The average lactation length calculated from records obtained between 1989 and 2012 was found as 313.23 ± 28.47 days (Table 3).

Lactation length calculated in this study shown similarity to the results obtained by the researchers (Southern, 1971; Bakır and Çetin, 2003; Koç, 2006; Özçakır and Bakır, 2003).

Table 3. Lactation length by the years 1989-2012

Years	N	Mean	SE	Min.	Max.	CV
1989	7	304.94^{c}	36.73	239.0	342.2	12.05
1990	155	309.96^{abc}	28.57	226.6	351.2	9.22
1991	267	311.02^{abc}	28.07	206.6	351.2	9.03
1992	358	311.49^{abc}	29.16	200.6	352.2	9.36
1993	316	306.86^{bc}	30.18	201.6	353.2	9.84
1994	258	308.61^{abc}	30.74	203.6	352.2	9.96
1995	252	313.47^{abc}	28.38	209.6	354.2	9.05
1996	245	314.12^{abc}	28.01	207.6	353.2	8.92
1997	268	312.63^{abc}	27.60	207.6	353.2	8.83
1998	273	314.64^{abc}	29.27	200.6	352.2	9.30

1999	248	316.03^{ab}	27.66	201.6	353.2	8.75
2000	297	310.35^{abc}	30.86	204.6	351.2	9.94
2001	348	315.06^{abc}	25.99	210.6	355.2	8.25
2002	311	316.47^{ab}	27.69	204.6	353.2	8.75
2003	317	318.11^{a}	24.09	211.6	352.2	7.57
2004	274	315.86^{ab}	26.88	212.6	352.2	8.51
2005	332	316.54^{ab}	26.38	206.6	351.2	8.33
2006	313	317.67^{a}	23.40	244.0	352.2	7.37
2007	354	312.67^{abc}	28.31	201.6	352.2	9.05
2008	307	313.33^{abc}	27.02	200.6	352.2	8.62
2009	314	311.68^{abc}	31.01	198.6	352.2	9.95
2010	205	314.27^{abc}	29.41	201.6	350.2	9.36
2011	120	308.43^{abc}	36.62	198.6	350.2	11.87
2012	26	284.01^{d}	42.34	198.6	348.2	14.91
	6155	**313.23**	**28.47**			

P<0.05

Lactation length in this study (313.23 days) was lower than the reports of Soysal and Özder (1989), Gündoğdu and Özder (1993), Yener et al. (1994), Özcan and Altınel (1995), Atay et al. (1995), Kumlu and Akman (1999), Yaylak (2003), Yener et al. (1994), Şahin (2009).

Lactation length in this study (313.23 days) was higher than the reports of Özcan and Pekel (1976), Şekerden and Pekel (1982), Özkütük and Pekel (1986), Kumlu et al. (1989), Kumlu et al. (1991), İpek (1993), Erdem (1997), Kaygısız (1997), Bilgiç and Yener (1999), Duru and Tuncel (2002), Özçelik and Arpacık (2000), Pelister et al. (2000a), Pelister et al. (2000b), Bilgiç and Alıç (2005), Sehar and Özbeyaz (2005), Çilek (2009).

Lactation length has shown fluctation between the years 1998-2015 (Figure 2).

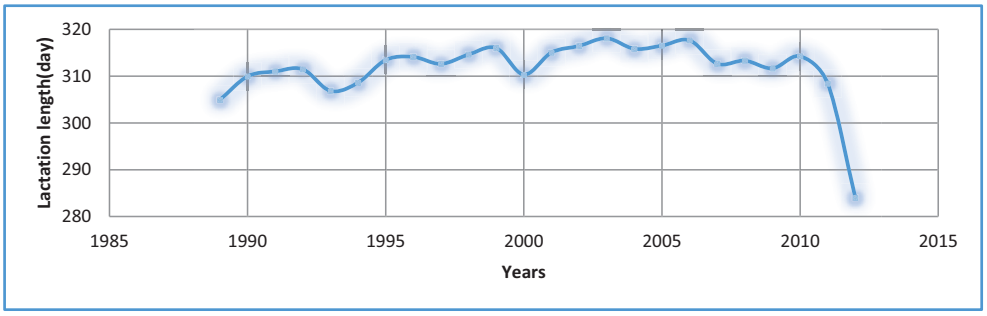

Figure 2. Changes of lactation length according to years

The Trend Components

The Trend Components was shown in Table 4. The genetic trends of cow breeding values were calculated by taking regression into cow birth years were found as -0.19 kg/year for 305-day milk yield.

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Table 4. Estimates of Trend Components

Years	N	Phenotypic trends	Environmental trends	Genetic trends
1989	7	7175.9	7443.74	-267.79
1990	155	7652.9	7796.67	-143.74
1991	267	7368.2	7467.25	-99.01
1992	358	6878.2	6923.90	-45.65
1993	316	6546.4	6523.05	23.40
1994	258	6504.5	6438.48	65.99
1995	252	6132.7	6085.45	47.22

1996	245	5937.1	5918.63	18.46
1997	268	5980.3	5987.91	-7.58
1998	273	6075.6	6137.80	-62.16
1999	248	6285.1	6356.53	-71.44
2000	297	6560.9	6602.96	-42.05
2001	348	6238.8	6275.63	-36.87
2002	311	6590.2	6616.19	-25.95
2003	317	6526.9	6522.04	4.84
2004	274	6371.3	6243.95	127.31
2005	332	6516.6	6370.23	146.33
2006	313	5470.2	5295.73	174.45
2007	354	5025.8	4872.02	153.77
2008	307	4715.9	4665.50	50.41
2009	314	5487.2	5608.03	-120.80
2010	205	5348.1	5589.18	-241.03
2011	120	5831.1	6108.88	-277.83
2012	26	6340.7	6572.38	-231.64
		Regressions of the corrected milk yield for 305 days		
		Y=7115.73-70.72X	Y=7149.23-70.53X	Y=-33.48-0.19X

X=Year

The Phenotypic trends as regressions of the corrected milk yield averages on years for 305-days milk yield were found -70.72 kg/year ($P<0.01$). The environmental effect on the phenotypic trend was estimated by using corrected milk yield records of cows for 2 consecutive years. The difference between the first and second year milk records of a cow was assumed to be a result of environmental fluctuations.

The environmental change for 305-days milk yield per year was estimated as -70.53 kg/year (Figure 3).

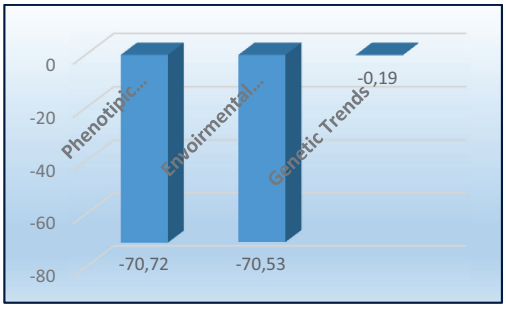

Figure 3. Distribution of Trends Components

The annual genetic trends value (-019 kg/year) obtained in this study was higher than the reported by Yener et al. (1978) -2.3 kg/year, Tonhati and Lobo (1997) -10.20 kg/year, Kaygısız (2000) -78 kg/year.

This value was found lower than Mc Daniel et al. (1961) reported 71.7 kg / year, Verde et al. (1974) 33 kg/year, Siam and Düzgüneş (1984) 78 kg/year, Lee and Freeman (1985) 55 kg/year, Akar and Pekel (1988) 53.6 kg/year, Tusuruta et al. (1990) 73.2 kg/year, Gürdoğan and Alpan (1990) 149 kg/year , Avandano et al. (1992) 74 kg/year, Zuk et al. (1994) 10.5 kg/year, Kaygısız (1996) 83,7 kg/year, Hansen (2000) 116 kg/year, Posadas et al. (2001) 29 kg/year, Duraes et al. (2001) 18.4 kg/year, Akman and Kumlu (2004) 84 kg/year, Abou-Bakr (2009) 2.19 kg/year, Gaidarska (2009) 26.48 kg/year, Golverdi et al. (2011) 6.79 kg/year, Yaeghoobi et al. (2011) 19,61 kg/kg and Katok and Yanar (2012) 3.73 kg/year.

When studies conducted by different researchers in different countries were examined, it was found that the trends of the annual genetic trends were positive except for some of the values calculated by some researchers (Yener et al., 1978; Tonhati and Lobo, 1997; Kaygısız, 2000) It was also observed that the value of genetic trends gradually decreased in recent years.

CONCLUSIONS

This result indicates that there are deficiencies in the environmental conditions such as management, nutrition and herd management applied in the cattle enterprises. Breeders have to make continuous selection regardless of their genetic and environmental trends. In the enterprises, these assessments give the opportunity to measure the success of applications up to now. A negative phenotypic trend in terms of milk yield in the farm may be due to insufficient environmental factors. Despite the right choice in the selection of the enterprise, environmental factors have led to a decrease in productivity. Annual fluctuations for these traits, maybe due to sudden changes in climate condition, management changes, nutrition and hygienic levels or interaction between genetic and environment. In this context, it is proposed to improve the environmental conditions of maintenance feeding and barn.

ACKNOWLEDGEMENTS

This study was derived from my Phd Thesis aceppted in 2015 by Ankara University Graduate School of Natural And Applied Sciences. Thanks to Ceylanpınar State Farm Autority and Supervisor Prof.Dr. Sadık Metin Yener.

REFERENCES

Abou-Bakr S., 2009. Genetic And Phenotypic Trends Of 305-Day Milk Yield of Holstein Cows Raised At Commercial Farm In Egypt. Egyptian Journal of Animal Production, 46(2), 85-92.

Akar M., Pekel E., 1988. Türkiye Şeker Fabrikaları A.Ş. Eskişehir Tohum Üretme Çiftliğinde Yetiştirilen Saf ve Melez Esmer Sığırların Süt Verimindeki Genetik ve Fenotipik Yönelimler. Ziraat Fakültesi Dergisi, 3, 51-65, Adana.

Akkaş Ö., Şahin E.H., 2008. Holştayn Irkı Sığırlarda Bazı Verim Özellikleri, Kocatepe Vet. Dergisi, 1, 25-31.

Akman N., Kumlu S., 2004. Türkiye Siyah Alaca Populasyonunda 305-Gün Süt Verimine Ait Genetik ve Fenotipik Parametreler Tarım Bilimleri Dergisi,10 (3), 281-286.

Alpan O., Arpacık R., 1998. Sığır yetiştiriciliği. Ankara. Medisan.

Arslan S., Cak B., 2013, Yozgat İli Boğazlıyan İlçesinde Özel Bir Süt İşletmesinde Yetiştirilen Holştayn İneklerin Süt Verim Özellikleri. YYU Veteriner Fakültesi Dergisi, 24 (3), 101 – 104.

Avandaño L.R., Mondragón I.V., Ochoa G.P., 1992. Genetic And Environmental Trends in Milk Production of First Lactation Holstein Cows In Mexico. Journal of Dairy Science, 75 (1), 150.

Bakır G., Çetin M., 2003. Reyhanlı Tarım İşletmesinde yetiştirilen Siyah Alaca Sığırlarda Döl ve Süt Verim Özellikleri. Turkish Journal of Veterinary and Animal Science, 27, 173-180.

Bilgiç N., Yener M., 1999. Ankara Üniversitesi Ziraat Fakültesi Zootekni Bölümü Sığırcılık İşletmesinde yetiştirilen Siyah Alaca İneklerde bazı süt ve döl verimi özellikleri. Ankara Ün. Ziraat Fakültesi Tarım Bilimleri Dergisi, 5, 81-84.

Bilgiç N., Alıç D., 2005. Polatlı Tarım Đşletmesinde yetiştirilen Siyah Alaca ineklerin bazı süt verim özellikleri. S.Ü. Ziraat Fak. Derg., 19, 36, 116-119.

Burnside E.B., J.E. Legates, 1967. Estimation of genetic trends in dairy populations. J. Dairy Sci., 50, 1448-1457.

Cilek S., 2009. Milk Yield Traits of Holstein Cows Raised at Polatli State Farm in Turkey. Journal of Animal and Veterinary Advances, 8 (1), 6-10.

Duraes M.C., Fereitas A.F., Valenta J., Teixeira N.M., Barra R.B, 2001. Genetic Trend for Milk and Fat Productivity for Holstein Cattle in Minas Gerais State. Revista Brasileira de Zootecnia, 30(1), 66-70.

Duru S., Tuncel E., 2002. Koçaş Tarım İşletmesinde Yetiştirilen Siyah Alaca Sığırların Süt Ve Döl Verimleri Üzerine Bir Araştırma. 1. Süt Verim Özellikleri. Turkish Journal Of Veterinary And Animal Science, 26, 97-101.

Erdem H., Atasever S., Kul E., 2007. Gökhöyük Tarım İşletmesinde Yetiştirilen Siyah Alaca Sığırların Süt Ve Döl Verim Özellikleri, 1. Süt Verim Özellikleri. OMÜ Ziraat Fakültesi Dergisi, 22(1), 41-46.

Falconer D.S., Mackay T.F.C., 1996. Introduction to Quantitative Genetics. 4th Edn., Longman, England, 464.

Gaidarska V., 2009. Evaluation Of Genetic Trend of The Bulgarian Dairy Population. Biotechnology in Animal Husbandry, 25 (5-6), 639-644.

Golverdi A.Y., Hafezian H, Chashnidel Y., Farhadi A., 2011. Genetic Parameters And Trends Of Production Traits İn Iranian Holstein Population. African Journal Of Biotechnology, 11(10), 2429-2435,

Gürdoğan T., Alpan O., 1990. Ankara Şeker Fabrikası Çiftliğinde Yetiştirilen Holştayn Sürüsünde Süt Verimine İlişkin Genetik Parametreler ve Genetik İlerleme Hızı. A.Ü. Vet. Fak. Derg., 37 (1), 101-115.

Hansen L.B., 2000. Consequences of Selection for Milk Yield from a Genetisist's Viewpoint. Journal of Animal Science, 83, 1145-1150.

Harville D.A., Hendeason C.R., 1966. Environmental and Genetic Trends in Production and Their Effects on Sire Evaluation. Department of Animal Husbandry, Cornell University, Ithaca, New York.

ICAR, 2003. International Agreement of Recording Practices. International Committee for Animal Recording ed., Interlaken, Switzerland.

Katok N., Yanar M., 2012. Milk traits and estimation of genetic, phenotypic and environmental trends for milk and milk fat yields in Holstein Friesian cows.

International Journal of Agriculture and Biology, 14(2), 311-314.
Kaygısız A., 1996. Kahramanmaraş Tarım İşletmesi Siyah Alaca Sürüsümde Süt Verimine İlişkin Yönelim Unsurlarının Tahmini, Tarım Bilimleri Dergisi Ankara, 2(3), 71-73.
Koç A. 206. Aydın İlinde Yetiştirilen Siyah-Alaca ve Esmer Irkı Sığırların Laktasyon Süt Verimleri ve Somatik Hücre Sayıları, Hayvansal Üretim Dergisi 47(2), 1-8.
Kovac M., Groeneveld E., 1990. Genetic and enviromental trends in German swine herdbook populations. J. Anim. Sci., 68, 3523-3535.
Kumlu S., 1989. Türkiye Damızlık Siyah Alaca Sürülerinin Süt Verimlerinde Genetik Ve Fenotipik Yönelimler. Akdeniz Üniversitesi Ziraat Fakültesi Dergisi, 12, 11-24.
Lee K.L., Freeman A.E., Johnson L.P., 1985. Estimation of genetic change in the registered Holstein cattle population. J. Dairy Sci., 68, 2629-2638.
Mashhadi M.H., Kashan N.E.J., Nassiry M.R., Torshizi R.V., 2008. Prediction breeding value and genetic parameter in Iranian holstein bulls for milk production traits. Pak. J. Biol. Sci., 11, 108-112.
Mc. Daniel B.T., Plowman, R.D., Davis R.F., 1961. Causes and Estimation of Environmental Change in a Dairy Herd. J. Dairy Sel., 44, 699-707.
Meinert T.R., Pearson R.E., 1992. Estimates of genetic trend in an artificial insemination progeny test program and their association with herd characteristics. J. Dairy Sci., 75: 2254-2264.
Özçakır A., Bakır G., 2003. Tahirova Tarım İşletmesinde Yetiştirilen Siyah Alaca Sığırların Döl Ve Süt Verim Özellikleri 1. Süt Verim Özellikleri. Atatürk Üniversitesi Ziraat Fakültesi Dergisi, Cilt 34, Yayın 2.
Özkök H., Uğur F., 2007. Türkiye' de Yetiştirilen Esmer ve Siyah Alaca Sığırlarda Süt Verimi, İlk Buzağılama Yaşı ve Servis Periyodu. Atatürk Üniversitesi Ziraat Fakültesi Dergisi, 38 (2), 143-149.
Pelister B., Altınel A., Güneş H., 2000a. Özel işletme koşullarında yetiştirilen değişik orijinli Siyah Alaca sığırların süt verimi özellikleri üzerinde araştırmalar. İstanbul Üniversitesi Veteriner Fakültesi Dergisi, 26(1), 201–214.
Pelister B., Altınel A., Güneş H., 2000b. Özel işletme koşullarında yetiştirilen değişik orijinli Siyah Alaca sığırların döl ve süt verimi özellikleri üzerinde bazı çevresel faktörlerin etkileri. İstanbul Üniviversitesi Veteriner Fakültesi Dergisi, 26(2), 543–559.
Posadas M.V, Lopez F.J., Waldenegro H.M., Keown J.F., Van Vleck L.D., 2001. Genetic Evalution for Milk Production in Holstein Cattle in Mexico. Tektran, United State Department of Agriculture.
Potocnik K., Stepec M., Krsnik J., 2007. Genetic trends for production and non-production traits in Simmental breed in Slovenia. Biotechnol. Anim. Husbandry, 23, 47-53.
Powell R.L., Norman H.D., Dickinson F.N., 1977. Trends in breeding value and production. J. Dairy Sci., 60, 1316-1326.
Siyam A., Düzgüneş E., 1984. Türkgeldi ve Sarmısaklı Devlet Kuruluşlarında Yetiştirilen Siyah Alaca Sığır Sürülerinde Süt Verimim Bakımından Genotipik ve Fenotipik Yönelimler, Ankara Üniversitesi Fen Bilimleri Enstitüsü, No: ZT-3, Ankara.
Şahin A., Ulutaş Z., 2010. Polatlı Tarım İşletmesinde yetiştirilen Siyah Alaca ineklere ait süt ve döl verim Özellikleri. Anadolu Tarım Bilimleri Dergisi, Cilt 25, Yayın 3
Şekerden Ö., 1987. Laktasyon Veriminin Hesaplanmasında Kullanılan Çeşitli Hesaplama Metodlarının Ve Değişik Süt Verim Kontrol Periyodlarının Karşılaştırılması. 19 Mayıs Üniversitesi Ziraat Fakültesi Dergisi, 2, 133-148.
Tapkı İ., Şahin M., Okyay M.S., 2007. Ceylanpınar Tarım İşletmesinde Yetiştirilen Siyah Alaca Sığırların Süt ve Döl Verim Özellikleri 1. Süt Verim Özellikleri. MKU Ziraat Fakültesi Dergisi, 12 (1-2), 1-8.
Tonhati H., Lôbo R.B., 1997. Estimation of The Genetic Trend For Milk And Milk Fat Yields in Holstein. Revista da Sociedade Brasileira de Zootecnia, 26(4), 714-718.
Tuna Y.T., Gürcan E.K., Savaş T., 2007. Sarımsaklı Tarım İşletmesi'nde yetiştirilen Siyah Alaca ırkı süt sığırlarının döl verim özellikleri. Tekirdağ Ziraat Fakültesi Dergisi, 4(3).
Uğur F., 2000. Kumkale Tarım İşletmesinde Yetiştirilen Siyah Alaca sığırların bazı süt verim özellikleri. Selçuk Üniversitesi Ziraat Fakültesi Dergisi, 14, 50-59.
Ulutas Z., 2002. Estimation of genetic and phenotypic trends of 305-day milk yield for Holsteins reared at Gelemen state farm in Turkey. Indian Journal of Animal Sciences, 72(10), 875-877.
Van Vleck L., Westell D.R.A., Schneider J.C., 1986. Genetic change in milk yield estimated from simultaneous genetic evaluation of bulls and cows. J. Dairy Sci., 69, 2963-2965.
Verde O.G., Wilcox C.J., Martin F.G., Reaves C.W., 1972. Genetic Trends in Milk Production in Florida Dairy Herd Improvement Association Herds. Journal of Dairy Science, 55(7), 1010-1012.
Wilson D.E., Willham R.L., 1986. Within-herd phenotypic, genetic and environmental trend lines for beef cattle breeders. J. Anim. Sci., 63, 1087-1094.
Yaeghoobi R., Doosti A., Noorian A.M., Bahrami A., 2011. Genetic Parameters and Trends of Milk and Fat Yield in Holstein's Dairy Cattle of West Provinces of Iran. International Journal of Dairy Science, 6(2), 142.
Yılmaz O., Bayrıl T., 2010. Kazova Vasfi Diren Tarım İşletmesinde yetiştirilen Siyah Alaca ırkı ineklerin süt verimi özellikleri. Yüzüncü Yıl Üniversitesi Veteriner Fakültesi Dergisi. 21 (2). 115-119.
Yener S.M., Tuncel E., Eker M. 1978. Atatürk Orman Çiftliğinde Yetiştirilen Siyah Alaca Sığırların Süt Veriminde Genetik Yönelim. Ankara Üniversitesi Ziraat Fakültesi Yıllığı, Cilt:28, s:820. Ankara.
Żuk B., Łoś P., Łukaszewicz M., Sobek Z., Zwolin´ska, Bartczak I., 1994. Genetic trend of Dairy Traits in the Polish Cattle. Animal Science Papers and Reports - Polish Academy of Sciences, Institute of Genetics and Animal Breeding Jastrzębiec, 12(2), 87-98.

Scientific Papers. Series D. Animal Science. Vol. LXI, Number 1, 2018
ISSN 2285-5750; ISSN CD-ROM 2285-5769; ISSN Online 2393-2260; ISSN-L 2285-5750

CARCASS PERFORMANCE OF HEIFERS AND BULLS OF DIFFERENT BREEDS

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Abstract

This study aimed to evaluate and compare performance of body weight (BW) at slaughter, hot carcass weight (HCW) and dressing-out percentages (DP%) of bulls and heifers of different breeds. The data from heifers (84) and bulls (90) for sex; and for breeds of Simmental (58), Holstein (62) and their crosses (S×H) (54) were used as174 animals in total. BWs of each breed were 486.5, 485.6 and 462.2 for Holstein, Simmental and crosses, respectively. BWs of sex were 482 kg for both bulls and heifers. There were significant differences (P<0.05) in HCW and DP% between sexes. HCW of bulls and heifers were 257.68 and 245.58 kg, respectively. DP% for bulls and heifers were 53.68 % and 51.15 % respectively. There were significant differences (P<0.05) in HCW and DP% between breeds. HCW and DP% of Simmentals were greater than the other breeds while they were not significant (P>0.05) for Holstein and Crosses. There was no significant (P>0.05) breed and sex interaction for HCW and DP%. Simmental bulls and heifers were heavier than those of other breeds for HCW and DP%. It was observed that the performance of bulls and heifers of Crosses were better than Holstein bulls and heifers performance.

Key words: *carcass, performance, heifers, bulls, breeds.*

INTRODUCTION

Despite the continuing trend of growth in beef consumption in Turkey over the years, beef producers are increasingly facing problems of decrease in profitability of production, which in turn requires them to research more deeply into factors associated with the success of their business. Breeds and sex type of animals continue to be one of the key components of such production facilities. Numerous studies confirm that breeds and genotypes of cattle have unequal adaptability and productivity in different natural environmental conditions (Aslam et al., 2002; Charles et al., 2012; Demircan et al., 2007).

Beef production is an important sector of agriculture in many countries. The type of beef industry that develops in any country depends largely on climate and terrain types. It is also related to the size of agricultural holdings and the general structure of the cattle industry, in particular the association between beef and milk production (Allen and Kilkenny, 1984)

Beef production methods have changed significantly since the Second World War towards more planned beef production systems. The main reason for the change is that it makes it difficult for the old systems to be economically viable in their land and labor needs.

This allowed to concentrate on an increase in the production scale and, to keep the optimum number of animals in a smaller area and thus to use more land for other agricultural enterprises (King, 1978).

Various published reports on beef performance of different breeds have been made available and compared feedlot and carcass characteristics of different breeds of bulls and heifers finished under different feeding conditions and slaughtered at different bodyweights (Steen, 1995; Steinwidder et al., 2002).

It appears that the bulls and heifers of even the same breed slaughtered at the same age might have different carcass performances. The results of such comparisons including different breeds are, however, limited in the literature.

Therefore, in this study it was aimed to evaluate and compare performance differences such as BW at slaughter, hot carcass and dressing-out percentages of bulls and heifers of different breeds.

MATERIALS AND METHODS

Experimental Location

This study was carried out in a commercial slaughterhouse in 2017 in Isparta province (37°45′N, 30°33′E, elevation 1035 m) located in the west Mediterranean region of Turkey.

Animals

Slaughter groups were formed according to breeds and sexes at almost the same weight from the animals slaughtered at the same abattoir. Total number of animals involved in the experiments was 174 head of bulls and heifers cattle which included 90 heads bulls and 84 heads heifers. The data for breeds used were Simmental (58), Holstein (62) and their crosses (Simmental×Holstein, S×H) (54) in the experiment. The age of the animals used in this study ranged from 17 to 19 months old at the time of slaughter.

Statistical Analysis

The analysis of variance of the data for breed and sex types were analyzed by GLM (General Linear Model) procedure by statistical software program (Minitab v.16), using the following model:

$$Y_{ijk} = \mu + \alpha_I + \beta_j + \gamma_k + \phi_l + \alpha\beta_{ij} + \varepsilon_{ijkl}$$

Where:

Y_{ijk} is the $_{ijk}$th observation of animal weight;

μ is the overall mean;

α_i is the effect of breed type;

β_j is the effect of sex type;

γ_k is the effect of BW at slaughter;

ϕ_l is the effect of age at slaughter;

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

$\alpha\beta_{ij}$, is the two-way interactions of breed × sex.

Breed and sex type were fitted as fixed effects, and although slaughter groups were designed to set up at the same weight, the age and BW at slaughter was included in the model as a covariate (479 kg approximately). The significance of differences between individual breed and sex means were examined using Scheffé's pair-wise comparison test.

RESULTS AND DISCUSSIONS

The average performance comparisons of breeds and sex types for BW at slaughter, HCW and DP% are shown in Table 1 respectively.

Average BW at slaughter of each breed were 486.5, 485.6 and 462.2 for Holstein, Simmental and crosses, respectively. Average BW at slaughter of sex were 482 kg for both bulls and heifers. There were significant differences ($P<0.05$) in HCW and DP% between sexes. Average HCW of bulls and heifers were 257.68 and 245.58 kg, respectively. Mean DP% for bulls and heifers were 53.68% and 51.15% respectively. A higher carcass performance ability for bulls compared to heifers, as shown in this study, has been well reported previously and the results were in line with those found in literature (Tanner et al., 1970; Steen, 1995; Link et al., 2007; Bures and Barton, 2012; Bozkurt, 2012). As expected, the lower dressing-out proportion for heifers was mostly due to their markedly higher deposition of internal fat compared to bulls. This is in agreement with other studies comparing carcass traits in bulls and heifers (Steen, 1995; Frickh et al., 2002; Velik et al., 2008; Bures and Barton, 2012).

There were significant differences ($P<0.05$) in average HCW and DP% between breeds. HCW and DP% of Simmental breeds were greater than the other breeds while there were no significant differences ($P>0.05$) in HCW and DP% between Holstein and Crosses. Average HCW of Simmental, Holstein and crosses were 257.39, 246.39 and 251.11 kg, respectively. Mean DP% for breeds of Simmental, Holstein and crosses were 53.62%, 51.41% and 52.21%, respectively.

Table 1.Performance comparison means of breeds and sex types

BREED TYPES	N	BW (kg)	HCW (kg)	DP (%)
Simmental	58	485.6	257.39^{A}	53.62^{a}
Holstein	62	486.5	246.39^{B}	51.41^{b}
Crosses (S×H)	54	462.2	251.11^{B}	52.21^{b}
SEX TYPES				
Bulls	90	482.2	257.68^{C}	53.68^{e}
Heifers	84	482.1	245.58^{D}	51.15^{f}

BW: Body Weight at Slaughter; HCW: Hot Carcass Weight; DP: Dressing-out Percentages.

The least square means and interaction effects of breed and sex typeonperformance (BWat slaughter, HCW and DP%) are shown in Table 2.

Table 2. Least square means and interaction of breed and sex types on performance

BREED TYPES	SEX TYPES							
	Bulls				Heifers			
	N	BW (kg)	HCW (kg)	DP (%)	N	BW (kg)	HCW (kg)	DP (%)
Simmental	30	482.2	261.42^{A}	54.51^{a}	28	489.0	250.35^{B}	52.74^{b}
s.e.		(1.68)	(2.08)	(0.431)		(13.0)	(2.16)	(0.448)
Holstein	30	482.1	255.14^{A}	53.21^{a}	32	490.9	237.65^{C}	49.60^{c}
s.e.		(0.186)	(2.08)	(0.431)		(9.06)	(2.03)	(0.420)
Crosses (S×H))	30	482.2	256.48^{A}	53.31^{a}	24	442.2	245.75^{BC}	51.11^{bc}
s.e.		(0.517)	(2.08)	(0.431)		(16.4)	(2.43)	(0.503)
Total Means		482.2	257.68	53.68		482.1	245.58	51.15

Standard errors (s.e.) of the means are shown in brackets
BW: Body Weight at slaughter; HCW: Hot Carcass Weight; DP: Dressing-out Percentages.
Significant differences for HCW are shown as capital superscripts, significant differences for DP% are shown as lowercase superscripts at 5% significance level.

The body weights of bulls and heifers were taken as covariate in general linear models.

There was no significant (P >0.05) breed and sex interaction for HCW and DP%. In another study carried out by Bozkurt (2012) found that both breeds performed similarly; moreover, there was no significant interaction between breeds.

It was found that there were no significant differences (P>0.05) in HCW and DP% of bulls of breeds. Average HCW and DP% of Simmental bulls, Holstein bulls and bulls of Crosses were 261.4, 255.1 and 256.5 kg, respectively and 54.51%, 53.21% and 53.31%, respectively.

It was observed that there were also significant differences (P <0.05) in HCW values of heifers of breeds. Average HCW for Simmental heifers, Holstein heifers and Crosses heifers were 250.35, 237.65 and 245.75 kg respectively. While the differences (approximately 13 kg) in HCW values between Simmental and Holstein heifers were significant (P<0.05), there were no significant differences (P>0.05) in HCW (approximately 5 kg) between Simmental and Crosses heifers (S×H) and also between Holstein heifers and crosses heifers (8.1 kg)

Similarly, there were significant differences (P <0.05) in DP% values of heifers of breeds. Average DP% for Simmental heifers, Holstein heifers and Crosses heifers were 52.74%, 49.6% and 51.11%, respectively. While the differences (approximately 3%) in DP% values between Simmental and Holstein heifers were significant (P <0.05), there were no significant differences (P >0.05) in DP% (approximately 1.5 %) between Simmental and Crosses heifers (S×H) and also between Holstein heifers and crosses heifers (1.5%).

Although there was no significant (P >0.05) breed and sex interaction for HCW and DP%, Simmental breed of bulls and heifers were heavier than those of other breeds for HCW and DP%.

There are many well documented reports of breed comparisons however, as Keane et al. (1989), Keane and More O'Ferrall (1992) suggested that the results of such comparative studies, including those results of performance of breeds presented in this study, are not necessarily applicable outside the countries where the experiments were conducted due to the differences in factors such as production systems, slaughter weights, climate and management conditions.

CONCLUSIONS

It was observed that Simmental breed animals regardless of sex types were superior to those of other breeds. However, the performance of bulls and heifers of crosses were better than Holstein bulls and heifers performance.The results of this study confirmed that carcass performanceof bulls of all breeds was found to be higher than the heifers of the breeds studied. However, carcass performances of Simmental×Holstein crosses were higher than those of Holstein heifers.

In addition, other measures, such as growth rate, feed conversion efficiency and carcass and bodyweight at slaughter including management conditions, are important parameters that need to be considered for further study to ensure comprehensive breed comparisons.

ACKNOWLEDGEMENTS

This study was presented here as a part of project number 111O269 and 114O778 financially supported by TUBITAK (The Scientific and Technological Research Council of Turkey).

REFERENCES

Allen D. and Kilkenny B., 1984. Planned Beef Production. Collins, London.

Aslam M., Nawaz M., Khan M.S., 2002. Comparative Performance of Some Cattle Breeds Under Barani Conditions of Pakistan. International Journal of Agriculture & Biology, 1560–8530, 565–567.

Bozkurt Y., 2012. Seasonal performance of different breeds of feedlot beef cattle grown under the Mediterranean conditions. Bulg. J. Agric. Sci., 18: 443-445.

Bureš D. and Bartoň L., 2012. Growth performance, carcass traits and meat quality of bulls and heifers slaughtered at different ages. Czech J. Anim. Sci., 57(1): 34–43.

Charles L., Robert N., Mutaka1 J.O., 2012. The Growth Performance Evaluation of Cattle Breeds in the South Western Agro-Ecological Zone (SWAEZ) of Uganda/A Bird's-Eye View of Veterinary Medicine Edited. 321-336.

Demircan V., Koknaroglu H., Yılmaz H., 2007. Effect of Season on Beef Cattle Performance and Profitability, Agricultura Tropıca et Subtropıca, Vol. 40 (1), 19-23.

Frickh J.J., Steinwidder A., Baumung R., 2002. Effect of ration, sex and slaughter weight on slaughtering performance of Simmental cattle. Zuchtungskunde, 74,362–375.

Keane M.G. and More O'Ferrall G.J., 1992. Comparison of Friesian, Canadian Hereford × Friesian steers for growth and carcass composition. Anim. Prod. 55: 377-387.

Keane M.G., More O'Ferrall G.J., Connoly J., 1989. Growth and carcass composition of Friesian, Limousin × Friesian and Blonde Daqutaine × Friesian steers. Anim. Prod. 48: 353-365.

King J.O.L, 1978. An Introduction to Animal Husbandry. Blackwell Scientific Publications Ltd., Oxford.

Link G., Willeke H., Golze M., Bergfeld U., 2007. Fattening and slaughter performance of bulls and heifers of beef breeds and the cross breed German Angus ×Simmental. Archiv Für Tierzucht, 50, 356–362.

Minitab, 2016. Minitab User's Guide. Release 13 for windows. Inc.

Steen R.W.J., 1995. The effect of plane of nutrition and slaughter weight on growth and food efficiency in bulls, steers and heifers of three breed crosses. Livestock Production Science, 42, 1–11.

Steinwidder A., Guggenberger T., Schauer A., Romer A., Ibi G., Frickh J., 2007. Effect of ration, sex and breedingon fattening performance of cattle from sucklercow systems. Zuchtungskunde, 79, 128–141.

Tanner J.E., Frahm R.R., Willham R.L., Whiteman J.V., 1970. Sire × sex interactions and sex diffrences in growth and carcass traits of Angus bulls, steers and heifers. Journal of Animal Science, 31, 1058–1064.

Velik M., Steinwidder A., Frickh J.J., Ibi G., Kolbe-Romer A., 2008. Effect of ration, sex and breed on carcass performance and meat quality of cattle from suckler cow systems. Zuchtungskunde, 80, 378–388.

Scientific Papers. Series D. Animal Science. Vol. LXI, Number 1, 2018
ISSN 2285-5750; ISSN CD-ROM 2285-5769; ISSN Online 2393-2260; ISSN-L 2285-5750

ECONOMIC WEIGHT OF PRODUCTION TRAITS FOR ROMANIAN BUFFALO

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Abstract

The paper aimed to present the economic weights of milk yield, fat and protein percent estimated in the population of Romanian Buffalo from Șercaia Research and Development Station. A total 609 milk yield and associated characters records, belonging to 87 females, which coming from 11 sire families, for 7 lactations were analysed. The method used was multiple linear regression, and as a global indicator the „mozzarella index" was used. The economic values for milk yield, fat and protein percent in seven lactations were calculated as: € 0.9636, € 0.1367, € -0.0974; € 0.9729, € 0.0912, € -0.0661; € 0.9948, € 0.1978, € -0.1935; € 0.9922, € 0.1506, € -0.1452; € 0.9932, € 0.2691, € -0.2645; € 0.9891, € 0.1454, € -0.1352; € 0.9890, € 0.1708, € -0.1597. Results indicated that a major weight should be given to milk yield and fat percent. Negative value associated with protein percent suggest that the fat had a higher price compared to protein, and the payment system should be based on milk yield and fat percent for mozzarella production.

Key words*: economic weight, milk yield, fat and protein percent, Romanian Buffalo.*

INTRODUCTION

In Romania, the buffalo entered with the invasion of the Huns and Avars in the Carpatho-Danubian area. It found the good pedo-climatic conditions and so, in our country, has developed a buffalo population which had its own evolutionary path as a result of reproductive isolation (Vidu et al., 2008). The Romanian Buffalo is one of the most important genetic resources for milk and meat production.
Worldwide, in countries where milk production is ensured by buffalo milk, the population of buffalo increased numerical because of the demographical growth of the human population. In Europe, the main country that exploiting buffalo is Italy, the main production being mozzarella type soft cheese. In 2004, Romania ranked in Europe in terms of breeding buffalo, with 100,000 heads (Vidu et al., 2008). Consequence of the lack of supportive policies in the area of buffalo, herd showed a decreasing trend in Romania, FAO estimating that there are 70,000 heads in 2006 and Vidu (2007) estimated a population of about 64,000 heads.
At present, the buffalo herds in Romania have fallen further, reaching about 14,000 heads (personal estimation from the National Institute of Statistics data). In our country, buffalo is predominantly grown in individual subsistence households, with a maximum of 5 heads. Romania has a tradition of growing this species, but with the aging and biological disappearance of the rural population, the species is vulnerable. Also, the vulnerability of the Romanian buffalo is generated by the lack of financial aid, the low milk price, the lack of strong associations of breeders that protect farmers' interests in recent years.
However, Romania has the major advantage of the existence of a research station in the field of buffalo breeding, which has an extremely valuable breeding nucleus.
Increasing the economic efficiency of buffalo production and developing a breeding program are keys to actively conserving of this genetic structure.
The buffalo is a species with remarkable quality, of which we can remember: high percentage of milk fat, meat with exceptional taste qualities, resistance to diseases and heavy environmental conditions, good valorization of poor quality feeds. On the international market, the main product obtained from buffalo milk is Mozzarella, a cheese specialty. The amount of Mozzarella is closely related to the quantity and

quality of milk (Popa et al., 2014) and is a criterion for the selection of buffaloes.

Enormous advantage of exploitation of this species for characters associated with milk production, compared with cows and sheep, is the lower cholesterol content of milk and Mozzarella cheese type, despite higher values of the constituents (Zicarelli, 2004).

Compared with cows, buffalo milk has quality parameters with higher values. The fat percentage range between 6.87 to 8.59% (Rosati and Van Vleck, 2002; Tonhati et al., 2000), protein percentage between 4.13 to 4.55% (Macedo et al., 2001; Rosati and Van Vleck, 2002). In Romania, Velea and Mărginean (2004) specifies that buffalo's milk production falls in to the following parameters: average milk yield 1111.11 kg/lactation, average fat yield 82.10 kg (7.39%), and average protein yield 46.21 kg (4.23%).

So far, no breeding program related to this species has been developed in our country. The genetic improvement of the Romanian buffalo for the characteristics of milk production can increase the attractiveness of this species and thus preserve it.

Animal breeding addresses only useful economic characters. The economic value of the characters is dictated by the market and quantified by different statistical methods. Depending on the relative economic importance, the weight to be given in the selection of each character is determined (Grosu, 2003). Basically, the weight given in the character selection is determined by the impact of each attribute on the profit. The economic value of a character is defined as the relative effect that it is expected to have its increase with one unit on the per capita income (Hazel, 1943, quotet by Grosu, 2003). In other words, the contribution of genetic improvement of a character to increasing production efficiency is called the economic value or economic weight (Gibson, 1987; Groen, 1989).

There are a number of studies showing estimates of economic weight related to milk yield and milk quality parameters for cattle (Gibson, 1989; Van Arendonk and Brascamp, 1990; Bekman and Van Arendonk, 1993; Vargas B et al., 2002; Komlosi et al., 2010 etc.), but very few related to buffalo (Bahareh T.D et al., 2011).

The objective of this study was to estimate economic weight for milk yield, fat and protein milk content, using "Mozzarella index" as an indicator of profitability, using a methodology that gives the maximum accuracy in conditions of the existence an inconsistent data.

MATERIALS AND METHODS

In order to estimate economic weight values, were used the data resulting following control milk production in females belonging Şercaia Research and Development Station. To analyze parameters in dynamic were included in the analysis only animals presenting records to an equal number of lactations.

A total 609 milk yield and associated characters records, belonging to 87 females, which coming from 11 sire families, for 7 lactations were analysed.

The traits studied were: milk yield per lactation, percent of fat and protein, and Mozzarella index as an indicator of profitability.

In control milk production, records with length greater than 270 days were truncated at this point, as suggested by Tonhati et al. (2008) and Aspilcueta-Borquisetal (2010).

The amount of Mozzarella was estimated using the relationship proposed by Altiero et al. (1989) and used in the national genetic evaluation in Italy:

$$MP\ (kg) = MY * \{[(3.5*\%P) + (1.23*\%F) - 0.88] / 100\}$$

In which:

MP is Mozzarella yield (accumulated at 270 days);

MY is milk yield;

%P is protein percent;

%F is fat percent.

The method used to estimate economic weights is based on the multiple linear regression proposed by Hazel in 1943 (Grosu, 2003, 2005). In the case of multiple linear regression, the dependent variable (Y) is the income per head, and the determination of economic weights implies calculating the partial regression of Y relative to each character that it depends on. Y is also called global indicator.

For the production characters analyzed in this paper, the global indicator used (Y) represents the total amount of Mozzarella on lactation

(Mozzarella index) multiplied by 9.5 euro / kg (average market price at the time of analysis):

$$Y(MP \times 9.5\ Euro/kg) = b_1 \times X_1(MY) + b_2 \times X_2(\%P) + X_3(\%F)$$

The global indicator depends directly on each character associated with milk production.

After solving the equation system, in order to obtain comparable economic weights, the partial regression coefficients were standardized by the formula:

$$v_i = b_i \times \sqrt{\frac{\sum x_i^2}{\sum y^2}}$$

For data characterization, the classical statistical method was used: average ($\overline{X} \pm s_{\overline{X}}$), standard deviation (s) and variability coefficient (V%) (Sandu, 1995).

RESULTS AND DISCUSSIONS

Data collected from 87 females were used to derive phenotypical characterization of sample. The results on the average performance of milk production traits and for mozzarella yield are presented in Tables 1, 2, 3 and 4.

Table 1. Descriptive statistics for milk yield

Specification	**U.M.**	**n**	$\overline{X} \pm s_{\overline{X}}$	**s**	**V%**
Lactation 1	kg	87	942.32 ± 41.01	382.53	40.59
Lactation 2	kg	87	1038.21 ± 44.50	415.05	39.98
Lactation 3	kg	87	1181.47 ± 49.72	463.79	39.25
Lactation 4	kg	87	1274.73 ± 47.58	443.78	34.81
Lactation 5	kg	87	1371.47 ± 51.31	478.59	34.90
Lactation 6	kg	87	1479.17 ± 55.46	517.31	34.97
Lactation 7	kg	87	1421.32 ± 50.51	471.14	33.15

Table 2. Descriptive statistics for fat percent

Specification	**U.M.**	**n**	$\overline{X} \pm s_{\overline{X}}$	**s**	**V%**
Lactation 1	kg	87	6.9834 ± 0.1082	1.0090	14.4492
Lactation 2	kg	87	7.0102 ± 0.1071	0.9994	14.2572
Lactation 3	kg	87	6.8664 ± 0.1039	0.9695	14.1196
Lactation 4	kg	87	6.8274 ± 0.1005	0.9375	13.7310
Lactation 5	kg	87	6.8297 ± 0.0814	0.7595	11.1203
Lactation 6	kg	87	6.8672 ± 0.0809	0.7550	10.9949
Lactation 7	kg	87	6.9446 ± 0.1052	0.9810	14.1264

Table 3. Descriptive statistics for protein percent

Specification	**U.M.**	**n**	$\overline{X} \pm s_{\overline{X}}$	**s**	**V%**
Lactation 1	kg	87	4.2749 ± 0.0342	0.3189	7.4593
Lactation 2	kg	87	4.1795 ± 0.0392	0.3656	8.7479
Lactation 3	kg	87	4.5051 ± 0.0289	0.2700	5.9939
Lactation 4	kg	87	4.1625 ± 0.0288	0.2683	6.4471
Lactation 5	kg	87	4.4572 ± 0.0181	0.1692	3.7954
Lactation 6	kg	87	4.1549 ± 0.0249	0.2321	5.5862
Lactation 7	kg	87	4.2976 ± 0.0243	0.2267	5.2751

Table 4. Descriptive statistics for Mozzarella yield

Specification	U.M.	n	$\overline{X} \pm s_{\overline{X}}$	s	V%
Lactation 1	kg	87	213.11 ± 9.22	85.97	40.34
Lactation 2	kg	87	233.04 ± 10.41	97.13	41.68
Lactation 3	kg	87	276.78 ± 12.12	113.08	40.86
Lactation 4	kg	87	282.57 ± 11.16	104.06	36.83
Lactation 5	kg	87	318.21 ± 12.44	116.04	36.46
Lactation 6	kg	87	327.53 ± 12.65	117.96	36.01
Lactation 7	kg	87	323.30 ± 11.85	110.48	34.17

The data presented in Tables1-4 shows that the average values associated with milk production traits are characteristic of a buffaloes population. The values obtained are similar to those reported by Tonhati et al. (2000) and Sarubbi et al. (2012), but lower than those obtained by Malhado et al. (2007).

The variability is high for milk and Mozzarella yield, most likely due to human error associated with the measurement, without neglecting individual variation caused by various factors (genetic and environmental), but for the percent of fat and protein, variability is low. These values indicate that, at least for milk and Mozzarella traits, the population can constitute object of a breeding program with a sufficiently large field for action of artificial selection. The phenotypical homogeneity for fat and protein percent is an advantage for analyzed population because, at first side, effort must be channelled towards genetic improvement of milk yield.

The economic weight of the characters occupies an important place in the improvement decisions. It is closely related to the establishment of the selection objective and genetic improvement technology. When the objective is complex, setting the economic weight is very important for animal breeding as it determines the weight to be given in the selection of the different traits that contribute to the complex character (Drăgănescu and Grosu, 2003).

The economic weights calculated according to the described model for milk yield, fat and protein percent are presented in Table 5.

Table 5. The economic weights for milk yield, fat and protein percent

Specification	U.M.	Milk yield (kg)	Fat percent	Protein percent
Lactation 1	euro	0.9636	0.1367	-0.0974
Lactation 2	euro	0.9729	0.0912	-0.0661
Lactation 3	euro	0.9948	0.1978	-0.1935
Lactation 4	euro	0.9922	0.1506	-0.1452
Lactation 5	euro	0.9932	0.2691	-0.2645
Lactation 6	euro	0.9891	0.1454	-0.1352
Lactation 7	euro	0.9820	0.1708	-0.1597

The values presented in Table 5 show that an increase of average of milk production with one kilo in one lactation period, the income will be increase with approximatively 1 euro. Also, the unitary increase of fat percent (one percent per lactation) will be a positive effect to the income of farm.

Very interesting are the economic weights obtained for the percentage of protein. The negative values associated with this character indicate that, at least in the analyzed population, it is not desirable to increase the percentage of protein, but all the efforts should be directed towards the genetic improvement of the milk quantity and the percentage of fat.

Economic weights obtained for milk yield and fat percent indicates the importance of these traits for Mozzarella production and for analyzed herd.

Negative value associated with protein percent suggest that the fat had a higher price compared to protein, and the payment system should be based on milk yield and fat percent for Mozzarella production. These indicate that, in the first instance, genetic improvement should be directed to increasing milk quantity and fat percentage to increase the economic efficiency of buffalo exploitation. Once the desired level of production is reached, it may eventually improve the quality of the protein.

It is very clear that the economic weights of the analyzed traits are influenced by the obtained quantity of Mozzarella. In this way, a decrease in the cost of obtaining one kg of mozzarella, ie an improvement in the economic efficiency of the exploitation (increase of the feed conversion, decrease of the cost of obtaining the fodder) can lead to an increase of the economic weight for the milk quantity and the percentage of fat. However, this requires a more detailed analysis using other estimation methods with a higher accuracy (profit function).

Similar results have been obtained by other authors, but in dairy cattle. Thus, Gibson (1989) and Bekman and Van Arendonk (1993) obtain negative economic weights for protein.

Seno et al. (2007) in a study regarding economic values of milk production traits, reported positive economic weight for milk yield, and negative for fat and protein yield, but in a system in which milk is sold to diary industry. In other system, in which produce Mozzarella in the farm, the situation is radically changed, all economic weight being positive. This draws attention to the need for deeper analysis, but the lack of data has prevented us from doing so.

CONCLUSIONS

The results regarding economic weights for studied traits indicates that, in analyzed population and according to our available data, a major weight should be given to milk yield and fat percent in relation to genetic improvement program. Genetic improvement of the quantity of Mozzarella will be made only on account of milk and fat. We mention that is absolutely necessary a complex analysis of this topic according to production system, using a more accurate method, but additional data is required for this which we did not have access to in this paper. The results of the animal breeding work depend directly on the accuracy and complexity of the primary data.

ACKNOWLEDGEMENTS

The paper work was elaborate based on researches financed by two grants: BIOBUFFALO no 169/2014 and ADER 8.1.1./2015.

REFERENCES

Altiero V., Moio L., Addeo F., 1989. Previsionedellaresa in „Mozzarella" sulla base del contenuto in graso e proteine del latte di bufala. Sci.Tec. Lattiero-Casearia, 40, 425-433.

Aspilcueta-Borquis R. et al., 2010. Genetic parameter estimates for buffalo milk yield, milk quality and Mozzarella production and Bayesian inference analysis of their relationships. Genet. Mol. Res. 9 (3), 1636-1644.

Bahareh T.D. et al., 2011. Economic weight of milk production traits for buffalo herds in the southwest of Iran using profit equation. World Applied Sciences Journal, 15(11), 1604-1613.

Bekman H., van Arendonk J.A.M., 1993. Derivation of economic values for veal, beef and milk production traits using profit equations. Livestock Production Sci., 34, 35-56.

Drăgănescu C., Grosu H., 2003. Ameliorarea animalelor. Ed. AgroTehnica, Bucureşti.

Gibson J.P., 1987. The option and prospects for genetically altering milk composition in dairy cattle. Animal Breeding Abstract, 55, 231-243.

Gibson J.P., 1989c. Selection on the major components of milk: alternative methods of deriving economic weights. J. Dairy Science, 72, 3176-3189.

Groen A.F., 1989. Cattle breeding goals and production circumstances. PhD thesis, Wageningen Agriculture Univ., Netherlands, p. 176.

Grosu H., 2003. Programe de ameliorare. Ed. AgroTehnica, Bucureşti;

Grosu H. et al., 2005. Programe de ameliorare genetică în zootehnie. Ed. Ceres, Bucureşti;

Komlosi M.J. et al., 2010. Economic weight of production and functional traits for Holstein-Friesian cattle in Hungary. J. Animal Breeding and Genetics, 127(2), 143-153.

Macedo M.P., Wechsler F.S., Ramos A.A., Amaral J.B. et al., 2001. Composiçãofísico-química e produção do leite de búfalas da raçaMediterrâneo no oeste do Estado de São Paulo. Rev. Bras. Zoot., 30(1), 1084-1088.

Malhado C.H. et al., 2007. Parâmetros e tendências. Rev Brasil de Zoot, 37, 376-79.

Popa R., Popa Dana, Vidu Livia, Diaconescu Cristiana, Băcilă V., Bota A., Dronca D., 2014. Genetic parameters estimates for milk yield, milk quality and mozzarella production of Romanian Buffalo. Bulletin of University of Agricultural Sciences and veterinary Medicine Cluj-Napoca. Animal Science and Biotechnologies, 72(2), 236-241, ISSN print 1843-5262, Electronic 1843-536X.

Rosati A., Van Vleck L.D., 2002. Estimation of genetic parameters for milk, fat, protein and Mozzarella cheese production for the Italian River buffalo *Bubalis bubalis* population. Livest. Prod. Sci.,74, 185-190.

Sandu Gh., 1995. Modele experimentale în zootehnie. Ed. Coral Sanivet, Bucureşti.

Sarrubi F. et al., 2012. Milk Yield and Quality to Estimate Genetic Parameters in Buffalo Cows. Journal of Buffalo Sci., 1. 102-106.

Seno L.O. et al., 2007. Economic values for milk production traits of buffaloes in the State of Sao Paulo. Rev. Brasileira de Zootecnia, 36(6), 2016-2022.

Tonhati H. et al., 2000. Genetic aspects of productive and reproductive traits in a Murrah buffalo herd in Sao Paulo, Brazil. Journal of Animal Breeding and Genetics, p. 331-336.

Tonhati H. et al., 2008. Test-day milk yield as a selection criterion for dairy buffaloes (*Bubalus bubalis* Artiodactyla, Bovidae). Genet. Mol. Biol., 31, 674-679.

Van Arendonk J.A.M., Brascamp E.W., 1990. Economic consideration in dairy cattle breeding. Proceedings of 4th World Congress on Genetics Applied to Livestock Production, Edinburgh, Germany, 14, 78-85.

Vargas B. et al., 2002. Economic values for production and functional traits in Holstein cattle of Costa Rica. Livestock Prod. Sci., 75(2), 101-116.

Velea C., Mărgineanu Gh., 2004. Producţia, reproducţia şi ameliorarea taurinelor. Ed. Tehnică Agricolă, Bucureşti.

Vidu Livia, Georgescu Gh., Udroiu Alina, Ungureanu Maria, Vlăsceanu Florentina, 2007. Studiu asupra parametrilor calitativi ai laptelui de bivoliță la o populație din zona de sud a României. The 36th International Session of Scientific Communications, November, 14 –16.

Vidu Livia, Popa R, Gras M., 2008. Stadiul, direcțiile și obiectivele ameliorării bubalinelor din România. Ed. Alpha Mdn, Buzău.

Zicarelli L., 2004. Buffalo milk: its properties, dairy yield and Mozzarella production. Vet. Res. Commun., 28(1), 127-135.

Scientific Papers. Series D. Animal Science. Vol. LXI, Number 1, 2018
ISSN 2285-5750; ISSN CD-ROM 2285-5769; ISSN Online 2393-2260; ISSN-L 2285-5750

GENETIC CHARACTERISTICS OF THE CATTLE POPULATION OF THE ABERDEEN-ANGUS BREED

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Abstract

In the article are presented the results of studied animals of the Aberdeen-Angus breed by blood groups. The highest frequency of antigens B_2, G_3, Y_2, G ', Q', G" (EAB locus), antigens C_1, C_2, E, W and X_2 (EAC locus) was detected. There is a high incidence of allele B_1G_1 (0.0913), which is accessible to us from literary sources, is met only at Holstein cattle of the Yaroslavl breed and the red Estonian breed. The concentration of the main alleles in the herd PF "Juliana Gore" was 59.3%, rare - 30.4%, respectively. The homozygosity of the analyzed population of Aberdeen-Angus cattle is the lowest in comparison with the data available in the literature and is 0.31. This indicates a very high genetic diversity of this herd, the confirmation which serves the genophond of various breeds - German and Romanian, more detailed research will follow.

Key words*: blood groups, antigen, allele, frequency, Aberdeen-Angus breed.*

INTRODUCTION

The increased interest in meat cattle breeding in recent years has contributed to an increase in the number of beef cattle in many countries around the world. The share of livestock meat in the total number of cattle in Europe and North America ranges from 40 to 85% (Legoshin, 2003).

The most specialized competitive meat breed of the world importance is the Aberdeen-Angus breed.

Aberdeen-Angus breed - one of the classic British breeds, created in Scotland, in the mountainous part of the country with a harsh climate, enters into the number of the fastest meat breeds of world importance.

It was formed from two offspring of local cattle: Aberdeen with a more pronounced meat type of constitution and early maturity, and Angusian - more than the first, tall and with higher milkiness (Bailey, 1981, 1988). Adaptation to pasture content is an important economic value of the breed (Lasley, 1979). Since in Scotland fattening of beef cattle was not practiced,, the animals were walking for 2-3 years on pastures, and then sold for fattening in England.

The maintenance on pastures has developed at animals the ability to consume in a considerable quantity the green weight. They are characterized by a high precocity, early finish the growth and manifest a tend to earlier obesity in comparison with other breeds of beef cattle. Aberdeen-Angus acclimatizes well in a temperate and cold climate. Meat quality of animals is high: the meat is tender, fine-grained, with good marbling.

A small batch of Aberdeen-Angus cattle was brought to the Republic of Moldova from Romania (2011) and Germany (2015). In 2017, the number of Aberdeen-Angus cattle in the herd of Peasant farming „Juliana Gorea" amounted 149 heads, including 70 cows, 43 heifers and 36 bull-calves.

In the available literature sources there are single publications on the research of the blood groups of the Aberdeen-Angus breed (Nakhushev et al., 2015), were analyzed the herds of Aberdeen-Angus cattle in Kabardino-Balkaria according to the frequency of 26 antigens of blood group systems, were revealed differences in antigens Q', W, F', L, indicating a sufficient diversity of livestock.

The purpose of our studies was to give an immunogenetic characterization of the Aberdeen-Angus cattle population imported into the Republic of Moldova.

MATERIALS AND METHODS

The material used for the study was blood selected from the Aberdeen-Angus breed in the herd of cattle PF „Juliana Gorea" (peasant farming) (n = 115).

Taking blood from animals, setting the reactions of hemolysis of erythrocytes, as well as studying blood groups were carried out according to the generally accepted method, 1983. Blood groups were determined by hemolytic tests using 49 bovine reagents standardized in international comparative trials, which were detected by antigens controlled by allelic genes of 9 genetic systems.

The frequency of occurrence of antigens and alleles of the EAB locus (q) was determined by a conventional method. Identification of the EAB-locus alleles and subsequent analysis of the allelophond was carried out according to the following genetic indicators: total number of alleles of the EAB locus; total frequency of occurrence of alleles: basic, rare; degree of homozygosity (Cα) (Merkurieva et al., 1983). The obtained materials were processed on a personal computer.

RESULTS AND DISCUSSIONS

As a result of studies and analysis of the antigen spectrum of the blood groups of animals of the Aberdeen-Angus breed, it was established that 9 animals were carriers with antigen A_2, with a frequency of 0.0783. Carriers of Z′ antigen were not detected, although according to some sources, the presence of Z′ antigen is characteristic for meat breed animals (Cherkashchenko, 1984; Ukhanov et al., 1990).

It should be noted that, according to the AEB locus, carriers of antigens P_2, Q, T′, B″ were not detected in the analyzed sample of animals, and carriers of antigens P_1 and J'_2, I_1 and I′ were about 1, 2 animals, respectively.

The highest frequency of occurrence in the AEB locus have the antigens B_2, G_3, Y_2, G′, Q′, G″, Figure 1, and according to the AEC locus, the antigens C_1, C_2, E, W and X_2, Figure 2.

According to the AEC locus, three antigens with the lowest frequency of occurrence were detected: R_1 (0.0348), L′ (0.0348) and C' (0.0696).

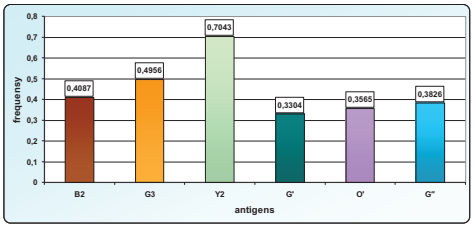


Figure 1. Frequency of occurrence of some antigens of AEB locus

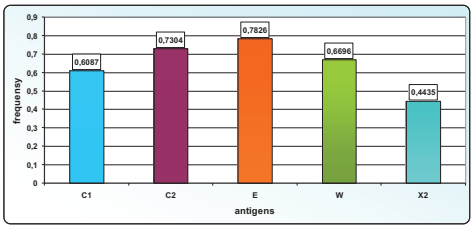


Figure 2. Frequency of occurrence of some antigens of AEC locus

According to the F-V- locus, the frequency of occurrence of antigens F and V amounted 0.5565 and 0.1739, respectively, but for some breeds of meat direction of productivity, such as Buryat and Kalmyk cattle, the frequency of their occurrence varies between 0.8940-0.9760 and 0.3230-0.5080, respectively (Cherkashchenko, 1984).

In single-factor AEJ-, AEL-, AEM-, AEZ-locuses, all antigens were detected in the analyzed animal population, the lowest frequency of antigen M (0.0173), Figure 3.

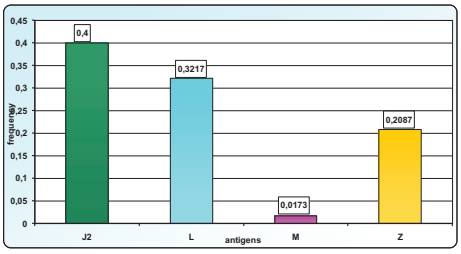
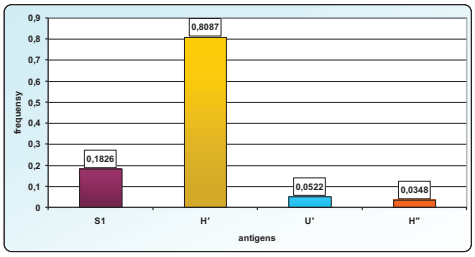


Figure 3. Frequency of occurrence of antigens in single-factor loci

According the AES locus of the 6 studied antigens, the antigens U and U″ could not be detected, among others the highest frequency was observed at the animals of the H′ antigen carrier (0.8087), Figure 4.



4. Frequency of occurrence of antigens of the AES locus

Saturation with antigenic factors of animals of Aberdeen-Angus breed was at the level of 23.5%.

As a result of the studies, were also identified the AEB locus alleles, which to a greater extent reflect the hereditary characteristics of the animals.

In the herd of the Aberdeen-Angus breed, 77 alleles were identified according to the AEB locus, Table 1.

Table 1. Allelophond of the AEB locus of Aberdeen-Angus cattle

No.	Allele	n	Frequency	No.	Allele	n	Frequency
1.	B_1	3	0.0130	40.	$Y_2E'_2Y'G''$	1	0.0043
2.	**B_1G_1**	21	0.0913	41.	$Y_2E'_2Q'$	2	0.0087
3.	$B_1G_1I_1$	1	0.0043	42.	$Y_2E'_2O'G''$	2	0.0087
4.	B_1P'	1	0.0043	43.	Y_2G'	2	0.0043
5.	$B_2G_2O_2$	5	0.0217	44.	$Y_2G'O'$	1	0.0043
6.	$B_2G_2T_1$	2	0.0087	45.	$Y_2G'O'Q'G''$	1	0.0043
7.	$B_2G_2Y_2$	2	0.0087	46.	$Y_2G'P'Q'G''$	1	0.0043
8.	$B_2G_2Y_2E'_2O'$	1	0.0043	47.	$Y_2G'K'O'Q'G''$	1	0.0043
9.	$B_2Y_2G'O'P'Q'G''$	1	0.0043	48.	$Y_2G'O'Y'G''$	1	0.0043
10.	B_2O_1	4	0.0174	49.	$Y_2G'O'G''$	4	0.0174
11.	$B_2O_1Y_2D'$	3	0.0130	50.	$Y_2G'O'Q'G''$	1	0.0043
12.	$B_2O_1Y_2G'P'Q'G''$	1	0.0043	51.	$Y_2G'Y'G''$	2	0.0087
13.	B_2G'	1	0.0043	52.	$Y_2G'G''$	6	0.0261
14.	G_1	3	0.0130	53.	$Y_2G'Q'$	3	0.0130
15.	$G_1I_1T_1$	1	0.0043	54.	$Y_2G'Q'G''$	4	0.0174
16.	$G_1O_1I_1$	1	0.0043	55.	Y_2K'	1	0.0043
17.	$G_1T_1O_1$	1	0.0043	56.	Y_2O'	3	0.0130
18.	G_2O_1	1	0.0043	57.	$Y_2O'P'$	1	0.0043
19.	$G_2O_2T_1$	1	0.0043	58.	$Y_2O'P'Q'G''$	1	0.0043
20.	**$G_2Y_2E'_1Q'$**	12	0.0522	59.	$Y_2O'Q'$	8	0.0347
21.	G_3T_1	5	0.0217	60.	$Y_2O'G''$	2	0.0087
22.	I_2	16	0.0696	61.	$Y_2P'Q'G''$	1	0.0043
23.	O_1	5	0.0217	62.	Y_2Q'	1	0.0043
24.	O_1Y_2D'	1	0.0043	63.	Y_2Y'	2	0.0087
25.	$O_1Y_2E'_2$	1	0.0043	64.	$Y_2Y'G''$	2	0.0087
26.	$O_1Y_2G'G''$	1	0.0043	65.	E'_2	1	0.0043
27.	$O_1E'_2Q'$	1	0.0043	66.	E'_2O'	1	0.0043
28.	O_2Y_2Q	1	0.0043	67.	$E'_2O'G''$	1	0.0043
29.	$O_2G'G''$	1	0.0043	68.	E'_2Q'	2	0.0087
30.	$P_1E'_2I'J'_2O'P'$	1	0.0043	69.	$G'Q'$	1	0.0043
31.	T_1Y_1	1	0.0043	70.	$G'Q'G''$	1	0.0043
32.	T_1Y_2G'	1	0.0043	71.	$I'Q'$	1	0.0043
33.	$T_1Y_2G'O'G''$	1	0.0043	72.	O'	5	0.0217
34.	Y_2	3	0.0130	73.	$O'Q'$	2	0.0087
35.	$Y_2D'G'G''$	1	0.0043	74.	$O'G''$	1	0.0043
36.	$Y_2E'_2$	1	0.0043	75.	**Q′**	20	0.0870
37.	$Y_2E'_1G'G''$	2	0.0087	76.	G''	3	0.0130
38.	$Y_2E'_2O'Q'$	1	0.0043	77.	"b"	2	0.0087
39.	$Y_2E'_2O'Y'$	1	0.0043				

Perhaps most alleles are specific and unique to the breed, such as, for example, $G_1I_1T_1$, $G_1T_1O_1$, $G_2O_2T_1$, $O_2G'G''$, $T_1Y_2G'O'G''$, $Y_2E'_2Q'$, Y_2K' and several others, further studies can confirm, or to refute our assumptions. The analysis found that the

spectrum of alleles is quite wide, since the estimated population includes animals from two different breeding - German and Romanian.

It is observed a high frequency of occurrence of the allele B_1G_1 (0.0913), which from available literature sources to us is met only at holsteinized cattle of the Yaroslavl breed (Popov, 1996) and the red Estonian breed (Konstandoglo et al., 2010). It should be noted that the allele B_1P' is common for Limousine, brown Carpathian breeds, specific for the Caucasian brown breed. Allele $B_2G_2O_2$ is common for Kalmyk, Yakut Simmental and Hereford breeds (Ukhanov et al., 1990). A number of alleles specific for other breeds were identified: $G_2O_2T_1$ and E'_2O' alleles for the Kholmogory breed, allele T_1Y_1 for the red Gorbatov breed, allele $Y_2E'_1G'G''$- specific for the Simmental breed population of the Tambov region (Sorokova et al., 1988).

The allele $G_2Y_2E'_1Q'$, with a frequency of occurrence of 0.0522, as it is known, characterizes many breeds of the black-motley root of dairy direction of the productivity, also was found at Kalmyk cattle. It is high the frequency of the occurrence and of the allele Q′ (0.0870), which is common for another breed of meat direction of productivity - Hereford. It should be noted that the neutral allele "b" is common to the Kalmyk, red Gorbatov, gray Ukrainian, other meat breeds of livestock, is present in the allelophond of the breed of the black and motley root.

The objective genetic characteristics of the Aberdeen-Angus animal population reflect also such indicators as the homozygosity coefficient (Ca), the number of effective alleles (Na), the degree of genetic variability (coefficient V), Table 2.

Table 2. Genetic variability of Aberdeen-Angus cattle

No.	Indices	Value
1.	Total investigated, heads	115
2.	Number of established alleles:	
	total	206
	main	136
	rear	70
3.	Total frequency of alleles:	
	main	0.5913
	rear	0.3043
4.	Homozygous coefficient, Cα	0.0031
5.	The number of effective alleles, Na	322
6.	Degree of genetic variability, V	100.6

As can be seen, the concentration of the main alleles in the analyzed sample was 59.3%, the rare - 30.4%, respectively. The homozygosity of the analyzed cattle population is the lowest in comparison with the data available in the literature. Thus, in comparison with the Kalmyk breed, where the homozygosity at the EAB locus was the lowest of all the breeds listed in the collection (Popov and Eskin, 2000) - 1.9%, the homozygosity of the Aberdeen-Angus breed is 0.31%.

This indicates a very high genetic diversity of this herd, as evidenced by the genofond of different breeding - German and Romanian, more detailed studies will follow.

The condition of the allelophond of breed according by the level of homozygosity is reflected by the index of the number of effective alleles. Studies have shown that in the Aberdeen-Angus animal population, the number of effective alleles reaches 322, which corresponds to the maximum possible "homozygous" structures in the herd and reflects the state of heterozygosity at this locus. The degree of realization of the possible genetic variability (V) is 100.6.

Thus, the allelophond of Aberdeen-Angus cattle is diverse for breeding with the participation of blood groups, and such a high level of homozygosity (0.31%) will ensure the existing genetic variability in the improvement of the main selectable characteristics of this breed.

CONCLUSIONS

Antigens B_2, G_3, Y_2, G′, Q′, G″ have the highest frequency of occurrence in the AEB locus, antigens C_1, C_2, E, W and X_2 in the AEC locus. The frequency of occurrence of antigens F and V was 0.5565 and 0.1739, respectively.

The spectrum of the alleles of the AEB locus of the analyzed Aberdeen-Angus cattle population is quite wide, 71 alleles are identified. Most alleles are specific and unique for this breed: $G_1I_1T_1$, $G_1T_1O_1$, $G_2O_2T_1$, $O_2G'G''$, $T_1Y_2G'O'G''$, $Y_2E'_2Q'$, Y_2K'.

It is observed a high frequency of occurrence of the allele B_1G_1 (0.0913), which occurs only in Holstein cattle of Yaroslavl and red Estonian breeds.

The homozygosity of the analyzed population of Aberdeen-Angus cattle is the lowest in comparison with the data available in the literature and is 0.31.

REFERENCES

Bailey D. R., 1981. Caff survival and prewedning grivt in divergent beef dreedsand. Crosses.-J. Anim.Sc., Vol.52, 1244-1245.

Bailey D.R., Swierstra E.E., Entz T., 1988. Postpartum interval in 10 firstgrosses of beef cows inderdrylot and range conditions. Canad. J. Anim. Sc., Vol.4, 1027-1033.

Cherekaev A.V., Zelepukhin A.G., Levakhin V.I. et al., 2000. Meat cattle breeding. Orenburg. 340.

Cherkashchenko V.I., 1984. Genetic features of the cattle of the Turan-Mongolian group on polymorphic blood systems in connection with the preservation of the genofond: thesis Dr.Sci.Biol. Leningrad-Pushkin, 145.

Konstandoglo A.G., Foksha V.F., Aleksandrova T., 2010. Immunogenetic characteristics of the cattle population of the red Estonian breed. J. Biology of animals, Lvov. Vol.12, 1, 240-246.

Lasley J., 1979. Further information on the inheritance of horns and sears. Bull- O-Vram., 40-43.

Legoshin G.P., 2003. Increasing the efficiency of beef cattle breeding in Russia. J. Zootechny. 3, 24-26.

Merkuryeva E.K., Shangin-Berezovsky G.N., 1983. Genetics with the fundamentals of biometrics. Moscow: Kolos, 400.

Methodical recommendations on the use of blood groups to enhance breeding and breeding work in dairy cattle, 1983, Leningrad.

Nakhushev R.B., Tleynsheva M.G., Vologirova F.A., 2015. Immunogenetic characteristics of Aberdeen-Angus cattle in Kabardino-Balkaria. Agrarian Russia: Scientific Prod. Journal. 3, 6-17.

Popov N.A., 1996. Evolutionary features of the red lear species according to alleles of the B-locus of blood grains. Reports of the Russian Academy of Agricultural Sciences, 2.

Popov N.A., Eskin G.V., 2000. Allelophond of cattle. Reference catalog. Moscow, 300.

Sorokoy P.F., Popov N.A., Osipova T.I., 1988. Genetic analysis of crossing of Simmental and red-motley Holstein cattle according by blood types. "Increasing productivity in dairy and beef cattle", Bulletin of Scientific Works, All-Russian Institute of Animal Husbandry. Dubrovitsy, vol. 92, 84-89.

Ukhanov S.V., Berendyaeva Z.I., Kovalenko V.P., Istomin A.A., 1990. Genetic features of the Yakut aboriginal cattle and its hybrids. J. Genetics. Vol.26(3), 525-530.

Scientific Papers. Series D. Animal Science. Vol. LXI, Number 1, 2018
ISSN 2285-5750; ISSN CD-ROM 2285-5769; ISSN Online 2393-2260; ISSN-L 2285-5750

EXTERIOR OF HOLSTEIN COWS OF DUTCH AND GERMAN BREEDING

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Abstract

Were investigated the exterior features on the basis of sampling and the calculation of the physique indexes of Holstein cows of Dutch and German breeding in the herd of Joint-Stock Company „Aydyn", Comrat, Administrative and Territorial Unit Gagauzia, Republic of Moldova. Cows of German breeding in many measurements had superiority in comparison with contemporaries. A cow of Dutch breeding were significantly inferior to the peers of the German selection for high-altitude measurements - the height at the withers and in the sacrum was 2.6 cm (P<0.05) and 2.0 cm (P<0.01), respectively. The body in length was better developed at cows of Dutch breeding - according to the index of stretching; they exceeded the peers of German breeding by 2.1 %.

Key words*: exterior, measurements of exteriors, body indexes.*

INTRODUCTION

In recent years, in the breeding of dairy cattle, an enormous role is given to the exteriors of animals. The exterior is one of the necessary elements of an integrated assessment of animals, in particular cattle. Animals with good exteriors are less exposed to diseases of the udder, limbs, they have less difficult calving, they are able to eat more forages needed to ensure high milk yields (Kochetkov et al., 2003; Pereverzev et al., 1990; Hamoen, 1995).

Assessment of the exterior is important and necessary for understanding the biological and economic characteristics of animals. The study of appearance, external forms of the animal's constitution is important, as the exterior serves as an external expression of the constitution of animals, characterizes the state of their health, determines the individual features of the build, predisposes to a certain type of productivity (Eisner, 1984; Liskun, 1949). Correct constitution and strong constitution can testify to the resistance of animals to unfavorable external influences, ability to prolong economic use (Abrampolsky et al., 2005; Sokolova et al., 2013).

Many scientists (Ali, 1984; Misik, 2003; Sokolova, 2013) note that the assessment of the exterior is necessary for judging the strength of the animal's constitution and the conformity of this body to the conditions in which the animal exists in that productivity for which it is bred. Underestimation of the exteriors in this attitude can lead to overdevelopment, weakening of health, and, consequently, to a decrease in the productivity and acclimatization abilities of animals. In modern selection, the assessment of the exterior and the constitution has acquired special relevance.

The complication of the selection process is accompanied by deterioration in the conditions of feeding and maintenance, as a result of which the role of animals with good stress resistance, strong health and the constitution is significantly increased (Spivak et al., 1987; Reshetnikova et al., 1995; Hamoen, 1994).

The genetic potential of Holstein cattle breeding of Dutch and German breeding allows receiving high milk yields in the herd of Joint-Stock Company „Aydyn", which are quite comparable with the achievements of Holland, Germany and other countries.

The aim of the research was to study the exteriors of Holstein cows of different breeds in the herd of the Joint-Stock Company „Aydyn".

MATERIALS AND METHODS

The objects of research were Holstein cows of Dutch and German selection of the third lactation. The studies were conducted in Joint-Stock Company „Aydyn" (J.S.K. „Aydyn"), Comrat, Administrative and Territorial Unit Gagauzia, where the animals were in the same conditions of feeding and keeping in accordance with the accepted technology in the farm.

Exterior-constitutional features of cows were studied using the method of taking measurements and calculating the indices of their physique.

The belonging of cows to various breeding was determined on the basis of analysis of the genealogical structure of the herd, using pedigree certificates, pedigree cards, artificial insemination logs and other documents of primary zootechnical accounting.

Measurements of animals were taken on the second, third month of a lactation. The milk ratio was determined by the formula:

MR = M/LW,

where: MR - milk ratio, kg; M - milk for 305 days or a shortened lactation, kg; LW - live weight, kg.

The genetic potential of the productivity of heifers was determined on the basis of the parental index of cows (PIC) according to the formula:

PIC = (2M + MM + MO):4,

where: M - the productivity of the mother; FM - the productivity of the father's mother; MM - the productivity of mother's mother.

Statistical processing of the results of the data was carried out by the method of Merkurieva (1983), Plokhinsky (1978) on a PC with the use of software.

RESULTS AND DISCUSSIONS

When studying the exterior-constitutional characteristics of cows of Dutch and German selection on the third lactation in J.S.K. „Aydyn", it was found that they were generally characterized by a relatively strong constitution, proportionally developed and slightly elongated trunk (Table 1, Figures 1, 2).

Table 1. Indicators of linear measurements of the body of cows of the herd J.S.K. „Aydyn" of various selections of Holstein breed

Measurements	Dutch Selection, n=31		German Selection, n=51	
	M± m, cm	Cv, %	M± m, cm	Cv, %
Height at withers	143.4±0.68*	2.64	146.0±0.78*	3.84
Height in sacrum	149.2±0.82**	3.08	151.2±0.87**	4.15
Depth of chest	76.1±0.63	4.65	77.4±0.58	5.42
Breast width behind the shoulder blades	46.5±0.82	9.87	48.3±0.71	10.6
Croup width at hips	57.2±0.67	6.52	58.5±0.69	8.48
Width in sciatic tubercles	37.4±0.61	9.2	36.4±0.41	8.2
Slanting length of body	171.0±1.02	3.32	170.4±1.17	4.9
Girth of chest behind the shoulder blades	206.3±1.45	3.93	209.3±1.34	4.6
Girth of the pastern	19.8±0.17	4.65	19.7±0.09	3.61

Note: * - P<0,05; ** - P<0.01

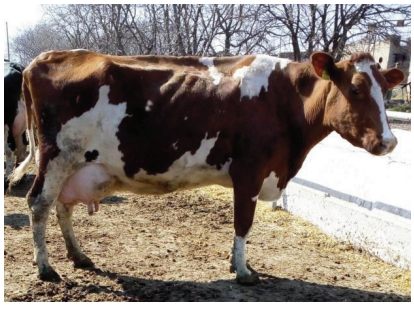

Figure 1. The cow of German breeding

Figure 2. The cow of Dutch breeding

Our studies have established that the cows of Dutch and German breeding proved to be quite large, as evidenced by the indicators of high-altitude measurements.

The height at the withers at cows of German breeding averaged 146 cm (a minimum of 136 cm, a maximum of 162 cm), the Dutch selection 143.4 cm (minimum 135 cm, maximum 152 cm).

Nevertheless, cows of German breeding in many measurements had superiority in comparison with contemporaries. Thus, the cows of Dutch breeding were significantly inferior to the peers of the German selection for altimetry measurements-the height at the withers and the sacrum was 2.6 cm ($P<0.05$) and 2.0 cm ($P<0.01$), respectively. By the depth and width of the chest behind the shoulder blades, croup width at hips and the girth of the chest behind the shoulder blades, of the cows of German breeding also had an advantage, the revealed difference was insignificant and unreliable.

On the width of the sciatic hillocks, the oblique length of the trunk and the girth of the pastern, the cows of German breeding, on the contrary, were inferior to those of the Dutch breeding by 2.7-0.4-0.5%, respectively. It is exposed a manifestation of a similar pattern of the basic exterior and constitutional features of the Holstein cattle, established by a number of authors (Katmakov et al., 2010).

For a more objective evaluation, the body index was calculated in Table 2.

Table 2. Indices of the constitution of Holstein cows of Dutch and German breeding

Index	Dutch Breeding	German Breeding	Stan-dard
High-legged	46.9	47.0	46.5
Lengthiness	119.2	116.7	120.0
Pelvic thoracic	81.3	82.6	80.2
Thoracic	61.1	62.4	61.8
Consistency	120.6	122.8	118.0
Outgrown	104.0	103.5	100.9
Osseous	13.8	13.5	14.6

It should be noted that the body was developed in length better at cows of Dutch breeding – they exceeded the indices of German breeding by 2.1% in the index of stretching (Figure 3).

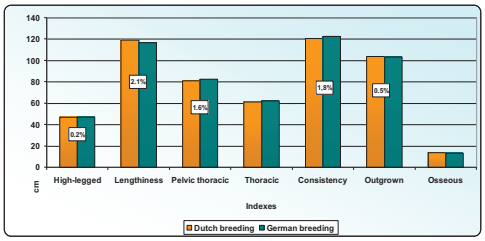

Figure 3. Indexes of the physique of the cows under evaluation

In most other indices, the superiority of German breeding cows is observed: high-legged - by 0.2%, pelvic thoracic - by 1.6% thoracic - by 2.1%, consistency - by 1.8% and outgrown by 0.5%. Thus, the cows of German breeding have a relatively better development of the chest in depth, respectively, of the chest organs.

Consequently, the more developed organs of the chest provide a higher metabolism, which causes higher milk production. This is confirmed by an analysis of the level of dairy productivity of animals.

The obtained values of the indices were also compared with the dairy-type standard. The values of the high-legged and thoracic indices for cows of both selections were within the standard for animals in the dairy direction of productivity.

The obtained values of the outgrown index indicate about a flat top line in all analyzed animals. The osseous index in comparison with the standard was 5.8 and 8.1% less, due to the high height at the withers of cows of Dutch and German breeding, in general, Holstein cattle. Our data are consistent with the results of studies carried out in the experiment on the study of the exteriors of Holstein full-aged cows of different breeds (Australian, Russian) by Shatalov et al. (2013) and studies of the exterior features of black and motley Holstein cows of Dutch and German breeding (Kibkalo et al., 2015).

Studies have established some advantage over the live weight of cows, depending on their origin. Thus, the cows of German breeding exceeded the cows of Dutch breeding by an average of 25 kg, the difference is highly reliable ($P<0.001$) (Tables 3, 4).

Table 3. Dairy productivity of cows of Dutch breeding

Body weight, kg	Milk, kg / day	Milk, kg	Fat, %	Fat, kg	Coefficient of milk, kg
First lactation, n=65					
641.7±7.8	26.4±0.37	7853.8±117.5	3.79±0.05	296.3±3.97	1225.9±23.6
Second lactation II, n=59					
637.4±6.7	27.6±0.50**	8612.2±146.2***	3.77±0.02	325.2±5.2***	1354.6±27.6
Average					
639.7±5.1	26.9±0.31	8228.0±100.9	3.78±0.02	310.1±3.5	1294.1±18.8

Note: ** P<0.01 *** P<0.001

Table 4. Milk productivity of cows of German breeding

Body weight, kg	Milk, kg / day	Milk, kg	Fat, %	Fat, kg	Coefficient of milk, kg
First lactation, n=91					
666.9±3.1	23.8±0.03	7261.3±93.8	3.80±0.02	275.5±3.0	1092.7±14.4
Second lactation, n=28					
657.9±6.8	28.7±0.62***	8740.9±188.9***	3.74±0.04	325.6±5.5***	1330.9±17.3
Average					
664.8±2.9	24.3±0.32	7594.4±104.2	3.79±0.02	287.2±3.28	1144.1±16.4

Note: *** P<0,001

Analysis of milk productivity of cows of different breeding in the herd J.S.K. „Aydyn" showed that from the cows of Dutch breeding for the first lactation was received on average 7853.8 kg of milk, which is by 592.5 kg more than from the cows of German breeding, the difference is highly reliable (P<0.001).

A comparative analysis of daily average milk yield for a number of lactations showed that cows of Dutch selection for the second lactation had an average of 1.11 kg of milk/day more than on the first by P<0.01. Compared with the first lactation, the increase of milk productivity for the second lactation was 758 kg of milk; the difference is highly reliable at P<0.001. A similar increase occurred in the amount of milk fat - by 28.9 kg at P<0.001. It should be noted a significant increase in milk yields at cows of German breeding - the milk productivity for the second lactation was 8740.9 kg of milk, which is by 1479.6 kg more milk than at the first lactation, the difference is highly reliable (P<0.001), while daily milk yield increased by 4.9 kg of milk (P<0.001). According to the second complete lactation, cows of German breeding on average, exceeded peers of Dutch breeding by 128.7 kg of milk, the difference is not reliable.

On average, cows of Dutch breeding signify-cantly exceed their peers of German breeding by milking for 305 days of lactation by 633.6 kg of milk (P<0.001), average daily yield of milk for 2.7 kg, milk fat yield for 22.9 kg at P <0,001.

The coefficient of milk yield at cows of Dutch breeding averaged 1294.1 kg of milk, which is more by 150 kg than at cows of German breeding, the difference is highly reliable (P<0.001). A higher coefficient milking was found at animals of Dutch and German breeding in the second lactation, which amounted to 1354.6 kg and 1330.9 kg of milk, respectively, with a difference of only 23.7 kg of milk.

The indicators of the genetic potential of the descendants of cows of Dutch and German breeding are given in Table 5.

As it can be seen from the data in table, the 5 parental index of cows (PIC) for milk yield was the highest at German heifers (10416.9 kg) - the superiority was 369 kg of milk, the difference is reliable (P<0.01), and on fat, on the contrary, Dutch breeding significantly exceeded the peers of German breeding by 0.23% (P<0.001).

Realization of the genetic potential (RGP) for 305 days of lactation was higher at heifers of Dutch breeding and amounted to 78.15%, which is by 8.45% more than the average of German heifers. The realization of the genetic potential for fat was higher at heifers of

German breeding - 93.4% or 5.3% more than at their peers of Dutch breeding.

Thus, the evaluation of the physique according to the values of the indices indicates, on the whole, that at the analyzed animals the type of dairy cattle is expressed. They have proportional forms of physique, large, elongated head, light bones, long thin neck, deep long chest, body stretched, muscles are moderately developed. The back of the cows is long, wide, straight. The limbs are straight, strong, short hoofs, pasterns strong, flexible hocks. Realization of the genetic potential of the heifers of Dutch and German breeding for 78.1 and 69.7% respectively confirms their high level of milk productivity.

Table 5. Realization of the genetic potential of the calves

Indicators		Breeding	
		Dutch	German
Parent index of cows	milk yield, kg	10047.9±134.3	10416.9**±128.6
	fat, %	4.30***±0.037	4.07±0.039
Own productivity	milk yield, kg	7853***±117.51	7261±93.8
	fat, %	3.79±0.05	3.80±0.02
Realization of genetic potential, %)	milk yield	78.15	69.70
	fat	88.10	93.40

Note: ** - P <0.01; *** - P <0.001

CONCLUSIONS

The height at the wither for cows of German breeding averaged 146 cm (a minimum of 136 cm, a maximum of 162 cm), the Dutch selection was 143.4 cm (minimum 135 cm, maximum 152 cm).

In most other indices, the superiority of German breeding cows is observed: high - legged by 0.2%, tight chest by 1.6%, thoracic by 2.1%, consistency by 1.8% and outgrown by 0.5%.

Milk productivity for the second lactation at cows of German breeding was 8740.9 kg of milk, which is by 1479.6 kg more milk than the first lactation, the difference is highly reliable (P<0.001). According to the second complete lactation, cows of German breeding on average exceeded the peers of Dutch breeding for 128.7 kg of milk, the difference is not reliable.

REFERENCES

Abrampolsky N.F., Abylkasymov D.A., 2005. Evaluation of the type of physique of cows and its relation with milk productivity. „Modern technological and selection aspects of the development of livestock in Russia". Scientific works of the All-Russian institute of livestock production. Dubrovitsy. Vol.1(63), 128-132.

Ali T.E., Burnside E.B, Schaeffer L.R., 1984. Relationship between external body measurements and calving difficulties in Canadian Holstein – Frisian cattle. J. Dairy Sci. Hb.64, 12, 3034-3044.

Eisner F.F., 1984. Constitution and exterior. Cattle breeding. Moscow. Kolos, 60-71.

Hamoen A., 1994. Type classification (l) Veepro Holland, December, 21, 16-17.

Hamoen A., 1995. Type classification (2). Veepro Holland, April, 22, 14-16.

Katmakov P.S., Anfimova L.V., Paramonov A.G., Fadeeva N.V., 2010. Exterior-constitutional features and milk productivity of cows of different genetic origin. Materials of the international scientific and practical conference „Agrarian science and education on the present stage of development: experience, problems and ways to solve them", vol. 2. Ulyanovsk, 41-45.

Kibkalo L.I., Tkacheva N.I., Goncharova N.A., 2015. Exterior features and milk productivity of Holstein cows of Dutch and German breeding. Bulletin of the Kursk Agricultural Academy, 5, 54-58.

Kochetkov A.A., Savachaev V.N., 2003. Strategy of development of dairy cattle breeding technology: state and perspectives. Union of Russian Cattle Breeders. Moscow, 23-52.

Liskun E.F., 1949. Exterior of farm animals. Moscow. Selkhozizdat, 173.

Merkuryeva E.K., Shangin-Berezovsky G.N., 1983. Genetics with the fundamentals of biometrics. Moscow, Kolos. 400.

Mysik A.T., 2003. The development of livestock in the countries of the world. J.Zootechny. 1, 9-12.

Pereverzev D.B., Dunin I.M., Privalikhin G.M., 1990. Perfection of Kholmogory cattle in Russia. Moscow. Rosagropromizdat, 207.

Plokhinsky N.A., 1978. Mathematical Methods in Biology. Moscow. Moscow State University. 265.

Reshetnikova T.M., Moroz T.A., Malinovsky V.A., 1995. Reproductive characteristics of cows of Holstein herd. Improvement of economic and biological indicators of native breeds of livestock.

Moscow. All-Russian Research Institute of breeding business, 45-48.
Shatalov S.V., Shatalov V.S., Tomilin V.K., Kochueva Y.V., 2013. Exterior of high-intensity dairy cattle. Scientific journal Kuban state agricultural university, 91 (07). http://ej.kubagro.ru/2013/07/ pdf/50.pdf
Sokolova I.B., Strekozov N.I., Gusev I.V., 2013. Influence of feeding of beta carotene (preparation GoKar) on biochemical indicators of blood of highly productive cows. J. Zootechny. 10, 7-8.
Spivak M.G., Milyukov A.K., 1987. Use of Holstein bulls in the creation of a new breed of red-and-motley dairy cattle. The use of Holstein breed for the intensification of breeding of dairy cattle. Theses of reports. Kyiv, 11-13.

Scientific Papers. Series D. Animal Science. Vol. LXI, Number 1, 2018
ISSN 2285-5750; ISSN CD-ROM 2285-5769; ISSN Online 2393-2260; ISSN-L 2285-5750

A REVIEW OF THE ADAPTATION OF THE NEWBORN CALF TO ITS ENVIRONMENT

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Abstract

During the first months of life, the calf needs to adapt physiologically to three challenges: extra-uterine life, maintaining the prolonged pre-ruminant stage and weaning. This paper aims to detail the newborn calf's adaptation to extra-uterine life, namely changes occurring at the digestive level, and less at the endocrine or immunological levels, knowing that the calf is born hypo- or a-gammaglobulemic. At birth, the digestive system of the calf is structurally complete (rumen, reticulum, omasum and abomasum), but functionally incomplete, as the abomasum is the only active compartment in the digestion of the pre-ruminant calf. From this moment and up to two weeks of life, the calf can be considered monogastric, as a result of the existence of an anatomical structure that ensures the passage of the colostrum/milk replacer to the abomasum.

The transition from intrauterine life to extra-uterine life is very demanding for the calf, as immediately after birth it has to adapt to new environmental and nutritional conditions. At birth, the young ruminant becomes dependent to the extra-uterine environment regarding food intake. Concerning the environment, the greatest adaptation efforts are related to thermoregulation, as the calf transitions from 38.8°C in utero to below 20°C in the shelter. After birth, changes in the newborn's energy metabolism determine the production of endogenous glucose and the use of fats to compensate for the continued loss of glucose. In the meat industry, young calves are generally fed by their mother and are weaned progressively. Instead, calves coming from dairy cows are separated from their mother immediately after birth and receive colostrum during the first two days of life and then milk or milk replacer for the next weeks. Ingestion of colostrum is essential for the morphological and functional development of calves. Maintaining the calves in a prolonged pre-ruminant phase (up to 3-5 months or more) is done in some European countries producing approximately 750 000 tons of veal, consumed annually in the European Union. Some dairy calves are maintained in a precursor stage for about a month and then weaned over a two-week period. At present, artificial colostration, due to the establishment of colostrum banks, is gaining more and more ground, thus giving up the direct contact of the calf with the mother.

Key words*: newborn calves, colostrum, thermoregulation, extra-uterine life.*

INTRODUCTION

Response mechanisms to environmental challenges of the newborn calves' population has been going on for thousands of years. Adaptation must be successful and populations must be capable of sustained production, but there are a few reasons for which adaptation might be difficult. Management systems are changing more rapidly, typically in the direction of greater intensification. Compared to only a few decades ago, for example, cows now produce their first calf at two rather than three years of age, animals are maintained at higher density per unit of land area and cattle are fed on higher energy diets (Hohenboken et al., 2004). In many instances, management systems and environments are changing more rapidly than animal populations can adapt through natural selection. Stress is a fact of life. Fortunately, response mechanisms have evolved to stressors commonly encountered in a population's evolutionary past. These physiological, immunological, metabolic and behavioral responses generally are sufficient to maintain biological integrity and physical well being. However, when responses are inappropriate or inadequate, stress can lead to distress, defined here as ill health or compromised well being (Moberg, 1999). In a maladapted population, inherent response mechanisms to prevailing environmental challenges do not maintain the well being of many individuals. An adapted population is one

in which most individuals cope successfully with those stressors most commonly encountered in their environment.

MATERIALS AND METHODS

We searched scientific databases for relevant articles identified by the keywords: newborn calves, colostrum, thermoregulation, extra-uterine life and we selected those articles which discuss the most important problems of the calves in their first days of life in regard to the productivity of the farmer. The different authors mention three critical points in the life of the neonate calf: the digestive system, heat stress and colostrum intake. Also, other problems like pneumonia, diarrhea, omphalitis, are mentioned in the sources consulted, from birth until weaning.

This research tries to evaluate the issues which healthy newborn calves face from birth up to 30 days of life, a period marked by organic adaptations to the environment and immunological immaturity.

RESULTS AND DISCUSSIONS

Newborn calves are very susceptible to lethal digestive, respiratory infections in the first days of life and also environmental stress might be a reason of newborn calve's pathology (Godfrey et al., 1991). Cattle management systems often dictate that calving occur at a time of the year when cold, fluctuating temperatures and increased precipitation are prevalent, usually in spring. Several researchers have observed that exposure to cold, wet weather has been involved in augmenting the problems observed in Weak Calf Syndrome (WCS) (Bull et al., 1978; Olson et al., 1980; Kvasnicka, 1982). During the fetal to neonatal transition, the newborn calf goes through severe thermolysis that is aggravated by the evaporation of fetal fluids and severe weather conditions. Maintaining homeothermy during the neonatal period requires a strong and sustained thermogenic response by the newborn calf. In regard to the environment, the greatest adaptation efforts are required for thermoregulation, as the calf transitions from 38.8°C in utero to below 20°C in the shelter (Kirovski, 2015). It is accepted that this thermogenic response is derived from both shivering thermogenesis in muscle tissue and nonshivering thermogenesis in brown adipose tissue (BAT) and it is critical that newborn calves possess functional BAT during the neonatal period (Carstens, 1994).

During the first few weeks of life, calves are functionally monogastric and milk is the primary source of nutrition. Upon drinking milk, the oesophageal (reticular) groove (sulcus reticuli) is activated and the milk is shunted past the forestomachs to the abomasum (Sjaastad et al., 2010). A number of factors trigger this oesophageal reflex: sucking behaviour, warm milk, the position of the calf's head while drinking and familiarity with the feeding method (Abe et al., 1979). For newborn and young calves, milk passage through the forestomachs is usually not problematic. The rumen, along with the reticulum and omasum, is not yet developed and empties into the abomasum within hours (Lateur-Rowet, 1983). The abomasum is able to gradually extend and accommodate different quantities of colostrum/milk (2-6.8 liters) without changes in the calves behaviour, without any indicative of abdominal pain (Ellingsen et al., 2015).

It is strongly recommended that calves be clinically examined immediately after birth in order to monitor cardio-respiratory function (heart rate, respiratory rate), evaluate the metabolic pathways (rectal temperature) and correct the irregularities (Uystepruyst et al., 2002).

Feeding also has a key role. It is essential that the newborn calves receive an adequate supply of colostrum as soon as possible, as both the concentration of immunoglobulins and permeability of the gut decrease rapidly over the first 24 hours following parturition (Weaver et al., 2000; Moore et al., 2005). Colostrum, the first milk neonates receive after birth, is rich in nutrient and non-nutrient biologically active factors. Colostrum feeding has an impact on postnatal development and possibly glucose homeostasis in several species (Koldovský, 1989, 1994; Kelly, 1994; Burrin et al., 1995; Savino et al., 2011).

The formation of colostrum in the cow's udder starts from the first day after calving. Its composition is similar to that of blood and differs significantly from milk (McGrath et al., 2015). Colostrum contains both nutrients

(proteins, fats, lactose, essential fatty acids and amino acids) and non-nutrients (biologically active substances). This is the first food the calves ingest after parturition that provides them with all necessary nutrients. Also, colostrum is particularly important for the passive immunization of the newborn, through the combination of various specific (immunoglobulins) and non-specific (humoral and cellular) antibacterial factors that pass to the offspring and protects them against infection during the first days after birth (Tomov, 1984; Medvezki, 1989; Iliev and Tomov, 1992; Blum and Hammon, 2000; Playford et al., 2000).

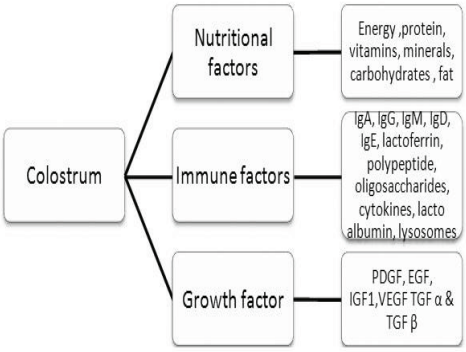

Figure 1. Composition of colostrum (Godhia, 2013)

Most nonnutritional components of colostrum are accumulated in the mammary gland during the prepartum period, so alimentation of the the mother in the dry-off period plays a key-role in the colostration period. Given the importance of physiological changes related to the critical period of adaptation of the newborn in the environment and high morbidity rate, collecting blood samples from calves could be a loial indicator of the health status of the animals (Silva et al., 2016; Windeyer et al., 2014).

A number of hormones are detected in colostrum and milk. Their concentrations in the first colostrum are many times higher than in milk (Table 1; Levieux, 1999).

The neonatal period of calves is characterized by an increase of disease susceptibility, especially diarrhea, umbilical cord inflame-mation and bronchopneumonia due to the immunological immaturity and a-gamma-globulinemic status of newborns at birth (Fontes Novo et al., 2015).

Table 1. Contents of some hormones in colostrum and milk (Levieux, 1999)

Hormone	Concentration
Insulin	colostrum: 4.2-34.4 ng/mL milk: 0.042-0.34 ng/mL
Total cortisol	colostrum: 4.4 ng/mL milk: 0.35 ng/mL
Free cortisol	colostrum: 1.8 ng/mL milk: 0.3 ng/mL
Prolactin	colostrum: 150 ng/mL milk: 50 ng/mL
Progesterone	colostrum: 2.6 ng/mL milk: 0.8 ng/mL

During this period, they are dependent on the maternal immunity transference by colostrum intake (Chase et al., 2008).

In newborn calves with diarrhea, the intestinal losses of bicarbonate and the formation of L-lactate by anaerobic glycolysis as a result of tissue hypoperfusion have been considered for a long time to be the main causes of metabolic acidosis. However, since Grude et al. (1999) first reported high serum concentrations of D-lactate in calves with neonatal diarrhoea that did not have abnormal ruminal contents, further evidence has been obtained that hyper-D-lactataemia frequently occurs in diarrhoeic calves (Omole et al., 2001; Lorenz, 2002). In human beings, substantial amounts of D-lactic acid are produced after the resection of large portions of the small intestines, when undigested carbohydrates are transported into the large intestine. The similarity of the clinical symptoms of the so-called short-bowel syndrome (ataxia, loss of memory, disorientation, headaches, slurred speech and alterations in consciousness up to coma) described by Uribarri et al. (1998) to the clinical signs observed in diarrheic calves with metabolic acidosis led to the assumption that these signs are influenced more by the concentration of D-lactate than by the degree of acidosis. In a study of calves with naturally acquired diarrhea, Lorenz (2004) has shown that changes in behaviour, and particularly in posture, can be better explained by an increase in serum D-lactate concentration than by the decreased base excess. The disturbance of the palpebral reflex is due almost completely to high levels of D-lactate (Lorenz, 2005). In that study all the calves had base excess values less than-10 mmol/litre and the study aimed to

investigate whether the clinical signs could be induced by hyper-D-lactataemia in the absence of acidosis. Previous studies consulted of the metabolism of D-lactate in ruminants have used adult animals (Stangassinger, 1977), and their additional objective was to investigate the ability of young calves to eliminate D-lactate from the blood (Lorenz et al., 2005).

Rupture of the umbilical cord that occurs during calving is characterized by hypoxia, responsible for the decreased blood oxygenation and increased blood concentrations of carbon dioxide, a substance that stimulates the gasping reflex, responsible for high lung compliance and establishment of final lung air volume. Increased oxygen tension in the blood and increased peripheral vascular resistance initiate closure of the *ductus arteriosus*, *foramen ovale* and *ductus venosus*, also preparing the neonatal cardio-vascular system for extra-uterine life (Nagy D.W., 2009). Umbilical cord inflammation is the most common affection in newborn calves, due to the environmental factors. Even if it is well treated with antibiotics, sometimes it could be also dangerous or fatal for the newborn calf (Kasari, 1994; Quigley and Drewry, 1998).

Respiratory disease in new born calves is a constant challenge for dairy replacement heifer rearing systems, and is responsible for 21.3% of mortality in pre-weaned calves and 50.4% of deaths in weaned heifers (Poulsen and McGuirk, 2009). Pneumonia known as shipping fever or Bovine Respiratory Disease (BRD), is the second most common cause of calf death. Calves that develop pneumonia before weaning are subjected to the same risk factors as those that become diarrheic: failure or incomplete passive transfer of immunity from colostrum, exposure to adult cattle, and/or the deficient ventilation in warm housing. The three most important infectious agents causing pneumonia in young calves are: *Pasteurella haemolytica, Pasteurella multocida* and *Mycoplasma dispar*, which can act individually or grouped (McGuirk, 2008). Calves have very small lungs compared to their body size so any episode of pneumonia causes a certain degree of permanent lung damage, making it difficult for the calf to thrive (Kasari, 1994). Calves most commonly get affected by pneumonia after a period of stress, such as weaning, dehorning, castrating, or transportation. Early detection of the disease is challenging. In the newborn calf, mucous membrane color, thoracic auscultation character and frequency of the respiratory effort and ability to oxygenate are critical elements of the clinical examination in order to determine whether or not respiratory disease is present (Poulsen and McGuirk, 2009). According to those mentioned before, it is desirable to take all the necessary measures as soon as possible to treat this episodes of disease, due to its very expensive cost of treatment and to decreased weight gain of the calves (Amir et al., 2013).

CONCLUSIONS

The neonatal period is the most critical phase in the dairy farming system, due to high morbidity rates (from 10.5 to 21.6%) and a mortality of 3.5%. Most issues are due to the inability of the newborn to adapt to the extra-uterine environment and take over the vital functions which were previously performed by the mother, such as thermoregulation, basic acid balance, cardiorespiratory functions, nutrition and development of the immune system.

Given that this is the most sensitive and fragile period of adaptation of the newborn to the environment, it is essential to possess knowledge of the physiology of the calf and mother in order to monitor the health and well-being of the calf. Thus, it is hypothesized that monitoring the physiological parameters and adaptations of newborn calves during the first four weeks of life, as well as the management conditions used are critical to the development of healthy stock and the farmers' productivity and income.

REFERENCES

Abe M.., K. Kondoh, H. Shibui, 1979. Effects of nipple or bucket feeding of milk substitutes on rumen by-pass and on rate of passage in calves, Br. J. Nutr., 41, 175–181.

Amir Parviz Rezae Saber, Babak Farkhad Hoshyar, Asghar Ajdari, Hamed Manafi, Amir Reza Yousefi, Behnam Ali Asgharzade, Amir Dabagh, Javad Kkhanzade, 2013. Control of clinical pneumonia in calves by antibiotic therapy, European Journal of Experimental Biology, 3(4), 61-65.

Blum J.W., Hammon H., 2000. Colostrum effects on gastrointestinal tract, and on nutritional, endocrine

and metabolic parameters in neonatal calves. Livestock Production Science, 66, 1151−1159.
Borne J.J.G.C.van den, Alferink S.J.J., Gerrits W.J.J., 2004. Identifying ruminal drinking by measurement of respiratory quotient and methane production in preruminant calves. J. Anim. Sci., 82(S1), 365 (W209).
Bull R.C., Olson D.P., Stoszek M.J., Kvasnicka W.G., 1982. Cooperative project on the weakcalf syndrome, Beef Res. Prog. Rep. No. 1, 43-44. R. L. Hruska US. Meat Anim. Res. Center ARM-NC21.
Burrin D.G., Davis T.A., Ebner S., Schoknecht P.A., Fiorotto M.L., Reeds P.J., McAvoy S., 1995. Nutrient-independent and nutrient-dependent factors stimulate protein synthesis in colostrum- fed newborn pigs, Pediatr. Res., 37, 593–599.
Carstens G.E., 1994. Cold Thermoregulation in the Newborn Calf, Veterinary Clinics of North America: Food Animal Practice, Elsevier, PMID: 8199923.
Chase C.C.L., Hurley D.J., Reber A.J., 2008. Neonatal immune development in the calf and its impact on vaccine response, Veterinary Clinics of North America: Food Animal Practice, 24(1), 87-104.
Godfrey R.W., Smith S.D., Guthrie M.J., Stanko R.L., Neuendorff D.A., Randel R.D., 1991. Cold Stress in Newborn Calves. J. Anim. Sci., 69, 258-263.
Godhia M.L, Patel N., 2013. Colostrum - its Composition, Benefits as a Nutraceutical - A Review. Curr. Res. Nutr. Food Sci., 1(1), 37-47.
Grude T., Lorenz I., Rademacher G., Gentile A., Klee W., 1999. Levels of D- and L-lactate in rumen liquid, blood, and urine in calves with and without evidence of ruminal drinking. Proceedings of the 32nd Annual Conference of the American Association of Bovine Practitioners, Nashville, USA, September 23 to 25, 213-214.
Guilloteau P., Zabielski R., Blum J.W., 2009. Gastrointestinal tract and digestion in the young ruminant: ontogenesis, adaptations, consequences and manipulation. Journal of Physiology and Pharmacology, 60(3), 37-46.
Hammon H.M., Steinhoff-Wagner J., Flor J., Schönhusen U., Metges C.C., 2014. Role of colostrum and colostrum components on glucose metabolism in neonatal calves. Journal of Animal Science, 91(2), 685-695.
Hohenboken W., Jenkins T., Pollak J., Bullock D., Radakovich S., 2004. Genetic improvement of beef cattle adaptation in America, The National Beef Cattle Evaluation Consortium (NBCEC), Colorado State University, Georgia, http://www.nbcec.org/ producers/Beef_Cattle_Adaptation.pdf.
Iliev Y.I., Tomov T., 1992. Lysozyme as prophylactic and medicinal formulation in animal husbandry. Agricultural Science (Sofia), 30, 88–96 (BG).
Johnson K.F., Chancellor N., Burn C.C. et al., 2017. Prospective cohort study to assess rates of contagious disease in pre-weaned UK dairy heifers: management practices, passive transfer of immunity and associated calf health. Veterinary Record Open, 4:e000226. doi:10.1136/ vetreco-2017-000226.
Kasari T.R., 1994. Physiologic mechanisms of adaptation in the fetal calf at birth. Veterinary Clinics of North America: Food Animal Practice, 10, 127-136.
Kelly D., 1994. Colostrum, growth factors and intestinal development in pigs, Digestive physiology in pigs. European Federation of Animal Science (EAAP), Bad Doberan, Germany, 151–166.
Kirovski Danijela, 2015. Endocrine and metabolic adaptations of calves to extra-uterine life. Acta Veterinaria, Beograd, 65(3), 297-318, DOI: 10.1515/acve-2015-0025.
Koldovský O., 1989. Search for role of milk-borne biologically active peptides for the suckling. J. Nutr., 119, 1543–1551.
Koldovský O. 1994. Hormonally active peptides in human milk. Acta Paediatr., Suppl. 402, 89–93.
Ellingsen K., Mejdell Cecilie M., Ottesenb Nina, Larsen S., Grøndahl Ann Margaret, 2015. The effect of large milk meals on digestive physiology and behaviour in dairy calves. Physiology & Behavior, 154, 169–174.
Lateur-Rowet H.J.B., 1983. The failure of the oesophageal groove reflex, when fluids are given with an oesophageal feeder to newborn and young calves. Vet. Q., 5, 68–74.
Levieux D., 1999. Le colostrum, un lait particulièrement riche en de nombreux composants: peut-on en déceler la présence dans les livraisons de lait de vache? Le Lait, 79(5), 465−488.
Lorenz I., Gentile A., Klee W., 2005. Investigations of D-lactate metabolism and the clinical signs of D-lactataemia in calves. Veterinary Record, 156, 412-415.
Lorenz I., 2002. Untersuchungen zur Bedeutung der D-Laktatazidose bei Kälbern mit Neugeborenendurchfall. Proceedings of the 11 Jahrestagung der Fachgruppe 'Innere Medizin und Klinische Laboratoriums diagnostik', Munich, Germany, February 13 to 16, 19-20.
Lorenz I., 2004. Investigations on the influence of serum D-lactate levels on clinical signs in calves with metabolic acidosis. Veterinary Journal, 168**,** 323-327.
Marquart Fontes Novo Sylvia, Freitas Renata Leite, da Costa e Silva Cynthia Pereira, Baldacim Vinicius Alvim Passos, Baccili Camila Costa, dos Reis Juliana França, Hagiwara Mitika Kuribayashi, Gomes Viviani, 2015. Hematological adaptation in Holstein calves during the neonatal period. Braz. J. Vet. Res. Anim. Sci., São Paulo, 52(3), 212-216.
McGrath B.A., Fox P.F., McSweeney P.L.H., Kelly A.L., 2015. Composition and properties of bovine colostrum: a review. Dairy Sci. & Technol., 96, 133–158 DOI 10.1007/s13594-015-0258-x.
McGuirk S.M., 2008. Disease management of dairy calves and heifers. Veterinary Clinics of North America: Food Animal Practice, 24, 139–153.
Medvezki D., 1989. The Rearing of Calves, Zemizdat, Sofia.
Moberg G.P., 1999. When does stress become distress? Lab Animal, 28, 22-26.
Moore M., Tyler J.W., Chigerwe M., Dawes M.E., Middleton J.R., 2005. Effect of delayed colostrum collection on colostral IgG concentration in dairy cows. J. Am. Vet. Med. Assoc, 226, 1375–1377.

Nagy D.W., 2009. Resuscitation and critical care of neonatal calves. Veterinary Clinics of North America: Food Animal Practice, 25, 1-11.

Olson D.P., Papasion C.J., Ritter R.C., 1980. The effects of cold stress on neonatal calves. I. Clinical conditions and pathological lesions, Can. J. Comp. Med.

Omole O.O., Nappert G., Naylor J.M., Zello G.A., 2001. Both L- and D-lactate contribute to metabolic acidosis in diarrheic calves. Journal of Nutrition, 131, 2128-2131.

Penchev Georgiev I., 2008. Differences in chemical composition between cow colostrum and milk. Bulg. J. Vet. Med., 11(1), 3−12.

Playford R.J., Macdonald C.E., Johnson W.S., 2000. Colostrum and milkderived peptide growth factors for the treatment of gastrointestinal disorders. American Journal of Clinical Nutrition, 72, 5–14.

Poulsen Keith P., McGuirk Sheila M., 2009. Respiratory Disease of the Bovine Neonate. Vet. Clin. Food Anim., 25, 121–137, doi:10.1016/j.cvfa.2008.10

Quigley J.D., Drewry J.J., 1998. Pratical considerations of transition cow and calf management Nutrient and immunity transfer from cow to calf pre-and postcalving. Journal of Dairy Science, 81, 2779-2790.

Ross R.M., 1978. Maternal protein restriction nd cold stress during rearing on blood composition and pathologic lesions in neonatal calves. Roc. West. Sec. Am. Soc. Anim. Sci., 29, 339.

Sauter S.N., Roffler B., Philipona C., Morel C., Romé V., Guilloteau P., Blum J.W., Hammon H.M., 2004. Intestinal development in neonatal calves: effects of glucocorticoids and dependence of colostrum feeding. Biol. Neonate, 85(2), 94-101.

Savino F., Liguori S.A., Sorrenti M., Fissore M.F., Oggero R., 2011. Breast milk hormones and regulation of glucose homeostasis. Int. J. Pediatr., 803985.

Silva B.T., Henklein A., Marques R. De S., de Oliveira P.L., Leite S.B.P., Novo S.M.F., Baccili C.C., Reis J.F. & Gomes V., 2016. Vital parameters of Holstein calves from birth to weaning**.** Revista Brasileira de Medicina Veterinária, 38(3), 299- 304.

Sjaastad V., Hove K., Sand O., 2010. Physiology of Domestic Animals. 2nd ed. Scandinavian Veterinary Press, Oslo.

Swanson E.W., Harris J.D., 1958. Development of rumination in the young calf. J. Dairy Sci., 41, 1768–1776.

Tomov T.A., 1984. The Basis of Lactation in Ruminants. Zemizdat, Sofia.

Uribarri J., Carroll M.S., 1998. D-lactic acidosis - a review of clinical presentation, biochemical features, and pathophysiologic mechanisms. Medicine 77, 73-82.

Uystepruyst C., Coghe J., Dorts T., Harmegnies Natacha, Delsemme Marie-Hélène, Art Tatiana, Lekeux P., 2002. Sternal recumbency or suspension by the hind legs immediately after delivery improves respiratory and metabolic adaptation to extra uterine life in newborn calves delivered by caesarean section. Vet. Res., 33, 709–724, INRA, EDP Sciences, DOI: 10.1051/vetres:2002051

Weaver D.M., Tyler J.W., VanMetre D.C., Hostetler D.E., Barrington G.M., 2000. Passive transfer of colostral immunoglobulins in calves. J. Vet. Intern Med., 14, 569–577.

Windeyer M.C., Leslie K.E., Godden S.M., Hodgins D.C., Lissemore K.D., Leblanc S.J., 2014. Factors associated with morbidity, mortality, and growth of dairy heifer calves up to 3 months of age. Preventive Veterinary Medicine, 113, 231-240.

NUTRITION

Scientific Papers. Series D. Animal Science. Vol. LXI, Number 1, 2018
ISSN 2285-5750; ISSN CD-ROM 2285-5769; ISSN Online 2393-2260; ISSN-L 2285-5750

UTILIZATION OF COMPLETE DIET CONTAINING SUGARCANE PEELS MEAL USED IN FEEDING GROWING KANO BROWN GOATS

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Abstract

This study was conducted for the nutritional evaluations of sugarcane peels as the replacement for maize offal in the diet of growing Kano brown goats for ninety days. Four different diets were formulated containing sugarcane peels to replace maize offal at 0, 25, 50 and 75% coded as A, B, C and D respectively, were compared. Twenty (20) growing Kano brown goats were used for the study. The goats were allocated to each treatment and used to evaluate the effect of feeding sugarcane peels diets on performance, apparent digestibility and economics of production. Results of the performance shows the average initial body weight and average final body weight were not significantly affected (P>0.05). Total feed cost per kg and total variable cost of production reduced as the inclusion levels of sugarcane peels increased from 0 – 75% as replacement level for maize offal without any effect on the performance of goats. Therefore, this study recommends that sugarcane peels meal can be incorporated into the diets of goats up to 75% as replacement levels without affecting the performance and nutrients digestibility.

Key words*: evolution, sugarcane peels, maize offal, Kano brown goats.*

INTRODUCTION

Ruminants are essential to the livelihoods of millions of farmers and critical to human health, global food and nutritional security. (FAO, 2016). In Nigeria, livestock contributes 6-8% of agricultural gross domestic product. These estimates highlight the important contribution of livestock to sustainable agricultural development. Goats play multiple roles in the livelihoods of people in Nigeria, especially the poor. They provide food and nutrition, economic and social status, and ensure environmental sustainability by providing manure to the soil. Nigeria estimates 72.5 million goats' population and has the largest goats' population in Africa (Premium Times News Paper, 2017). The breeds of Nigerian goats are Sokoto red, Sahel or Desert Goat, and West African Dwarf Goat (WAD). The Kano brown is believed to be strains of the Sokoto red goat. Saleh (2017) indicated that feeds constitute about 80% of the cost of livestock production in Nigeria and the feed is generally inadequate to meet growth and production requirements. This problem is more critical during the dry season period and affects all categories of livestock. For ruminant animals there are inadequate availability of conventional grazing forages and very expensive industrial by-products such as wheat offal, maize offal and cotton seed cake. In this situation the search for alternative feed ingredient which is cheaper and easier to obtain as well as easier to process becomes of paramount importance. The use of sugarcane peels as feedstuff during the dry season will help in reducing the problem of feed shortage especially in the Northern part of the country where the sugarcane peels are available and unutilized at the period.

Sugarcane *Saccharum officinarum* by-product is one of such usable crop residues as ruminant feeds because of its nutritional components. Sugarcane peels is one of by-products of sugarcane and found in Nigeria as a result of local consumption of sugarcane which consist of sugarcane roots, wax, tips, leaves, fibrous materials, parenchyma cells and soil particles after sugarcane was peeled using sharp knife. Ayoade et al. (2007) revealed that, on dry matter basis sugarcane peels contained dry matter (DM) 87.6%, crude protein 6.5%, crude fibre 12.7%, ether extract 2.8%, ash 12.8% and

NFE 77.1%. However, Ochepo et al. (2012) reported the chemical composition of sugarcane peels as CP 6.56%, CF 15.22%, EE 4.79%, ash 7.31% and NFE 66.12%. The author also reported that sugarcane peels can be fed to goats at 40% dietary inclusion without significantly affecting the performance.

MATERIALS AND METHODS

Experimental location

Feeding trial was conducted at the Federal College of Education (Technical) Bichi, Department of Agricultural Education, Teaching and Research Farm, about 40 km west of Kano city in Bichi Local Government Area of Kano State. Kano is located within longitude 8°31'0.2"E and latitude 12°0'0.4"N and 13°N in the semi-arid zone of North-western Nigeria (KNARDA, 2001).

Experimental animals and their management

Twenty (20) growing Kano brown goat bucks used in the experiment and were purchased from Bichi market, Kano State. The animals were randomly distributed to 4 groups of 5 animals each. Each animal goat served as a replicate and an adjustment period of a week was allowed for the animals before data collection commences. The feeding trial lasted for a period of 90 days; 21 days used for metabolism study. Water and salt lick were also offered *ad libitum.* The animals were quarantine in the College Farm, for two weeks, vaccinated with *PPR* vaccine and given prophylactic treatment with Avomec® against *endo* and *exto* parasites and also treated with oxytetracycline HCl (a broad spectrum antibiotic). Prior to the experiment, the animals were managed intensively and group-fed with groundnut haulm and wheat offal.

Experimental feed preparation

The principal ingredient for the experimental feeds is sugarcane peels which was collected from the selling points within the Bichi local government area of Kano State. The peels were sun dried on a floor for a period of 3 - 4 days depending on sunlight intensity and finally milled with a hammer mill to produce sugarcane peels meals. Other feed ingredients include the following: maize offal, rice offal, groundnut haulm, cotton seed cake, bone meal and salt which were purchased from Kano and Bichi markets.

Four complete experimental diet were formulated to feed twenty (20) growing bucks using varying levels of sugarcane peels to replace maize offal at 0 (control), 25, 50, and 75% inclusion levels as presented (Table 1 and Table 2 present compositions and proximate analysis of sugarcane peel meals based diets fed to growing Kano brown goats). The diets were designated as diets A, B, C, and D representing experimental treatments.

Table 1. Composition of the sugarcane peels meal based diets

	Experimental treatments			
Ingredients (%)	**A**	**B**	**C**	**D**
SPM	0	8.75	17.5	26.25
Maize offal	35	26.25	17.5	8.75
Rice offal	20	20	20	20
Cotton seed cake	20	20	20	20
Ground nut haulm	20	20	20	20
Salt	2	2	2	2
Bone meal	3	3	3	3
Total	**100**	**100**	**100**	**100**
Calculated values				
CP (%)	17.11	16.71	16.29	15.89
CF (%)	16.19	17.37	18.56	19.76
ME (Kcal/Kg)	2,120	2,330	2,540	2,750

SPM = Sugarcane Peels Meal, CP = Crude Protein, CF = Crude Fibre, ME = Metabolisable Energy

Experimental design and statistical analysis

Completely randomized design was used. The data generated were subjected to analysis of variance (ANOVA) using General Linear Model in SAS (2000). Where differences in means manifest, the Fisher's least significance difference test (FLSD) was used to separate them at ($P<0.05$) level of probability.

Data collected

Daily feed intake was kept for the whole 90 days feeding trial.

Metabolism trial

At the end of the feeding trial, metabolism study was conducted using three (3) animals from each treatment. The animals were fed the same experimental diets used for the feeding

trial. The trial lasted for twenty one (21) days (14 days for adaptation and 7 days for collection of faeces). Daily feed intakes were kept. Harness bags were used to collect the faecal output. Total faecal output from each animal was recorded daily and 5% of it was oven-dried at 80^{o}C for dry matter determination and proximate components determination.

Sampling and analytical procedure

Thoroughly mixed representative samples of the experimental diets, and faeces were analyzed for proximate composition as outlined by the Association of Official Analytical Chemist (AOAC, 1990). Acid Detergent Fibre (ADF) was analysed in the samples as reported by Ranjhan and Krishna (1980).

RESULTS AND DISCUSSIONS

Proximate analysis of sugarcane peel meals based diets fed to growing Kano brown goats

The proximate composition of sugarcane peels meal based diets is presented in Table 2. The values of CP in this study (16.96 - 18.87%) are within the value reported by NRC (2001).

Table 2. Proximate analysis of sugarcane peels meal based diets

Para-meters %	Experimental diets				LSD
	A (0)	B (25)	C (50)	D (75)	
DM	94.87^{a}	94.74^{a}	93.94^{b}	93.35^{b}	0.440
CP	18.87	18.86	18.86	16.96	2.19
CF	9.97^{c}	10.60^{c}	12.56^{b}	14.97^{a}	1.099
EE	6.21^{d}	8.42^{c}	9..20^{b}	9.61^{a}	0.372
Ash	5.99^{d}	6.97^{b}	6.33^{c}	7.49^{a}	0.129
NFE	58.978^{a}	55.34^{b}	53.06^{c}	51.00^{d}	41.74

a, b, c, d = Means in the same row with different superscripts are significantly different (P<0.05) and LSD = Least Significant Difference, DM = Dry matter, CP = Crude Protein, CF = Crude fibre, EE = Ether Extract.

Effect of feeding sugarcane peels meal based diets on performance of growing Kano brown goats.

The effect of feeding sugarcane peels meal based diets on performance of growing Kano brown goats is presented in Table 3.

The average initial body weight and average final body weight were not significantly affected (P>0.05) by the inclusion of sugarcane peels, while average daily feed intake and feed conversion ratio (FCR) were affected (P<0.05). Also affected (P<0.05) were average daily weight gain, total body weight gain and average daily water intake. Average daily weight gain (ADWG) of 32.22 - 45.89 g/day reported in this study is lower than ADWG of 90.58 g/day reported by Bawala et al. (2008) when they replaced sugarcane tops (grass) with *Leucaena leucocephala* (legume) foliage to West African dwarf sheep and 94.9 g/day reported by Ramli et al. (2005) for goats fed fermented sugarcane bagasse feed.

Also the present values were higher than the values of 7.50 - 24.30 g/day reported by Ochepo et al. (2012) on the utilization of complete diet containing sugarcane peels by goats. The values for average daily feed intake (ADFI) 280.74 - 349.77 g/day is lower than 723.1 g/day reported by Saleh (2010) for 30% sugarcane peels diet fed to Yankasa sheep.

The variation in the above mentioned is that, feed intake could be as a result of individual differences in the feeding habits of the animals. Values of average daily water intake ranged from 665.59 ml to 838.55 ml/day in this study. These values are within 0.8520 - 1.257 liters/day reported by Ochepo et al. (2012) and 680 ml/day reported by Devendra and Mc Leroy (1982) for pen fed *Katjang* goat raised for meat production.

The values of feed to gain ratio (FGR) of 7.67 - 9.32 (intake/kg gain) reported by this study is lower than 11.12 - 28.36 reported by Ochepo et al. (2012) and 13.3 reported by Ramli et al. (2005) for goats fed fermented sugarcane bagasse feed. In this study, FGR was best obtained in treatment A (7.67) and C (7.98). The lower value of feed to gain ratio in treatments A and C in this study is an indication that the diets were better utilized by animals fed and that the animals on the diets had better ability to convert feed to meat.

Dry matter and nutrient digestibility of growing Kano brown goats fed sugarcane peels based diets

The dry matter and nutrient digestibility of growing Kano brown goats fed sugarcane peels based diets is presented in Table 4. The crude protein digestibility was significantly affected by the level of sugarcane peels inclusion (P<0.05), while the other digestibility

parameters were not significant (P>0.05). Values for dry matter digestibility which ranged from 69.32% (D) - 74.32% (A) as reported in this study were within the values of 68.85 - 78. 35 reported by Ashiru (2014) who fed Yankasa rams with sugarcane waste based diets. Values of crude protein digestibility (CPD) ranged from 86.09% (D) - 91.84% (A) in this study and were higher than the values of 80.63 - 86.76% reported by Ashiru (2014) for Yankasa rams fed sugarcane waste based diets.

The high CP digestibility values reported in this study indicates that dietary protein was highly utilized by animals. Another reason for efficient CPD in this study is that all the experimental diets contained the recommended CP levels of 16 - 18% recommended by NRC (2001).

Values for crude fibre digestibility (CFD) ranged from 57.3%2 (B) - 61.77% (D) reported in this study were lower than 24.2 - 54.21% reported by Ochepo et al. (2012). McDonald et al. (1988) also indicated that fibre fraction of a food as well as the species of animal concerned have the greatest influence on digestibility. The high digestibility of all nutrients agreed with the report of FAO (1990) which classified digestibility of feeds as high (>60), medium (40 - 60) and low (<40). Therefore, the digestibility of all the nutrients was high and this indicates that, the quality of the feeds in all the treatments could be which for goat production.

Effect of feeding sugarcane peels based diets on economics of production of growing Kano brown goats

The effect of feeding sugarcane peels based diets on economics of production of growing Kano brown goats is presented in Table 5.

Feed cost per kg gain, gross margin and cost benefit ratio were highly significant (P<P0.05) and so also the rest of their parameters. Values for feed cost per kg ranged from ₦39.25 (D) - ₦57.10 (A) reported in this study and was within the range of ₦21.43 - ₦43.19 reported by Saleh (2010) for sugarcane peels based diets fed to Yankasa sheep.

Thus, the control diet (0% sugarcane peels) was more expensive in terms of cost when compared to others and this shows that sugarcane peels based diets were less expensive hence of reduce in cost of production.

The feed cost per kg gain ranged from ₦323.42 (D) - ₦403.12 (A); thus goat was higher than ₦112.74 - ₦274.58 reported by Saleh (2010) for sugarcane peels based diets fed to Yankasa sheep. This indicates that there was a reduction of cost per kg gain as the levels of sugarcane peels is increased in all the treatments. The total feed cost values obtained from this study ranged from ₦1133.02 (D) - ₦1797.51 (A) and total variable cost of production ranged from ₦5317 (D) - ₦5990 (A). This is an indication that incorporating sugarcane peels in the diet of goats will yield more profit since the treatment D which has the highest level of sugarcane peels has the least cost of production.

Gross margin ranged from ₦2573.7 (A) - ₦3533.4 (D) and also indicates that more profit was obtained from each diet as sugarcane peels increased. The cost benefit ratio ranged from 1.43 (A) - 1.66 (D) and showed decrease in cost (₦/kg) of feed production as inclusion levels of sugarcane peels increased from 0 - 75% as replacement level of maize offal. Therefore more benefit can be generated by goats' farmers with increased level of sugarcane peels. This trend was in confirms with the reports of Adesoji (2012) on the study of sugarcane peels in diet of growing rabbits *(Oryctologus cuniculus)* and Ochepo et al. (2012) on the utilization of complete diet containing sugarcane peels by goats.

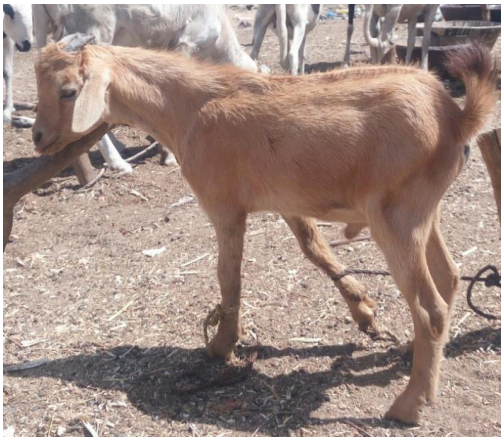

Figure 1. A typical Kano brown goat buck

Table 3. Effect of feeding sugarcane peels meal based diets on performance of growing Kano brown goats

Parameters	Experimental treatments				LSD
	A	B	C	D	
Average initial Body Weight (kg)	w	8.75	8.37	8.21	3.20
Average final Body Weight (kg)	12.33	11.65	12.2	11.38	3.02
Total Body Weight Gain (Kg)	4.13^{a}	2.9^{b}	3.83^{a}	3.17^{a}	36.51
Average Daily Weight Gain (g/day)	45.89^{a}	32.22^{c}	42.56^{ab}	35.22^{b}	8.21
Average Daily Feed Intake (g/day)	349.77^{a}	280.74^{b}	338.19^{b}	310.94^{c}	79.14
Average Daily Water Intake (ml)	838.55^{a}	782.42^{c}	752.09^{ab}	665.59^{b}	8.21
Feed Conversion Ratio (kg intake/kg gains)	7.67^{b}	9.11^{a}	7.98^{b}	8.82^{a}	2.19

a, b, c, = Means on the same row with different superscripts are significantly different(P<0.05); LSD = Least Significant Difference.

Table 4. Dry matter and nutrient digestibility of growing Kano brown goats fed sugarcane peels meal based diets

Parameters %	Treatments diets				LSD
	A (0)	B (25)	C (50)	D (75)	
Dry matter (DM)	74.32	69.87	70.99	69.32	5.18
Crude Protein (CP)	91.84^{a}	87.88^{b}	90.11^{a}	86.09^{b}	2.12
Crude Fibre (CF)	61.34	57.32	60.71	61.77	7.36
Ether Extract (EE)	70.13	68.25	68.86	68.20	5.46

a, b =Means in the same row with different super scripts are significantly different (P<0.05); LSD = Least Significant Difference.

Table 5. Effect of feeding sugarcane peels meal based diets on economics of production of growing Kano brown goats

Parameters	Experimental diets				LSD
	A	B	C	D	
Feed Cost per kg (₦ /Kg)	57.10^{a}	51.15^{b}	45.20^{c}	39.25^{d}	N/A
Feed Cost per kg Gain (₦ /Kg)	403.12^{b}	527.02^{a}	358.74^{b}	323.42^{b}	99.71
Total Feed Cost (₦)	1797.51^{a}	1292.39^{bc}	1375.59^{b}	1133.02^{c}	165.44
Total Variable Cost of Production (₦)	5990^{a}	5485^{c}	5568^{b}	5317^{d}	N/A
Gross Margin (₦)	2573.7^{b}	2681.8^{ab}	2948.6^{ab}	3533.4^{a}	925.34
Cost Benefit Ratio	1.43^{b}	1.49^{b}	1.52^{ab}	1.66^{a}	0.162

a, b, c, d = Means in the same row with different super scripts are significantly different (P<0.05). LSD = Least Significant Difference. N/A = Not Analyzed.
**Feed cost/kg was calculated on the bases of prevailing market prices of ingredients as at December, 2017 ($1Dollar = ₦360.00).*

CONCLUSIONS

The results of the study clearly showed that inclusion levels of sugarcane peels from 0 - 75% as replacement for maize offal in goats' diet enhanced performance and improved nutrient digestibility without affecting the performance and health challenge to the animals. Therefore, sugarcane peels can be used as a feeds under smallholder farmers' condition, where accesses to conventional energy sources are limited. Based on this study, it can therefore be recommended that farmers should incorporate sugarcane peels at 75% inclusion level for small ruminants as replacement for maize offal during the period of scarcity to alleviated body weight losses usually experienced during the dry season. Moreover, the result of the experiment could be used to improve sugarcane production and utilizations.

REFERENCES

Adesoji A.T., 2012. Utilisation of Sugarcne Peels in diet of Growing Rabbits (*Oryctologus cuniculus*). Unpublished M.Sc. Dissertation, Department of Animal Science, Bayero University, Kano, Nigeria. 75.

AOAC, 1990. Official Method of Analysis (15th edition). Association of Official Analytical Chemists. Vol. 1 Arlington, Virginia, USA.

Ayoade J.A., Carew S.N., Ameh A.E., 2007. The feed value of sugarcane scrapping meal for weaner rabbits: Growth, meat yield and cost of production: Proceedings of the 3rd annual conference of the

Nigerian Society for animal production, University of Calabar, March 18-21, 2007, 227 – 299.

Bawala T.O., Aina A.B.J., Onwuka C.F.L, Oni A.O., Adebisi Y., Alli M.A., 2008. Studies on performance of sheep fed sugarcane *Saccharum officinarum* tops supplemented with varying level of *Leuceana leucocephala* Foliage: Proceedings of Animal Production, Ayetoro, Nigeria, 575 – 578.

Chesworth J., 2002. Ruminant Nutrition: The Agriculturalist: Published in co-operation with the Technical Centre for Agriculture and Rural Co-operation, Wageningen, The Netherlands.

Devendra C., McLeroy G.B., 1982. Sheep and Goats Production in the Tropics, Longman Scientific Group Ltd. London.

FAO, 2016. Food and Agricultural Organization: Reducing enteric methane for improving food security and livelihoods. Rome, Italy, www.fao.org.

FAO, 1990. Upgrading crop residue for effective utilization by ruminant livestock Food and Agricultural Organization. Rome, Italy In: 46 1 APH202. Pdf. Htm.

McDonald P., Edward R.A., Greenhalgh J.F.D., Morgan C.A., 1998. Animal Nutrition 5th Edition, Longman, London.

NRC (National Research Council), 2001. Nutrient requirements of dairy cattle. 7th revised edition. National Academy Press, Washington, D.C.

Ochepo C.O., Ochepo G.O., Ayoade J.A., 2012. The utilization of complete diet containing sugarcane peels by goats. Proceedings of the 37th annual conference of the Nigerian Society for animal production, University of Agriculture, Makurdi, March 18-21, 507 – 511.

Premium Times News Paper, 2017. Premium Times News Paper, Thursday, May11, 2017, Lagos, www.premiumtimesng.com.

Ramli M.N., Higashi M., Imura Y., Takayama K., Nakanishi Y., 2005. Growth, feed efficiency, behaviour, carcass characteristics and meat quality of goats fed fermented Bagasse Feed. Asian-Aust. J. Anim. Sci., 18 (11), 1594-1599.

Ranjhan S.K., Krishna G., 1980. Laboratory Manual for Nutrition Research. Vicks Publishing House PVT, New Delhi.

Saleh G., 2010. Utilisation of Sugarcane Peels By Yankasa Lambs in Savannah Zone. Unpublished M.Sc. Dissertation, Department of Animal Science, Bayero University, Kano.

Saleh G., 2017. Nutritional Evaluation of Sugarcane Peels for Feeding Goats. Duetschland/Germany, LAMBERT Academic Publishing, ist ein imprint der / is a trade mark of Omni Sciptum GmbH & Co. KG

SAS Institute Inc., 2000. Statistical Analysis System SAS/STAT. Guide for Personal Computers, Version 6th edition, Cary N.C, USA, 967-978.

Scientific Papers. Series D. Animal Science. Vol. LXI, Number 1, 2018
ISSN 2285-5750; ISSN CD-ROM 2285-5769; ISSN Online 2393-2260; ISSN-L 2285-5750

PATH ANALYSIS FOR DETERMINATION OF RELATIONSHIPS BETWEEN SOME CARCASS PARTS AND CARCASS WEIGHT OF ROSS 308 BROILERS

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Abstract

Direct, indirect and total effects of some carcass parts on carcass weight of Ross 308 broilers were investigated by using path analysis in this study. Feather-sexed 1day old chicks were fed for 42 days. At the end of the feeding period, data of carcass weight (CW), breast weight (BW), thigh weight (TW) and wing weight (WW) were obtained from totally 36 birds (18female-18male), slaughtered at 42 days old. The results indicated that BW, TW and WW had statistically important effects on CW of the broilers. Total and direct effects of TW and BW were higher than the effect of WW. However the indirect effect of WW on CW was higher than the effect of BW. Based on the data, it can be concluded that breast and thigh weights have a high correlation with carcass weight but wing weight also can be used for predicting total carcass weight due to the indirect effect on CW.

Key words*: broiler, carcass parts, path analysis.*

INTRODUCTION

Main purpose of the broiler industry is to obtain carcasses having high quality and weight. Carcass can be defined as the part without head, internal organs, feather and feet (Atasoy and Aksoy, 2005; Anonymous, 1987). The breast muscle is the most valuable part of the carcass. Reducing abdominal fat and increasing the proportional weight of the breast muscle is the way of improving the profitability of broiler production (Bihan-Duval et al., 1999). Breast has received more attention in lots of studies. Thigh muscles followed the breast muscle. A few studies have interested other carcass parts, such as wings, shank and head (Ikeobi et al., 2004; Park et al., 2006; Gao et al., 2009).
Direct, indirect and total effects of some carcass parts on carcass weight of Ross 308 broilers were investigated by using path analysis in this study.

MATERIALS AND METHODS

All feeding and husbandry conditions were same with the advices of company, producing the genotypes. The diet was a typical corn-soybean based, which was formulated to meet all nutrient recommendations in the Ross rearing guidelines (Aviagen, 2007). Chicks were vaccinated against infectious bursal disease and Newcastle disease via drinking water at 9 and 13 days old, respectively. At day 42, 18 male and 18 female birds, representing the average body weight of the herd were selected and slaughtered to obtain the carcass parts.
Path analysis was used to explore the direct, indirect and total effects of some carcass parts on carcass weight.

RESULTS AND DISCUSSIONS

Descriptive statistics of data (carcass weight, thigh weight, breast weight and wing weight) were presented in Table 1.
Correlations, expressing the relationship between the carcass parts and carcass weight were presented in Table 2. All correlations between carcass parts and carcass weight were determined to be positive and significant ($P<0.01$) except the correlation between breast and wing.
The results of regression analysis, in which standardized regression coefficients, standard error, t, statistical significant levels, tolerance

and VIF values were presented to explain the relationships between the carcass weight and carcass parts in Table 3.

Table 1. Descriptive statistics for explored traits of the broilers

Traits	n	Mean ± SD	Min	Max	CV %
CW	36	2111.4± 125.9	1901.0	2396.0	5.96
TW	36	571.4 ± 47.9	436.0	652.0	8.38
BW	36	796.4 ± 56.3	682.0	889.0	7.07
WW	36	209.4 ± 14.2	188.0	243.0	6.77

SD: Standard deviation, CV: Coefficient of variation, n: Sample size

Table 2. Correlations for some carcass parts and carcass weight

	CW	TW	BW
TW	0.833**		
BW	0.784**	0.545**	
WW	0.555**	0.533**	0.202

**P<0.01, CW: Carcass weight, TW: Thigh weight, BW: Breast weight, WW: Wing weight

It is obvious that thigh weight had the largest effect on carcass weight, but wing weight had the least contribution to carcass weight. Preliminary analysis detected that the VIF values were smaller than 10 and the tolerance values was greater than 0.1 in all cases as stated in Table 3.

Path coefficients of the explanatory variables of Ross 308 broilers were presented in Table 4. Direct effect of breast weight was positive and higher than other traits. Moreover, direct effect of breast (0.497) on carcass weight was higher than total indirect of thigh weight. However, it appears that indirect effect of wing weight (0.338) was higher than indirect effect of breast. Total indirect effect of wing weight on carcass mainly arose from the effect to the thigh weight.

Table 3. Results of the regression analysis

	Traits		
	TW	BW	WW
Coefficient (b)	1.173	1.111	1.928
Std. error	0.222	0.164	0.644
t values	5.275	6.795	2.996
P values	<0.001	<0.001	0.005
Tolerance	0.516	0.692	0.704
VIF values	1.937	1.445	1.420

Carcass weight, TW: Thigh weight, BW: Breast weight, WW: Wing weight

Carcass weight is the most important economic trait for broiler production. So, which part of carcass is more effective on the carcass weight should be determined. Owing to this purpose, the path analysis is very important for determining factors effecting carcass weight (Çankaya and Abacı, 2012). Direct effects are very essential for predicting the carcass weight but indirect effects of explanatory variables on response variable should be considered beside the direct effects (Arı and Önder, 2013).

CONCLUSIONS

The results indicated that BW, TW and WW had statistically important effects on CW of the broilers. Total and direct effects of TW and BW were higher than the effect of WW. However the indirect effect of WW on CW was higher than the effect of BW. Based on the data, it can be concluded that breast and thigh weights have a high correlation with carcass weight but wing weight also can be used for predicting total carcass weight due to the indirect effect on CW.

Table 4. Direct and indirect effects of some carcass parts on carcass weight of Ross 308 broilers

Trait	Correlation coefficient with CW	Direct effect	Indirect effect			
			TW	BW	WW	Total
TW	0.833**	0.446	-	0.271	0.116	0.387
BW	0.784**	0.497	0.243	-	0.044	0.287
WW	0.555**	0.217	0.238	0.100	-	0.338

CW: Carcass weight, TW: Thigh weight, BW: Breast weight, WW: Wing weight, **P<0.01

REFERENCES

Anonim, 1987. Türk Standartları Enstitüsü (TSE) Yayınları. Tavuk Gövde Eti (Karkas) Parçalama Kuralları. Necatibey Cad. 112.- Bakanlıklar- Ankara.

Arı A, Önder H., 2013. Regression models used for different data structures. Anadolu J Agr Sci, 28 (3), 168-174.

Atasoy F., Aksoy T., 2005. Bir broiler sürüsünde cinsiyete göre ayrı büyütmenin ve erken dönemde yem kısıtlamasının karkas ve değerli parçalar üzerine etkisi. 53-56, Ankara Üniv Vet Fak Derg.

Aviagen, 2007. Broiler nutrition specifications. Aviagen Inc., Newbridge, Scotland, U.K.

Çankaya S., Abacı S.H., 2012. Path analysis for determination of relationships between some body measurements and live weight of German fawn x Hair crossbred kids. Kafkas Univ Vet Fak Derg, 18, 769-773.

Le E., Bihan-Duval Millet N., Remignon H., 1999. Broiler Meat Quality: Effect of Selection for Increased Carcass Quality and Estimates of Genetic Parameter. Poult Sci, 78, 822–826.

Gao Y., Du Z.Q., Wei W.H., Yu X.J., Deng X.M., Feng C.G., Fei J., Feng J.D., Li N., Hu X.X., 2009. Mapping quantitative trait loci regulating chicken body composition traits. Animal Genetics, 40, 952–954.

Ikeobi C.O.N., Woolliams J.A., Morrice D.R., Law A., Windsor D., Burt D.W., Hocking P.M., 2004. Quantitative trait loci for meat yield and muscle distribution in a broiler layer cross. Livestock Production Science, 87, 143–51.

Park H.B., Jacobsson L., Wahlberg P., Siegel P.B., Andersson L., 2006. QTL analysis of body composition and metabolic traits in an intercross between chicken lines divergently selected for growth. Physiological Genomics, 25, 216–23.

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Scientific Papers. Series D. Animal Science. Vol. LXI, Number 1, 2018
ISSN 2285-5750; ISSN CD-ROM 2285-5769; ISSN Online 2393-2260; ISSN-L 2285-5750

USAGE OF FOOD INDUSTRY BY PRODUCTS (FLAXSEED AND GRAPESEED MEAL) IN FATTENING PIGS' DIET

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Abstract

The 4 weeks study was conducted on 12 pigs divided in 2 groups (C, E). The average initial body weight for control group C was 66.42 kg±10.27 and 66.25 kg±9.88 for experimental group E, respectively. The C diet based on corn and soybean meal was characterized by: 17.46% crude protein and 3232 kcal/kg ME. Compared to diet C, the E diet had included 7.5% flaxseed meal and 1% grapeseed meal (17.60% CP, and 3200 kcal/kg ME). At the end of the experiment there were no significant differences (P≥0.05) among groups concerning the performances. All carcasses were assessed in E category, with no statistical differences between groups. Regarding meat quality, best results were noticed at E group for α-linolenic acid concentration (omega 3 fatty acid) of ham (1.29±0.04 g/100g total fatty acids) compare to C (0.51±0.01 g/100 g total fatty acids). Also α-linolenic acid concentration of tenderloin (1.12±0.03 g/100 g total fatty acids) in group E was higher compare to C group (0.6±0.06 g/100g total fatty acids), but without significant differences.

Key words*: pigs, flaxseed, grapeseed, carcass, meat, quality.*

INTRODUCTION

Flaxseed or Linseed (*Linum usitatissimmum*), represents one of the richest vegetarian source of α-linolenic acid (omega 3 fatty acid) and soluble mucilage, a novel high quality source of nutrition (Ganorkar and Jain, 2012).

The omega-3s and lignan phytoestrogens of flaxseed are in focus for their benefits for a wide range of health conditions and may possess chemo-protective properties in animals and humans, making flaxseed an important functional food ingredient (Singh et al., 2011). In general, the fatty acid profile of meat directly reflects the fatty acid profile in the pig diet (Eastwood, 2008). Also the content of insoluble fiber of flax helps improve laxation and prevent constipation, mainly by increasing fecal bulk and reducing bowel transit time (Greenwald et al., 2001) and water-soluble fiber helps in maintaining blood glucose levels and lowering the blood cholesterol levels (Kristensen et al., 2012).

Nowadays, consumers are interested in a higher content of PUFA by reason of healthier diet (in modern human diets, the ratio of n-6/n-3 PUFA is very high, 10- 15:1 and optimum is 4:1), while the increase in the PUFA brings producers complications in the durability of meat and fat (Warnants et al., 1999). Therefore, the dietary inclusion of a potentially antioxidant and low-cost ingredient such as grapeseed meal, concomitantly with flaxseed meal could improve the oxidative stability of omega-3 fatty acid. Grape skin and seed extracts exert strong free radical scavenging and chelating activities and inhibit lipid oxidation in various food and cell models *in vitro*. Also, grapeseed meal is seen as a potential source of protein (Fantozzi, 1981) improving protein digestibility significantly by removing the polyphenols.The objective of this study was to investigate the hypothesis that inclusion of flaxseed and grapeseed meal in fattening pigs' diet could enrich the Omega -3 fatty acids content of the pork meat in while improving the oxidative stability as well as obtaining the best carcass classification.

MATERIALS AND METHODS

The experimental protocol was approved by the Ethical Committee of the National Research-Development Institute for Animal Nutrition

and Biology, Balotesti, Romania (Ethical Committee no. 52/2014). The experiment was conducted on 12 crossbreed TOPIG hybrid [(Landrace × Large White)×(Duroc x Pietrain)] pigs with an average body weight of 68.92 kg±12.82, randomly assigned to two experimental groups (6 pigs per group) for a 30 days experimental period. The 9.80 m^2 concrete-floored pens were equipped with nipple drinkers. Feed and water were provided *ad libitum* during the experiment. The experiment was designed for two diets: a control diet based on corn, wheat, soybean meal and an experimental diet which included 7.5% of flaxseed meal and 1% grapeseed meal added to the basal diet (Table 1). The diets were formulated according to the requirements of TOPIGS guide management for fattening-finishing category pigs. The animals were cared for in accordance with the Romanian Law 43/2014 for handling and protection of animals used for experimental purposes and the EU Council Directive 98/58/EC concerning the protection of farmed animals.The animals were individually weighed at the start and at the end of the experiment. The main productive parameters: the average daily gain, average daily feed intake and feed conversion ratio were calculated per pen. The amount of feed given was weighed daily, as well as the leftovers (collected and weighed each morning). No veterinary interventions or pharmaceutical treatments were applied during the experiment. At the end of the experimental trial, pigs were slaughtered in an authorized slaughterhouse in according with EU legislation. The samples of ham and tenderloin (200 g weight) for laboratory analysis of fatty acid content were collected immediately after slaughtering, put in bags and frozen for further analyses.

Data on the lean percentage, muscle depth and back fat thickness were obtained from the slaughterhouse, where carcasses were classified according to EUROP, using an optical measurement system (device series A7509).

All the analyses concerning the meat samples, feed ingredients and compound feed were analyzed within the Laboratory of Chemistry and Animal Physiology of the National Research-Development Institute for Animal Nutrition and Biology, Balotesti.

Table 1. Formulation and chemical composition of compound feeds used for hybrid Topigs piglets

Ingredients (g/Kg as feed bases)	C	E
	(%)	
Corn	32.85	39.18
Flaxseed meal	-	**7.5**
Grapeseed meal	-	**1**
Rice bran	10	6.96
Wheat	30	23.07
Rapeseeed meal	12	12
Soybean meal	11.93	6.59
Monocalcium phosphate	0.82	0.9
Calcium carbonate	0.63	0.51
Salt	0.43	0.43
Methinonine	-	0.04
Lysine	0.26	0.49
Choline	0.08	0.08
Premix *	1.00	1.00
Biotronic SE^+	-	0.1
Biomin IMBO		0.15
Total	100	100
Calculated nutrients (g/kg feed)		
ME (Kcal/kg DM)	3232	3200
Dry matter (DMr)	88.21	90.28
Organic matter (OM)	83.03	84.90
Crude protein (g)	17.46	17.60
Crude fat (%)	3.07	4.30
Crude fibre (%)	5.18	6.48
Ash (%)	5.19	5.37
Non-protein nitrogen (or NPN)	57.31	56.52
Calcium (%)	0.80	0.80
Phosphorus (%)	0.65	0.65
Acid linolenic α C18:3n3	2.73	14.02

**1 kg premix content: 1500000 UI/g vit.A; 500000 UI/g vit.D3; 500 UI/kg vit.E; 200 mg/kg Vit.K; 200 mg/kg Vit.B1; 480 mg/kg Vit.B2; 1485 mg/kg Acid panthotenic; 2700 mg/kg acid nicotinic; 300 mg/kg Vitamin B6; 4 mg/kg vitamin B7; 100 mg/kg vitamin B9; 1.8 mg/kg vitamin B12; 2500 mg/kg vitamin C; 7190 mg/kg manganese; 6000 mg/kg iron; 600 mg/kg copper; 6000 mg/kg zinc; 50 mg/kg cobalt; 114 mg/kg iodine; 18 mg/kg selenium.*

The following determinations were performed: dry matter; crude protein; ether extractives; fatty acids; crude fiber; gross ash, expressed by 100 g dry matter. Samples of the studied products (flaxseed meal, grapeseed meal) and of the compound feeds were collected and assayed for the basic chemical composition: dry matter, crude protein, crude fibre and ash, using the chemical methods from Regulation (CE) nr. 152/2009 (Methods of sampling and analysis for the official inspection of feeds). The collected samples were prepared within the laboratory using standardized methods complying with ISO standards. The crude protein of the meat was determined using a semiautomatic classical Kjeldahl method using a Kjeltek auto 1030 – Tecator (SR ISO 973, 2007). The meat fat was extracted using an improved version of the classical method by continuous extraction in solvent, followed by fat measurement with Soxhlet after solvent removal (SR ISO 1444, 2008). The meat ash was determined by calcinations at 550°C (SR

ISO 936, 2009). The meat fatty acids composition was determined by gas chromatography. After lipid extraction from the samples, the fatty acids were transformed into methyl esters by transmethylation, and the components were separated in the capillary chromatograph column. The fatty acids were identified by comparison with blank chromatograms and were subsequently determined quantitatively as percent for 100 g fat. The analytical data were compared by variance analysis (ANOVA) using STATVIEW for Windows (SAS, version 6.0). The difference between the means was considered significant at $P<0.05$. The results were expressed as mean ± standard deviation.

RESULTS AND DISCUSSIONS

For the protein content value of flaxseed meal (Table 2), Panaite et al. (2016) reported 89.25% DM, 32.99% CP, 9.42% EE, 4.65% Ash and 11.99% CF and Petit (2003) reported 90.8% DM, 24% CP and 10.5% CF. Flax fibers include both soluble and insoluble dietary fibers.

Panaite et al. (2016) studied basic chemical composition of some Romanian by-products, including grapeseed meal, reporting 89.16% DM, 11.91% CP, 2.93% Ash, and 35.68% CF while Olteanu et al. (2017) reported for the same by-product: 88.44% DM, 10.64% CP and 40.66% CF.

It has been suggested that linseed meal could be used to best advantage at a level of up to 50% of the protein supplement (Seerley, 1991).

Table 2. Chemical composition of flaxseed and grapeseed meal (g/100 g DM)

Specification	Flaxseed meal	Grapeseed meal
Proximal chemical analysis		
Dry matter (DM), %	92.47	92.98
Organic matter (OM), %	87.38	90.13
Crude protein (CP) , %	31.95	13.26
Crude fat (EE), %	15.52	8.00
Crude fibre (CF), %	12.94	34.04
Non-protein nitrogen (NPN),%	26.98	34.82
Ash, %	5.09	2.85
Gross energy (GE), kcal/kg	1515.23	1763.55
Polyunsaturated fatty acid concentration (PUFA) from fat		
Alpha linolenic acid (C 18:3 omega 3), g FAME/100g total FAME	60.80	0.18
Fat degradation index		
Peroxide index, mlThiosulfate 0,01 Ng/gr	0.350	0.445
Fat acidity, mg KOH	9.10	11.24
KREISS reaction	negative	Negative

Flaxseed is the richest plant source of the ω-3 fatty acid i.e. α-linolenic acid (ALA) (Gebauer et al., 2006) and this is proven by data obtained in table 2 where α-linolenic acid is noticed having the highest percent among others PUFA, The bioavailability of ALA is dependent on the type of flax ingested (ALA has greater bioavailability in oil than in milled seed, and has greater bioavailability in oil and milled seed than in whole seed) (Austria et al., 2008). After manufacturing the mixed fodder, samples of combined feed were collected (aprox. 500 g/samples) and analysed from chemical and microbiological point of view.

Concerning the gross chemical composition of the compound feed (Table 1), we mention that crude protein ranged between 17.46% CP (C group) and 17.60% CP (E group). A higher content of fat were observed for group E diet (4.30%) compared to group C diet (3.07%). This increase is due to the use of flaxseed meal, high-fat ingredient of 16.33% EE.

In terms of fatty acid content, the experimental diet contains 14.02 g of α-linolenic acid/100 g of FAME compared to the control group containing 2.73 g of α-linolenic acid/100 g of FAME.Fat degradation indices were within the maximum admissible limits for compound feed, for both storage periods, 14 days and 28 days, respectively (Table 3).

Table 3. Fat degradation indices of feed compound

Specification	C	E
Peroxide index, ml Thiosulfate 0,01 Ng/gr		
Initial	0.389	0.446
At 14 days	0.465	0.504
At 28 days	0.764	0.836
Fat acidity, mg KOH		
Initial	9.12	8.47
At 14 days	13	14.1
At 28 days	19.44	22.02
KREISS reaction, %		
Initial	Negative	Negative
At 14 days	Negative	Negative
At 28 days	Negative	Negative

Neither the Kreiss Reaction, 28 days after manufacture, did not show any differences regarding lipid peroxidation in feed.

Initial average weight was similarly between the groups, 66.42±10.27 kg for C group and 66.25±9.88 kg for E group.

Table 4. Productive performances (average values/group)

Specification	C	E
Initial body weight (kg)	66.42 ±10.27	66.25 ±9.88
Final body weight (kg)	98.50 ±11.62	98.33 ±12.99
Average daily gain (kg/day)	0.972±0.06	0.972±0.103
Average feed intake (kg CF/head/day)	3.09	3.16
Feed consumption ratio (kg feed: kg gain)	3.46	3.54

Final average weight was 98.50±11.62 kg for C group, 98.33±12.99 kg for E group. The average daily gain was the same for both groups. The average feed intake and the feed efficiency were higher in group E compared to C group but the differences obtained were not significant (P>0.05) (Table 4). Romans et al. (1995a) fed ground flax at 0%, 5%, 10% and 15% of the diet to 48 barrows and noted no differences in ADG, hot carcass weight or percentage lean. In a companion study, Romans et al. (1995b) fed 15% ground flax to growing barrows and gilts for seven, 14, 21 or 28 days and again found no differences in performance or carcass traits. Kouba et al. (2003) fed growing pigs 6% crushed flax for 20, 60 or 100 days and found no differences in animal growth or carcass composition when compared with hogs fed wheat and soybean meal. It has been reported that an inclusion rate of 5% reduced efficiency of feed in growing pigs (Bell et al., 1993). However, flaxseed meal could be used at levels between 5 and 10% in diets for growing-finishing pigs provided that the diet has been balanced for digestible amino acids (Defa Li et al., 2000). Same author observed that the performances of growing-finishing pigs registered a liner decline in growth rate as the level of flaxseed in the diet increased, especially at the 15% inclusion. In the same trend, the feed intake showed a decrease concomitantly with the increasing levels of flaxseed. Defa Li et al. (2000) noticed also that an inclusion rate of 10% or greater of flaxseed meal produces declines of 5.7% and 20.0% in feed conversion, whereas the daily gain is reduced 18.9% for the 15% flaxseed meal inclusion. It is known that flaxseed meal contains some anti-nutritional factors so this could be the reason why the productive performances are so poor, when introducing high levels of flaxseed meal.

EC Regulations No. 3220/84 and No. 2967/85 require that classification of pigs has to be based on objective measurements that enable estimation of the lean meat percentage. This is calculated after dissections of the left carcass sides according to the EC reference method, which means complete separation of the muscles, including those of the head, as far as possible by knife. A lean meat percentage above 55 classifies it to E category, very important aspect taking into consideration that producers are being paid according to the lean meat percentage in pigs. All the pigs within the experimental trial satisfied the conditions for class „E".

Table 5. Carcass classification scale according to EUROPE

Specification	C	E
Carcass final weight warm (kg)	72.400±0.283	72.750±1.485
Fat thickness (mm)	13.350±1.202	12.950±0.636
Lean meat depth (mm)	48.300±1.697	50.00±0.424
Average lean meat percentage (%)	58.200±0.707	58.800±0.566
Quality carcass clasification according to EUROP scale	E	E

Data of table 5 show that there were no statistical differences (P>0.05) registered between the 2 groups concerning carcass final weight warm (kg), fat thickness (mm), lean meat depth (mm), average lean meat percentage (%) and quality carcass classification according to EUROP scale. The same result was published by Wiseman et al. (2006).

Table 6. Gross chemical composition of meat samples (average values/group)

Specification		C	E
Ham	Dry matter, %	30.39±1.047	33.67±2.135
	Crude protein , %	20.25±0.502	21.29±1.563
	Ether extract , %	8.05±0.29	8.99±3.642
	Ash, %	0.86±0.028	0.74±0.071
Tenderloin	Dry matter (DM), %	35.11±0.785	36.82±2.609
	Crude protein (PB), %	18.86±2.503	18.60±0.707
	Ether extract (EE), %	12.86±0.035	13.83±1.457
	Ash, %	0.84±0.12	0.74±0.255

Both, ham and tenderloin samples registered no differences between their nutrients (Table 6).
The values obtained for nutrients content of the edible parts mentioned above were according with those found by Gerber (2007).
The chemical composition of meat was found to be fairly constant, containing 62 to 75 %

water, 19 to 25 % protein and around 1 % ash, which is comparable with data reported by other food composition tables (Souci, Fachmann and Kraut, 2000).

Feeding flaxseed meal to pigs we noticed that the concentration of α-linolenic acid had increasing tendency for the edible parts analysed (Table 7).

Table 7. Fatty acids composition of ham and tenderloin samples (g/100g total fatty acids)

Specification		Ham		Tenderloin	
		C	E	C	E
Capric	C10:0	0.19±0.01	0.13±0.01	0.14±0.01	0.18±0.01
Lauric	C12:0	0.20±0.03	0.13±0.02	0.21±0.11	0.15±0.04
Miristic	C 14:0	1.51±0.04	1.43±0.01	1.72±0.09	1.86±0.09
Pentadecanoic	C 15:0	0.16±0.01	0.12±0.03	0.24±0.01	0.30±0.09
Pentadecenoic	C 15:1	0.42±0.07	0.71±0.01	0.22±0.03	0.26±0.01
Palmitic	C 16:0	23.73±0.94	23.67±0.10	26.73±0.21	26.53±0.02
Palmitoleic	C 16:1	3.10±0.02	3.04±0.03	2.66±0.06	2.59±0.08
Heptadecanoic	C 17:0	0.46±0.03	0.34±0.02	0.35±0.14	0.50±0.01
Heptadecenoic	C 17:1	0.47±0.03	0.33±0.02	0.30±0.03	0.30±0.02
Stearic	C 18:0	13.23±1.40	11.40±0.08	13.87±0.40	13.89±0.15
Oleic cis	C 18:1n9c	44.05±0.96	41.38±0.09	38.87±0.20	37.89±0.20
Linoleic cis	C 18:2n6	9.94±0.09	12.83±0.29	11.72±0.66	12.06±0.04
Linolenic α	C 18:3n3	0.51±0.01	1.29±0.04	1.06±0.06	1.12±0.03
Octadecatetraenoic	C 18:4n3	0.76±0.11	0.59±0.01	0.57±0.01	0.60±0.02
Eicosadienoic	C 20:2n6	0.41±0.03	0.52±0.07	0.41±0.01	0.50±0.14
Eicosatrienoic	C 20:3n6	nd	0.19±0.01	0.09±0.01	0.07±0.01
Eicosatrienoic	C 20:3n3	0.88±0.44	1.12±0.09	0.37±0.01	0.40±0.06
Arachidonic	C20:(4n6)	nd	0.05±0.01	0.05±0.01	0.07±0.01
Other fatty acids		nd	0.77±0.20	0.46±0.08	0.51±0.21
Total fatty acids determined		100	100	100	100

The best results were observed in the ham samples where we registered differences concerning the concentration of omega 3 fatty acid α-linolenic (LNA) for E group, who had 1.29 ± 0.04 compared to 0.51 ± 0.01 within the C group, but these differences were not significant statistically ($P>0.05$). In tenderloin samples the concentration of cis-oleic fatty acid was higher for C group (C group-38.87±0.20 vs. E group-37.89±0.20), but without significant differences. The ability to substantially alter the n-3 fatty acid content of pork was demonstrated in 1972 by Anderson et al., feeding 20% flaxseed oil for two months to six month old pigs, increasing the fat depot concentrations of LNA from 1% to 15%. Cunnane et al. (1990) fed 5% ground flaxseed to weaned pigs for 8 weeks and found several fold increases in LNA and its elongation and desaturation products in a number of tissues. Due to the fact that the experimental diet consisted of a combined feed rich in polyunsaturated fatty acids, it was necessary to analyze the degree of lipid degradation of fat in meat samples by determining TBARS. As a major degradation product of lipid hydroperoxides, malondialdehyde (MDA) was assessed as marker (Table 8).

Table 8. Malondialdehyde (mg/kg MDA) as a lipid peroxidation marker in meat samples

Meat sample	**C**	**E**
0 days(mg/kg MDA)		
Ham	0.028±0.003	0.028±0.021
Tenderloin	0.036±0.009	0.028±0.004
7 days(mg/kg MDA)		
Ham	0.177±0.026	0.098±0.045
Tenderloin	0.100±0.013	0.059±0.009

Table 8 contains data on the concentration of malondialdehyde (mg/kg) in the ham and tenderloin. The assessment of the meat oxidative stability during the storage depends on the concentration of the degradation

products, such as, for example, the malondialdehyde concentration (as by-product). The amount of malondialdehyde increases in proportion to the amount of fatty acids in the tissues. In the fresh samples (0 days) of tenderloin from the experimental group, a smaller amount of malondialdehyde was detected compare to group C. The amount of malondialdehydeof ham was at a similar level for both groups (Table 8). O'Grady et al. (2008) reported that the dietary grape pomace had no antioxidant effect on the lipids of the meat. On the other hand Mason et al. (2005) and Lahucky et al. (2010) demonstrated positive results regarding to oxidative stability of the meat or meat products when using various plant extracts, essential oils or parts containing antioxidant compounds to the diets of pigs.

Although only the experimental group diet was enriched in polyunsaturated fatty acids (flaxseed meal), the ham and the tenderloin contained a lower amount of malondialdehyde compared to conventional diet. This is due to the influence of grapeseed meal with proven antioxidant effect, added to diet E, in the process of inhibiting the propagation of oxidative reactions within muscle tissues.

At 7 days of refrigeration, the meat samples of the E group proved to have a higher oxidation stability 80.61% for ham and 69.49% for tenderloin compare tothe same meat samples of C group (Table 8).

Table 9 shows the antioxidant capacity of meat samples determined at 0 and 7 days of refrigeration. At 0 days, in the fresh ham and tenderloin of E group, the antioxidant capacity was superior compared to the C group.

Table 9. Antioxidant capacity of the meat samples

Meat samples	**C**	**E**
0 days(mM ascorbic acid equivalent)		
Ham	18.182±0.39	18.311±1.68
Tenderloin	16.945±1.30	21.218±1.22
7 days(Mm ascorbic acid equivalent)		
Ham	8.584±0.72	11.586±0.28
Tenderloin	9.470±0.11	10.473±2.39

Also, the antioxidant activity of the ham and tenderloin of E group at 7 days after refrigeration was higher than in the case of samples from group C.

CONCLUSIONS

The bioproductive performances of fattening-finishing pigs were not influenced by the inclusion of 7.5% of flaxseed meal and 1% grapeseed meal added to the basal diet, nor the carcass classification.

During the experimental trial a slight increasing in PUFA content was noticed but without any statistical significance both in ham and tenderloin. Also, it could be observed within E group the protective effect of grapeseed meal for ham and tenderloin suggesting a possible antioxidant effect of grapeseed meal.

ACKNOWLEDGEMENTS

This research work was carried out with the support of Ministry of Agriculture and Rural Development, financed from ADER Project.

REFERENCES

Austria J.A., Richard M.N., Chahine M.N., Edel A.L., Malcolmson L.J., Dupasquier C.M., Pierce G.N., 2008. Bioavailability of alpha-linolenic acid in subjects after ingestion of three different forms of flaxseed. Journal of the American College of Nutrition, 27(2), 214-221.

Bell J.M., Keith, M.O., 1993. Nutritional evaluation of linseed meals from flax with yellow or brown hulls, using mice and pigs. Animal feed science and technology, 43(1-2), 1-18.

Cunnane S.C., Ganguli S., Armstrong J.K., Stitt P.A., 1990. Raised omega-3 fatty acid levels in pigs fed flax. Canadian Journal of Animal Science, 70(1), 251-254.

Eastwood L., 2008. The nutritional value of flaxseed meal for swine (Doctoral dissertation).

EC-Regulation Nr. 3220184 for the assessment of the Community scale for grading pigs carcasses, d.d. November 13, 1984. 3pp.

EC-Regulation Nr. 2967185 laying down detailed rules for the application of the Community scale for grading pig carcasses, d.d. October 24, 1985. 2pp.

Fantozzi P., 1981. Grape seed: a potential source of protein. Journal of the American Oil Chemists' Society, 58(12), 1027-1031.

Ganorkar P.M., Jain R.K., 2013. Flaxseed-a nutritional punch. International Food Research Journal, 20(2).

Gebauer S.K., Psota T.L., Harris W.S., Kris-Etherton P.M., 2006. n-3 Fatty acid dietary recommendations and food sources to achieve essentiality and cardiovascular benefits. The American journal of clinical nutrition, 83(6), 1526S-1535S.

Gerber N., 2007. The role of meat in human nutrition for the supply with nutrients, particularly functional

long-chain n-3 fatty acids (Doctoral dissertation, ETH Zurich).
Greenwald P., Clifford C.K., Milner J.A., 2001. Diet and cancer prevention. European Journal of Cancer 37, 948–965.
Kouba M., Enser M., Whittington F.M., Nute G.R., Wood J.D., 2002. Effect of a high linolenic acid diet on lipogenic enzyme activities, fatty acid composition and meat quality in the growing pig. Journal of Animal Science.
Kristensen M., Jensen M.G., Aarestrup J., Petersen K.E., Søndergaard L., Mikkelsen M.S., Astrup A., 2012. Flaxseed dietary fibers lower cholesterol and increase fecal fat excretion, but magnitude of effect depend on food type. Nutrition & metabolism, 9(1), 8.
Lahucky R., Nuernberg K., Kovac L., Bucko O. Nuernberg G., 2010. Assessment of the antioxidant potential of selected plant extracts–in vitro and in vivo experiments on pork. Meat Science, 85(4), 779-784.
Leterme P., Londoño A.M., Muñoz J.E., Súarez J., Bedoya C.A., Souffrant W.B., Buldgen A., 2009. Nutritional value of aquatic ferns (*Azolla filiculoides* Lam. and *Salvinia molesta* Mitchell) in pigs. Anim. Feed Sci. Technol., 149, 135–148.
Mason L.M., Hogan S.A., Lynch A., O'Sullivan K., Lawlor P.G., Kerry J.P., 2005. Effect of restricted feeding and antioxidant supplementation on pig performance and quality characteristics of *Longissimus dorsi* from Landrace and Duroc pigs. Meat Sci., 70, 307–317.
O'Grady M.N., Maher M., Troy D.J., Moloney A.P., Kerry J.P., 2006. An assessment of dietary supplementation with the acatechins and rosemary extract on the quality of fresh beef. Meat Sci., 73, 132–146.
Olteanu M., Criste R.D., Panaite T.D., Ropota M., Vlaicu P.A., Turcu R.P., 2017. Bioproductive parameters and fatty acids profile of the meat from broilers treated with flax meal and grape seeds meal, Scientific Papers: Animal Science and Biotechnologies, 50 (1), 15-21
Panaite T.D., Criste R.D., Ropota M., Criste V.I., Mitoi M., Olteanu M., Vlaicu Al.P., 2016 Feeding quality of the meat from broilers treated with unconventional feed industry by-products, Scientific Papers Animal Sciences, Lucrari Stiintifice, Seria Zootehnie, vol. 68, 22, 136-145
Parliament R., 2014. Law 43/2014 on the protection of animals used for scientific purposes. Oficial Journal, (326 Part I), 2-4.
Petit H.V., 2003: Digestion, Milk Production, Milk Composition, and Blood Composition of Dairy Cows Fed Formaldehyde Treated Flaxseed or Sunflower Seed. J. Dairy Sci., 86, 2637–2646
Romans J.R., Wulf D.M., Johnson R.C., Libal G.W., Costello W.J., 1995. I Effects of ground flaxseed in swine diets on pig performance and on physical and sensory characteristics and omega-3 fatty acid content of pork: II. Duration of 15% dietary flaxseed. Journal of Animal Science, 73(7), 1987-1999
Seerley R.W., 1991. Major feedstuffs used in swine diets. In Swine nutrition, 451-481.
Singh K.K., Mridula D., Rehal J., Barnwal P., 2011. Flaxseed: a potential feed and fiber source of food, using mice and pigs. Animal feed science and technology, 43 (1-2), 1-18.
Souci S.W., Fachmann W., Kraut H., 1981. Food composition and nutrition tables 1981/82. Food composition and nutrition tables 1981/82., (Ed. 2).
Warnants N., Van Oeckel M.J., Boucque C.V., 1999. Incorporation of dietary polyunsaturated fatty acids into pork fatty tissues. Journal of Animal Science, 77(9), 2478-2490.
Wiseman J., 2006. Practice aspects regarding carcase quality of pigs and poultry. American Soybean Association.

Scientific Papers. Series D. Animal Science. Vol. LXI, Number 1, 2018
ISSN 2285-5750; ISSN CD-ROM 2285-5769; ISSN Online 2393-2260; ISSN-L 2285-5750

EFFECT OF THE DIETARY OREGANO (*Origanum vulgare* L.) POWDER AND OIL ON THE BALANCE OF THE INTESTINAL MICROFLORA OF BROILERS REARED UNDER HEAT STRESS (32°C)

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Abstract

An experiment on 90, COBB 500 broiler chicks (14-42 days), reared under heat stress, evaluated the effect of the dietary oregano (Origanum vulgare L.) powder and oil, on the balance of the intestinal microflora of broilers. The broilers, assigned to three groups (C, E1, E2), were housed in an experimental hall with 32°C constant temperature, humidity 36% and 23 h light regimen. The conventional diet C included monensin in the premix for the grower phase (14-35 days). Unlike the control group, the diet for the experimental groups included 0.01% oregano oil (E1), or 0.005% oregano oil plus 1% oregano powder (E2). Six broilers/ group were slaughtered in the end of the experiment (42 days), and samples of cecal and intestinal content were collected for bacteriological examination. The experimental results showed that the total Enterobacteriaceae, E. coli and Staphylococcus count was significantly (P≤ 0.05) lower both in the cecal microbiota and in the intestinal microbiota of the experimental groups than in group C, while the lactobacilli count was significantly (P≤ 0.05) higher in groups E1 and E2 than in group C.

Key words*: oregano, heat stress, broiler, intestinal microflora.*

INTRODUCTION

Along with the prohibition of antibiotics as feed growth promoters, there has been a growing interest in phytoadditives, recognized as safe substances that can replace synthetic substances. This new approach to monogastric nutrition has been imposed by the emergence of antibiotic resistance in the animal body as a result of their use as growth promoters (Windisch et al., 2008). Thus, there is a great interest in the development of innovative feeding strategies to stimulate the development of the digestive system and digestive tract health, especially in young animals, in order to improve the bioproductive performance even with the elimination of the presence of antibiotics. Considering the advances made in understanding how to use nutrients in the intestine and metabolism, a goal of nutrition may be to formulate monogastric diets with precise targets such as optimizing growth by maintaining healthy digestive tract to ensure good the development of physiological functions (Choct, 2009).

Fuller (1999) shows that young animals under stressful conditions suffer from changes in the composition and activity of the gut microbiota. Suzuki et al. (1983) demonstrated that heat stress resulted in a marked change of bacterial composition in chicken intestine, which was subsequently associated with depression of body-weight gain. The gastro intestinal tract is particularly responsive to stressors like heat stress, which modify the normal and protective microbiota (Bailey et al., 2004) and decreased integrity of the intestinal epithelium (Lambert, 2009) which, in turn, can affect its barrier function and the absorption of nutrients, impairing productive performance of animals (Liu et al., 2009).

Many natural compounds used as alternatives to antibiotics in animal feed have shown positive effects on growth performance and on

different health parameters (Jamroz et al., 2005; Steiner, 2009; Windisch et al., 2008). Globally, herbs play an important role in nutrition because they are important ingredients in numerous products (Black et al., 2010). This has caused plants and essential oils extracted from plants to attract attention as sources of natural compounds and be studied because of the potential they own. They can be used as alternative remedies for the treatment of many infectious and oxidative diseases and for the preservation of food against the toxic effects of bacteria and oxidants (Sepahvand et al., 2014). Several types of herbal products and their oils have improved the performance of broilers by growth promoting.

Oregano (*Origanum vulgare* L.) is an aromatic herb which, due to its very strong chemical nature, has been used primarily for the preservation of food quality, for the inhibition of microbial proliferation of poultry meat and recently as an alternative growth promoter in poultry feed (Halle, 2001; Modeva et al., 2003; Bampidis et al., 2005; Çabuk et al., 2006; LiHua et al., 2007). Various studies that investigated the influence of oregano (dried leaf and oil) on the performance of broilers have led to the conclusion that oregano contributes to improving the performance of broilers by promoting their growth (Giannenas et al., 2003; Halle, 2001; Modeva et al., 2003; LiHua et al., 2007) and can reduce bacterial gastrointestinal tract populations such as *Clostridium perfringens* and *Escherichia coli* (Giannenas et al., 2004; Fukayama et al., 2007). However, the prediction of the oregano broiler's response is not very simple, because it is influenced by the variety of the plant (Fritz et al., 1992; Lee et al., 2003; Demir et al., 2003; Hassan et al., 2004; Halle et al., 2004), the level of inclusion in diets (Giannenas et al., 2004; Alçiçek, et al., 2003; Alçiçek et al., 2004; Ertas et al., 2005), sanitary and environmental conditions, nutritional composition of diet (Fritz et al., 1992; Fritz et al., 1991; Jamroz et al., 2006) and possible interaction with other additives.

The literature data show distinct biological functions of the essential oils derived from aromatic plants, such as antibacterial, antimicrobial properties (Chen et al., 2016), antifungal (Hossain et al., 2016), antiviral (Gavanji et al., 2015), antioxidant (Shaaban et al., 2012) and antiproliferative properties (Park et al., 2014). Essential oils have positive effects on animal growth and health (Puvaca et al., 2013). The inclusion of oils in poultry diets had beneficial results on intestinal microflora (Helander et al., 1998) and digestive enzymes (Jang et al., 2004; Lee et al., 2003).

Criste et al. (2017) conducted a study on Cobb 500 broilers to investigate the effect of inclusion of 2% oregano and 3% rosehip powder on the health of intestinal microflora. The chicks were reared under in heat stress (32^{o}C). These researchers reported a beneficial action for maintaining the intestinal health of phytoadiatives used in the experiment by maintaining the balance of populations of microorganisms wich colonizing the intestine. In another study on maintaining the health of cecal microflora, Roofchaee et al. (2011) evaluated the effect of 300, 600 and 1200 mg/kg oregano oil (*Origanum vulgare* L.) inclusion in Ross 308 broilers diet. The chicks were housed at 34°C temperature, which gradually decreased to 28°C towards the end of the experiment. Although there were no significant differences ($P \geq 0.05$) for the *Lactobacillus* populations, the *Escherichia coli* populations were significantly decreased ($P \leq 0.05$) on broilers that received 300 and 600 mg/kg oregano oil in diets, compared to chicks from the control group and those who received 1200 mg/kg oregano oil. Peng et al. (2016) evaluated the effect of oregano oil inclusion on the intestinal morphology of chicks over a 42-day period. The broilers used in the experiment were from the Arbor Acres hybrid, and the control group received avilamycin in addition in diets. The results showed that oregano oil has a significant effect on the intestinal health of broilers, so it may be an alternative to antibiotics.

Within this context, the present paper aims to evaluate the effects of Oregano (*Origanum vulgare* L.), oil and powder, on the balance of intestinal microflora of broilers reared under heat stress (32°C).

MATERIALS AND METHODS

The trial was conducted within the experimental halls of the National Research-

Development Institute for Animal Biology and Nutrition (IBNA-Balotesti, Romania), according to the provisions of the protocol approved by the Ethics commission of the IBNA-Balotesti. 90, Cobb 500 broiler chicks (day-old) were purchased for the experiment. During the starter stage (1-14 days) all chicks received a conventional diet formulation with corn and soybean meal as basic ingredients. At 14 days, the broilers were weighed and assigned to three groups (C, E1, E2). The chicks were house in an experimental hall with 32°C constant temperature, humidity 36% and 23 h light regimen. They had free access to the feed and water. During the growing (14-35 days) and finishing (35-42 days) phase, the chicks from the control group (C) received a conventional diet based on corn and soybean meal (Table 1). Compared to the control diet (C), the experimental diets differed by the addition of 0.01% oregano oil (E1), or 0.005% oregano oil plus 1% oregano powder (E2). During the growth stage (14-35 days), only the conventional diet C included monensin in the premix. The oregano essential oil studied in this paper was purchased from China from Jiangxi Xuesong Natural Medicinal Oil Co. Ltd., and the oregano powder from an SME from Livezeni, Mureș County (46.55°N, 24.63°E).

Table 1. Diet formulation

Ingredients	Grower phase (14 - 35 days)			Finisher phase (35 - 42 days)		
	C	E1	E2	C	E1	E2
Corn, %	62	62	61	60.45	60.45	59.45
Soybean meal, %	26.58	26.57	26.575	25.54	25.53	25.545
Oil %	2.5	2.5	2.5	3.72	3.72	3.72
Oregano oil, %	-	*0.01*	*0.005*	-	*0.01*	*0.005*
Oregano powder, %	-	-	*1*	-	-	*1*
Gluten %	4	4	4	6	6	6
Methionine, %	0.26	0.26	0.26	0.25	0.25	0.25
Lysine, %	0.48	0.48	0.48	0.2	0.2	0.2
Carbonate, %	1.4	1.4	1.4	1.33	1.33	1.32
Monocalcium phosphate, %	1.36	1.36	1.36	1.13	1.13	1.13
Salt, %	0.37	0.37	0.37	0.33	0.33	0.33
Choline, %	0.05	0.05	0.05	0.05	0.05	0.05
Vitamino-mineral premix*with coccidiostatic, %	1	-	-	-	-	-
Vitamino-mineral premix without coccidiostatic, %	-	1	1	1	1	1
Total	100	100	100	100	100	100
Chemical composition determinated						
Dry matter, %	87.70	87.92	88.81	89.60	90.53	90.04
Organic matter, %	82.17	82.86	83.18	84.81	85.50	84.09
Crude protein, %	20.51	21.77	21.44	19.40	19.07	20.18
Ether extractives, %	4.13	4.30	4.27	5.59	5.65	5.69
Fibre, %	3.49	3.81	3.90	3.53	3.98	3.44
Ash, %	5.53	5.06	5.63	4.79	5.03	5.95
Nitrogen-free extractives, %	54.04	52.98	53.57	56.29	56.80	54.78
Calcium, mg/kg DM	0.84	0.85	0.86	0.85	0.84	0.84
Phosphorus, mg/kg DM	0.84	0.75	0.89	0.85	0.88	0.73

*1 kg premix vitamino-mineral contains: = 1.350.000 IU/kg vit. A; 300.000 IU/kg vit. D3; 2700 IU/kg vit. E; 200 mg/kg Vit. K; 200 mg/kg Vit. B1; 480 mg/kg Vit. B2; 1485 mg/kg pantothenic acid; 2700 mg/kg nicotinic acid; 300 mg/kg Vit. B6; 4 mg/kg Vit. B7; 100 mg/kg Vit. B9; 1.8 mg/kg Vit. B12; 2500 mg/kg Vit. C; 7190 mg/kg manganese; 6000 mg/kg iron; 600 mg/kg copper; 6000 mg/kg zinc; 50 mg/kg cobalt; 114 mg/kg iodine; 18 mg/kg selenium; 50 g sodium monensin /kg.

Throughout the experimental period (14-42 days) the following parameters were monitored: average daily feed intake (kg feed/chick/day), average daily weight gain (kg/chick/day), feed conversion ratio (kg feed/kg gain) and final weight (kg). In the end of the feeding trial (42 days broilers), six broilers per group were slaughtered, according to the working protocol. Samples of intestinal content were collected, in sterile tubes, from

the slaughtered chicks, for microbiological examination (determination of the *Enterobacteriaceae*, *E. coli, Salmonella*, and lactobacilli).

Gas chromatography coupled with a mass spectrometer was used to determinate the profile of volatile compounds of oregano oil and powder of the whole plant. For headspace analysis, 1.0 g of sample was placed in a 20 mL headspace vial sealed with silicone rubber septum and aluminum cap. The vial was heated to 80°C for 10 min before the injection. The essential oil and oregano herb samples diluted in hexane (1:100) (1 µL injection) and headspace gas (500 µL) were analyzed using a Thermo Electron system - Focus GC chromatograph coupled with a Polaris Q ion trap mass detector, both controlled with Xcalibur® software. A DB-5MS capillary column (25 m length, 0.25 mm i.d., and 0.25 µm of film thickness) was used. Both headspace and liquid samples were analyzed under the same chromatographic conditions. The GC oven temperature program was: initial temperature 60°C (3 min) followed by an increase of 10°C/min up to 200°C (2 min) and then 12°C/min to the final temperature of 240°C (2 min). The carrier gas (helium) flow rate was 1 mL/min. The source and interface temperature were 200°C and 250°C, respectively. Detector operated in electron impact mode (70 eV). Detection was performed in the range of m/z 35-300.The retention indices were determined using an alkane standard solution for GC (C8-C20 in hexane) (Sigma Aldrich Co., St. Louis, USA). Relative percent of individual components was calculated based on GC peak areas. All compounds were identified according to their retention indices and based on mass spectrum provided by electronic libraries (Wiley, NIST).

To determinate the basic chemical composition of feed, standardized methods were used in accordance with Regulation (EC) No. 152/2009 (Methods of sampling and analysis for official inspection of feeds).

The *Enterobacteriaceae* and *E. coli* were determined using a classical isolation medium, G.E.A.M. or Levine. The samples were first soaked in medium with lauryl-sulphate (enrichment medium), homogenized and left for 20-30 minutes are room temperature (23-24°C). Decimal dilutions were made up to 10^{-5} in the medium with lauryl-sulphate. The dilutions of 10^{-2} - 10^{-5} were used to seed 2 Petri dishes each per dilution, on Levine medium. The Petri dishes were incubated for 48 h at 37°C and the colonies were count. *E. coli* formed characteristic colonies on this medium (dark violet with metallic shine). The other *Enterobacteriaceae* formed either dark red opaque colonies (lactic-positive species) or pale pink semi-transparent or colourless colonies (lactic-negative species). The lactobacilli were determined on selective mediums (MRS broth and MRS agar), characteristic for the isolation and counting of these bacteria. The colony counter Scan 300, INTERSCIENCE (France) was used to determine the colony count of *Enterobacteriaceae*, *E. coli* and lactobacilli.

The experimental results are expressed as mean values ± standard error; StatView software and the analysis of variance (ANOVA and t test) were used for statistical processing of the data, the differences being considered statistically significant for $P \leq 0.05$.

RESULTS AND DISCUSSIONS

Table 2 shows the profile of the volatile compounds identified in oregano oil and powder of the whole plant. As can be seen, the major constituent of oregano oil is carvacrol, which, which according to Yanishlieva and Marinova (1995); Yanishlieva et al. (1999) accounts for about 78% to 82% of the total oil, with timol, being responsible for its antioxidant activity. Bampidis et al. (2005) studied oregano oil and recorded 85.49% carvacrol concentration. The oil used in this study showed a lower concentration by 59.08%.

At high concentrations, p-cymene (20.75%) and γ-terpinene (4.45%), were also identified, two monoterpenic hydrocarbons, which confirm the strong antioxidant properties of the oregano oil (Botsoglou et al., 2002) and which represents about 5%-7% of the total oil (Adam et al., 1998). These results are consistent with those reported by Kokkini et al. (2004), which identified high concentrations of p-cymene and γ-terpinene in oregano oil. Licina et al. (2013) also analysed the chemical composition of oregano oil, recording a concentration of 0.9% α-pinene, 1.2% β-pinene, 0.1% α-phellandrene,

4.0% p-cymene, 1.5% limonene, 5.6% γ-terpinene, 1.5% linalool, 0.1% camphor and 5.4% caryophyllene oxide.

Other volatile compounds were also identified in the oregano oil content used in experimental diets, as follows: 1.33% α-pinene, 0.21% α-phellandrene, 1.10% limonene, 1.19% caryophyllene oxide. Bampidis et al., (2005) analyzed oregano oil and obtained a concentration of 0.22% α-pinene, compared to the data presented in this paper, 0.08% alpha-phellandrene, 0.19% limonene and 0.29% caryophyllene oxide.

Table 2. Volatile compounds identified in oregano (essential oil and herb)

Compounds	CAS number	Esential oil (%)	Herb (%)
α-Pinene	80-56-8	1.33	3.44
Camphene	79-92-5		1.48
Sabinene	3387-41-5		56.57
β-Pinene	127-91-3	1.79	2.20
β-Myrcene	123-35-3		3.75
α-Phellandrene	99-83-2	0.21	
p-Cymene	99-87-6	20.75	3.98
Limonene	138-86-3	1.10	1.75
Eucalyptol (1,8-Cineole)	470-82-6		12.27
E-β-Ocimene	3779-61-1		3.26
γ-terpinene	99-85-4	4.45	1.75
cis-Sabinene hydrate	15826-82-1		0.64
α-Terpinolene	586-62-9	0.98	
Linalool	78-70-6	1.19	0.62
Camphor	76-22-2	0.14	3.90
Estragole	140-67-0	0.85	
Carvacrol	499-75-2	59.08	
Caryophyllene	87-44-5	6.95	2.74
Germacrene D	23986-74-5		1.46
Caryophyllene oxide	1139-30-6	1.19	0.21

Unlike oregano oil, the major compounds in the oregano powder were represented by sabinene and eucalyptol (1,8-cineole). Research done by Kokkini et al. (2004) and Nurzyńska-Wierdak (2009) also reported a 20.13% high concentration of sabine in oregano. In the same study, in which Nurzyńska-Wierdak (2009) assessed the chemical composition of oregano according to the stage of plant development, the researcher concluded that the developmental stage of plant in herb harvesting period have a significantly importance for their chemical composition, the best term for herb harvesting being on the full flowering phase. This also influences the chemical composition of essential oil of oregano, which depends by the development stage of plant at the extraction time. The researcher claimed that the oil obtained from plant on the flowering phase does not contain carvacrol. This statement may be an explanation for the fact that in this study carvacrol was not identified in the entire oregano plant analysed.

The parameters on feed conversion ratio (kg feed/kg gain) and final weight (kg) are shown in Figure 1. There were no significant differences ($P \geq 0.05$) between groups in terms of broilers feed conversion ratio (kg feed/kg gain) and final weight (kg). The results obtained are different from the Cobb Broiler Management Guide which indicates a final weight at 42 days by 2.857 kg and feed conversion ratio by 1.675 (kg feed/kg gain). Thus, in this study the final weight gains were lower than those established in the guide with 32.90% in the control group, with 25.51% in the E1 group, respectively with 36.61% in the E2 group. Regarding on feed conversion ratio of broilers for the entire experimental period, the chicks from the control group had 17.48% higher consumption to that mentioned in the guide, 28.11% in E1 group and 29.91%. in E2 group Contrary to the data obtained in this study, Roofchaee et al. (2011), which evaluated the effect of the oregano oil (*Origanum vulgare* L.) inclusion in Ross 308 broilers reared under heat stress conditions (34^{o}C), reported a significant improvement ($P \leq 0.05$) of feed conversion ratio (kg feed/kg gain). They also concluded that oregano oil used in broilers diet has beneficial effects on chicks growth parameters. Florou-Paneri et al. (2005) evaluated the effect of oregano essential oil and herb inclusion in turkeys' diet, reared under normal temperature conditions. The dietary supplementation was performed with 5 g/kg and 10 g/kg oregano herb, respectively 100/kg and 200 mg/kg oregano oil. The results showed that the inclusion of oregano oil and herb in

turkeys diets had no effect on average daily fed intake (kg/broiler/day) and average daily weight gain (g/broiler/day). Another study regarding the effect of oregano oil inclusion on the performance of broilers during the 42 days was undertaken by Peng et al. (2016). The recorded data revealed an improvement of final body weight (kg) and average daily weight gain (kg/broiler/day) In fact, feed conversion ratio (kg feed/kg gain) was significantly reduced ($P \leq 0.05$).

Karimi et al. (2010) investigated the effect of including different levels of dried oregano leaves in the starter phase of Cobb 500, aged one. The chicks were divided into 2 control groups, one with 55 mg/kg penicillin, and 8 experimental groups with an inclusion level of 2.5 to 20 g/kg of oregano leaves. The recorded results showed that diets supplemented with oregano leaves did not influence the final weight (kg), the feed conversion ratio (kg feed/kg gain) or the mortality rate. The researchers concluded that it is necessary to include a higher level of oregano in diets for obtaining strong positive results from chicks to this.

In another study on the effect of the inclusion of oregano in broilers diet, Halle et al. (2004) reported that the gradual introduction of the oregano plant and its oil in diets reduced the average daily fed intake and significantly improved the feed conversion ratio of chicks compared to the control group that received a conventional diet formulation. This finding is very similar to that of Amad et al. (2011) who investigated the effect of phytoaditives, respectively of oregano on the growth performance and ileal digestibility of nutrients of Cobb 500 broilers. Chicks were reared under heat stress conditions of 35°C, the temperature being gradually reduced to 25°C towards the end of the experimental period. The data obtained revealed a decrease in the average daily fed intake and an improvement in both the feed conversion ratio and final weight. Other studies have shown significant improvements on the bioproductive parameters for broilers along with the inclusion of oregano oil in diets (Bozkurt et al., 2009).

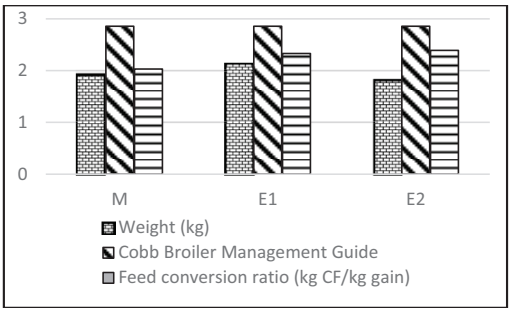

Figure 1. Broiler performance (average values/group)

The results recorded in this study on the inclusion of oregano (*Origanum vulgare* L.) (Table 3) oil (E1), respectively oil and powder (E2) in broilers diet on the intestinal microbiota revealed that the total number of *Enterobacteriaceae, E. coli* and staphylococci was significantly reduced ($P \leq 0.05$) in the experimental groups compared to the control group. Differences were also observed with regarding to the total number of Lactobacilli, which increased significantly ($P \leq 0.05$) in experimental diets, unlike the control diet. The determinations performed did not report the presence of *Salmonella* spp. in intestinal microflora. The results obtained in this study are in agreement with those obtained by Criste et al. (2017), who also reported a significant decrease ($P \leq 0.05$) of *E. coli* populations in a Cobb 500 broiler study, reared under heat stress (32^{o}C) and fed with diets that included 2% oregano powder. Mohiti-Asli and Ghanaatparast-Rashti (2017) investigated the essential oregano oil inclusion on intestinal properties, the number of *Escherichia coli* and lactobacilli in Ross 308 broilers diet. The supplementation of experimental diets with oregano oil resulted in a significant decrease ($P \leq 0.05$) of the *E. coli* populations compared to the control diet, but for the total number of Lactobacilli was not different between the treatments.

Other studies too, determined the inhibitory effects of essential oils against pathogens such as *C. perfringens* or *E. coli* (Zeng et al., 2015). The reduction of the number of pathogenic bacteria in the intestine increases the intestinal absorption capacity.

Table 3. The effect of *Origanum vulgare* L. (oil/ oil and powder) in the diet of broilers (14-42 d) on intestinal microbiota composition (log10 CFU*/g wet intestinal digesta)

Specification	C	E1	E2	SEM	Significance of treatment effect (p<)
Enterobacteriacea, lg10	7.349[a]	7.337[b]	7.273[c] 2	0.009	<0.0001
E. coli, lg 10	6.070[a]	5.994[b]	5.942[c]	0.014	<0.0001
Stafilococci, lg10	5.886[a]	5.863[b]	5.831[c]	0.006	<0.0001
Lactobacilli, lg 10	6.406[a]	6.978[b]	7.120[c]	0.082	<0.0001
Salmonella spp.	Absent	Absent	Absent	-	-
Where: *CFU-colony forming units; [a-c] Mean values within a row having different superscripts are significantly different by least significant difference test (P≤0.05); SEM: standard error of the mean; means in the same row no common superscript significantly different (P≤0.05).					

Moreover, the controlled pathogenic load contributes to the establishment of healthy microbial metabolites, the improvement of intestinal integrity and the protection against enteric diseases (Placha et al., 2013; Tiihonen et al., 2010; Oviedo-Rondón et al., 2006; Baker et al., 2010.

With regard to the cecal microbiota, the results obtained (Table 4) revealed that the inclusion of oregano (*Origanum vulgare* L.) oil (E1) and oil and powder (E2) in broilers diet reduced the total number of *Enterobacteriaceae, E. coli* and staphylococci, this being significant (P≤0.05) lower in the experimental groups compared to the control group. Concerning the total number of Lactobacilli, it was significantly (P≤0.05) higher in groups E1 and E2, compared to the control group. Horošová et al. (2006) pointed out the potential negative effects induced by essential oils included in diets on the healthy intestinal bacteria. They reported that the inclusion of oregano oil in broilers diet showed a strong bactericidal effect against isolated Lactobacilli in manure samples.

As with intestinal microflora, no populations of *Salmonella* spp. have been identified in cecum microflora. Roofchaee et al. (2011), which evaluated the effect of the inclusion of oregano oil (*Origanum vulgare* L.) in Ross 308 broilers diet, reared under heat stress (34°C), reported strong antibacterial effects of oregano oil against cecal *E. coli*.

Table 4. The effect of *Origanum vulgare* L. (oil/ oil and powder) in the diet of broilers (14-42d) on cecal microbiota composition (log10 CFU*/g wet cecal digesta)

Specification	C	E1	E2	SEM	Significance of treatment effect (p<)
Enterobacteriaceae, lg10	11.360[a]	11.331[b]	11.282[c]	0.009	<0.0001
E. coli, lg 10	10.106[a]	10.086[b]	10.044[c]	0.007	<0.0001
Stafilococci, lg10	8.804[a]	8.775[b]	8.734[c]	0.008	<0.0001
Lactobacilli, lg 10	11.257[a]	11.284[b]	11.365[c]	0.012	<0.0001
Salmonella spp.	Absent	Absent	Absent	-	-
Where: *CFU-colony forming units; [a-c] Mean values within a row having different superscripts are significantly different by least significant difference test (P≤0.05); SEM: standard error of the mean; means in the same row no common superscript significantly different (P≤0.05).					

CONCLUSIONS

The data obtained in this study confirm the inhibitory, antibacterial effect of oil and oregano powder against pathogens and for chicks reared under heat stress. The bio productive parameters of broilers, such as feed conversion ratio (kg feed/kg gain) and final weight (kg), did not differ between groups but were affected by heat stress.

ACKNOWLEDGEMENTS

The article presents part of the results obtained within project PN 1641_04.01, Contract 24 N / 2016, Nucleus Program.

REFERENCES

Alçiçek A., Bozkurt M., Çabuk M., 2003. The effect of an essential oil combination derived from selected herbs growing wild in Turkey on broiler performance. South African Journal of Animal Science, 33, 89–94.

Alçiçek A., Bozkurt M., Çabuk M., 2004. The effect of a mixture of herbal essential oils, an organic acid or a probiotic on broiler performance. South African Journal of Animal Science, 34, 217–222.

Amad A.A., Männer K., Wendler K.R., Neumann K., Zentek J., 2011. Effects of a phytogenic feed additive on growth performance and ileal nutrient digestibility in broiler chickens. Journal of Poultry Sci, 90 (12), 2811-2816.

Bailey M.T., Lubach G.R., Coe C.L., 2004. Prenatal stress alters bacterial colonization of the gut in infant monkeys. Journal of Pediatric Gastroenterology and Nutrition, 38, 414–421.

Baker J., Brown K., Rajendiran E., Yip A., Decoffe D., Dai C., 2012. Medicinal Lavender Modulates the Enteric Microbiota to Protect Against *Citrobacter rodentium*-Induced Colitis. G825–36.

Bampidis, V.A., Christodoulou V., Florou-Paneri P., Christaki E., ChatzopoulouP.S., Tsiligianni T., Spais A.B., 2005. Effect of dietary dried oregano leaves on growth performance, carcase characteristics and serum cholesterol of female early maturing turkeys. British Poultry Science, 46, 595–601.

Black C., Haughey S.A., Chevallier O.P., Galvin-King P., Elliott C.T., 2016. A comprehensive strategy to detect the fraudulent adulteration of herbs: The oregano approach. Food Chemistry, 210, 551–557.

Bozkurt M., Küçükyılmaz K.,ÇatlıA.U., Çınar M., 2009. The effect of single or combined dietary supplementation of prebiotics, organic acid and probiotics on performance and slaughter characteristics of broilers. South African Journal of Animal Science, 39, 3.

Çabuk, M., Bozkurt M., Alçiçek A., Akbaş Y., Küçükyilmaz K., 2006. Effect of an herbal essential oil mixture on growth and internal organ weight of broilers from young and old breeder flocks. South African Journal of Animal Science, 36, 135–141.

Chen Z., He B., Zhou J., He D., Deng J., Zeng R., 2016. Chemical compositions and antibacterial activities of essential oils extracted from *Alpini aguilinensis* against selected foodborne pathogens. Industrial Crops and Products, 83, 607-613.

Choct M., 2009. Managing gut health through nutrition. British Poultry Science, 50(1), 9-15.

Criste R.D., Panaite T.D., Tabuc C., Saracila M., Soica C., Olteanu M., 2017. Effect of oregano and rosehip supplementson broiler (14-35 days) performance, carcass and internal organs development and gut health. AgroLife Scientific Journal, 6(1), 75-83.

Demir, E., Sarica S., Özcan M.A., Suicmez M., 2003. The use of natural feed additives as alternatives for an antibiotic growth promoter in broiler diets. British Poultry Science, 44, S44–S45

Ertas O.N., Güler T., Çiftçi M., Dalkihç B., Simsek Ü.G., 2005. The effect of an essential oil mix derived from oregano, clove and anise on broiler performance. International Journal of Poultry Science, 4, 879-884.

Florou-Paneri P., Palatos G., Govaris A., Botsoglou D., Giannenas I., Ambrosiadis I., 2005. Oregano Herb Versus Oregano Essential Oil as Feed Supplements to Increase the Oxidative Stability of Turkey Meat. International Journal of Poultry Science 4(11), 866-871.

Fritz, Z., Schleicher A., Kinal S., Jarosz L., Majdanski F., 1992. Substitution of antibiotics by herbs in feed mixtures for broiler chicks. Roczniki Naukowe Zootechniki, 11, 315–325.

Fuller R., 1999. Probiotics for farm animals. In: Tannock, G. W. (ed), Probiotics: a critical review. Horizon Scientific Press, Wymondham, UK, 15-22.

Gavanji S., Sayedipour S.S., Larki B., Bakhtari A., 2015. Antiviral activity of some plant oils against herpes simplex virus type 1 in Vero cell culture. Journal of Acute Medicine, 5(3), 62-68.

Giannenas, I. A., Florou-Panera P., Papazahariadou M., Christaki E., Botsoglou N.A., Spais V., 2003. Effect of dietary supplementation with oregano essential oil on performance of broilers after experimental infection with *Eimeria tenella*. Archives of Animal Nutrition, 57, 99–106.

Giannenas, I.A., Florou-Paneri P., Papazahariadou M., Botsoglou N.A., Christaki E., Spais A.B., 2004. Effect of diet supplementation with ground oregano on performance of broiler chickens challenged with *Eimeria tenella*. Archiv fur Geflugelkunde, 68, 247–252.

Halle I., 2001. Effects of essential oils and herbal mixtures on growth of broiler chicks. Pages 439–442 in Proc. Symp. Vitamine und Zusatzstoffe in der Ernährung von Mensch und Tier, Jena, Thüringen, Germany. Inst. Ernähr. Biol. Pharm. Fak, Friedrich-Schiller-Univ., Jena, Germany.

Halle I., Thomann R., Bauermann U., Henning M., Kohler P., 2004. Effects of a graded supplementation of herbs and essential oils in broiler feed on growth and carcass traits. Landbauforschung Volkenrode, 54, 219-229.

Hassan I.I., Askar A.A., El-Shourbagy V., 2004. Influence of some medicinal plants on performance: Physiological and meat quality traits of broiler chicks. Egyptian Poultry Science Journal, 24, 247–266.

Helander I.M., Alakomi H.L., Latva-Kala K., Mattila-Sandholm T., Pol I., Smid E.J.,1998. Characterization of the action of selected essential oil components on Gram-negative bacteria. Journal of Agricultural and Food Chemistry, 46(9), 3590-3595.

Horošová K., Bujňáková D., Kmet V., 2006. Effect of oregano essential oil on chicken lactobacilli and *E. coli*.Folia Microbiologica, 51, 278–80.

Hossain F., Follett P., Vu K.D., Harich M., Salmieri S., Lacroix M., 2016. Evidence for synergistic activity of plant-derived essential oils against fungal pathogens of food. Journal of Food Microbiology, 53(Part B), 24-30.

Jamroz D., Wiliczkiewicz A., Wertelecki T., Orda J., Skorupinska J., 2005. Use of active substances of plant origin in chicken diets based on maize and locally grown cereals. Journal of British Poultry Science, 46, 485–493.

Jamroz, D., T. Wertelecki, M. Houszka, and C. Kamel. 2006. Influence of diet type on the inclusion of plant origin active substances on morphological and histochemical 483 characteristics of the stomach and jejunum walls in chicken. Journal of Animal Physiology and Animal Nutrition, 90, 255-268.

Jang I.S., Ko Y.H., Yang H.Y., Ha J.S., Kim J.Y., Kang S.Y., Yoo D.H., Nam D.S., Kim N.D.H., Lee C.Y., 2004. Influence of Essential Oil Components on Growth Performance and the Functional Activity of the Pancreas and Small Intestine in Broiler Chickens. Australian Journal of Plant Physiology, 394-400.

Karimi A., Yan F.,Coto C., Park J. H., Min Y., Lu C., Gidden J. A., Lay Jr J.O., Waldroup P.W., 2010. Effects of level and source of oregano leaf in starter diets for broiler chicks. The Journal of Applied Poultry Research, 19, 137–145.

Kokkini S., Karousou R., Handilou E., Lanaras T., 2004. Essential Oil Composition of Greek (*Origanum vulgare* ssp. *hirtum*) and Turkish (*O. onites*) Oregano: a Tool for Their Distinction. Journal of Essential Oil Research, 16(4), 334-338.

Lambert G.P., 2009. Stress-induced gastrointestinal barrier dysfunction and its inflammatory effects. Journal of Animal Science 87 (E. Suppl.), E101–E108.

Lee K.W., Everts H., Kappert H.J., Frehner M., Losa R., Beynen A.C., 2003. Effects of dietary essential oil components on growth performance, digestive enzymes and lipid metabolism in female broiler chickens. British Poultry Science, 44(3), 450-457.

Licina B. Z., Stefanovic O.D., Vasic S.M., Radojevic I.D., Dekic M.S., Comic L.R., 2013. Biological activities of the extracts from wild growing *Origanum vulgare* L. Food Control, 33, 498-504.

LiHua, C., Ying Y., Yi Fu L., Lei C., 2007. Effects of oregano oil on growth performance and carcass quality of broilers. China Poultry, 29, 9–11.

Liu Y., Yan G., Chen G., Zhang J., 2009. Efficacy trials of crude extraction from Artemisia Annul L. against newcastle disease virus in vivo in Xinjiang. Modern Applied Science, 3, 176-178.

Modeva T., Profirov Y. 2003. Influence of the oregano etheric oil on weight gain and some blood biochemical indices in chickens. Zhivotnoe Nauki, 40, 59–62.

Mohiti-Asli M., Ghanaatparast-Rashti M., 2017. Comparing the effects of a combined phytogenic feed additive with an individual essential oil of oregano on intestinal morphology and microflora in broilers. Journal of Applied Animal Research, DOI:10.1080/09712119.2017.1284074.

Nurzyńska-Wierdak R., 2009. Herb yield and chemical composition of common oregano (*Origanum vulgare* L.) essential oil according to the plant's developmental stage. Herba Polonica, 55(3), 55-62.

Oviedo-Rondón E.O., Hume M.E., Hernández C., Clemente-Hernández S., 2006. Intestinal microbial ecology of broilers vaccinated and challenged with mixed *Eimeria* species, and supplemented with essential oil blends. Poultry Science, 85, 854–60.

Park S.E., Shin W.T., Park C., Hong S.H., Kim G.Y., Kim S.O., 2014. Induction of apoptosis in MDA-MB-231 human breast carcinoma cells with an ethanol extract of *Cyperus rotundus* L. by activating caspases. Oncology Reports, 32(6), 2461-2470.

Peng Q.Y., Li D.J., Li Z., Duan Z.Y., Wu Y.P., 2016. Effects of dietary supplementation with oregano essential oil on growth performance, carcass traits and jejunal morphology in broiler chickens. Animal Feed Science and Technology, 214, 148-153.

Placha I., Chrastinova L., Laukova A., Cobanova K., Takacova J., Strompfova V., 2013. Effect of thyme oil on small intestine integrity and antioxidant status, phagocytic activity and gastrointestinal microbiota in rabbits. Acta Vet. Hung., 61, 197–208.

Puvaca N., Stanacev V., Glamocic D., Levic J., Peric L., Milic D., 2013. Beneficial effects of phytoadditives in broiler nutrition. World's Poultry Science Journal, 69(01), 27-34.

Roofchaee A., Irani M., Ebrahimzadeh M.A., Akbari M.R., 2011. Effect of dietary oregano (*Origanum vulgare* L.) essential oil on growth performance, cecalmicroflora and serum antioxidant activity of broiler chickens. African Journal of Biotechnology, 10(32), 6177-6183.

Sepahvand R., Delfan B., Ghanbarzadeh S., Rashidipour M., Veiskarami G.H., Ghasemian-Yadegari J., 2014. Chemical composition, antioxidant activity and antibacterial effect of essential oil of the aerial parts of *Salvia sclareoides*. Asian Pacific Journal of Tropical Medicine, 7(Suppl 1), S491-S496.

Shaaban H.A.E., El-Ghorab A., Shibamoto T., 2012. Bioactivity of essential oils and their volatile aroma components: Review. Journal of Essential Oil Research, Volume 24, Issue 2: Chemistry and Biology of Citrus Essential Oils.

Steiner T., 2009. Phytogenics in Animal Nutrition-Natural Concepts to Optimize Gut Health and Performance. Nottingham University Press, Nottingham, UK.

Suzuki K., Harasawa R., Yoshitake Y., Mitsuoka T., 1983. Effects of crowding and heat stress on

intestinal flora, body weight gain, and feed efficiency of growing rats and chicks. Japanese Journal of Veterinary Science, 45(3), 331-338.

Tiihonen K., Kettunen H., Bento M.H., Saarinen M., Lahtinen S., Ouwehand A.C., 2010. The effect of feeding essential oils on broiler performance and gut microbiota. British Poultry Sciece, 51, 381–92.

Windisch W., Schedle K., Plitzner C., Kroismayr A., 2008. Use of phytogenic products as feed additives for swine and poultry. Journal of Animal Science, 86, E140–E148.

Yanishlieva N. V., Marinova E. M., 1995. Antioxidant activity of selected species of the family *Lamiaceae* grown in Bulgaria. Die Nahrung, 39, 458–463.

Yanishlieva N.V., Marinova E.M., Gordon M.H., Raneva V.G., 1999. Antioxidant activity and mechanism of action of thymol and carvacrol in two lipid systems. Food Chemistry, 64, 59–66.

Zeng Z., Zhang S., Wang H. Piao X., 2015. Essential oil and aromatic plants as feed additives in non-ruminant nutrition: a review. Journal of Animal Science and Biotechnology, 6, 7.

***2015. The management guide of Cobb 500 hybrid (http:// www.cobb-vantress.com).

Scientific Papers. Series D. Animal Science. Vol. LXI, Number 1, 2018
ISSN 2285-5750; ISSN CD-ROM 2285-5769; ISSN Online 2393-2260; ISSN-L 2285-5750

EFFECT OF DIETARY SUPPLEMENTATION WITH DIFFERENT LEVELS OF L-CARNITINE ON PRODUCTIVE AND ECONOMIC PERFORMANCE OF BROILER CHICKENS

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Abstract

The experiment was conducted between March 8th, 2017 and April 26th, 2017 at the Poultry Farm of Animal Sciences Department, College of Agricultural Sciences, Sulaimani University to investigate the effects of dietary supplementation with different levels of L-carnitine on economic productivity and performance of broiler chickens. By using 260 one-day old of Ross 308 broiler chicks, divided into 5 treatments and 4 replicates based on completely randomized design for 49 days. Feed and water were providedas ad libitum. Chicks were divided into five treatments 52 birds for each treatment. Each treatment contained four replicates of 13 birds. Dietary L-carnitine was added to the diet from the first day to the end of experimental which lasted 49 days at levels of 0% (Control), 0.01% (T1), 0.02% (T2), 0.04% (T3) and 0.08% (T5). The body weight had significantly ($p<0.05$) affected by L-carnitine supplementation at period 6 and 7, feed intake at 6th, 7th and 8th period, L-carnitine had a significantly ($p<0.05$) effect on weight gain at 6th and 8th period, it had significant effect on feed conversion ratio at 5th and 6th period. While L-carnitine had no significant effect on the overall body weight, weight gain, feed intake and feed conversion ratio at the final of the experiment. However, L-carnitine had no significant effect on dressing percentage with and without giblets while it had a significantly ($p<0.05$) effect on abdominal fat at T5 compare to other treatments. In addition, there were no significant effects of treatments on the economic index (European Production Efficiency Factor and European Broiler Index).

Key words*: broiler chicks, diet, L-carnitine, performance, productive.*

INTRODUCTION

Poultry meat is nutritionally desirable because of its high-quality protein and low-fat content (Laudadio et al., 2012). Since poultry meat is an important source of high-quality protein, minerals, and vitamins to balance the human diet, poultry industry continues to play a positive role in the whole world as the major supplier for animal protein. Due to an increasing consumer demand for the lean tissue, the production of broiler meat that contains body fat is among one of the problems for the poultry meat industry (Daşkiran, 1996). This strategy increases the rate of growth and feed conversion but had undesirable influence in the form of increased abdominal fat, as a result, increased carcass fat levels can reduce the profits of poultry producers (Michalczuk et al., 2012). It is necessary to look at the development of chicksdiet that will meet the nutrient requirements of the bird more precisely for optimum growth and increased performance. The effect of nutrition and genetics on fat deposition is higher than environmental factors (Lin et al., 1980). Thus, livestock researchers and producers tend to evaluate ad try new feed additives that can be beneficial to poultry performance and production. This presents a large opportunity for the use of a recent physiological feed additive L-carnitine.

L-carnitine was needed to transport long-chain fatty acids into mitochondria, it takes part in β-oxidation which leads to theproduction of energy (Carter et al., 1995; Brooks, 1998). L-carnitine has two major functions. The best-known function is to facilitate the transport of long-chain fatty acids across the inner mitochondria membrane. L-carnitine also helps the removal of short and medium-chain fatty acids from the mitochondria that produced as a result of normal and abnormal metabolism (Matalliotakis et al., 2000; Buyse et al. 2001; Xu et al., 2003). Alterations in carnitine concentration or metabolism may significantly

affect energy production in mitochondria (Arslan et al., 2003). In addition, L-carnitine has secondary functions, including the containment, buffering and removal of potentially toxic acyl groups from cells, equilibratingthe ratio of free CoA and acetyl-CoA between the mitochondria and cytoplasm, participating in biological processes such as regulation of gluconeogenesis, stimulating fatty acid and the metabolism of ketones, branched-chain amino acids, triglycerides and cholesterol (Novotny, 1998; Corduk et al., 2007).

Some studies suggested that supplemental L-carnitine improved body weight gains and decreasedfat content deposition of chickens (Rabie et al., 1997a; Rabie and Szilagyi, 1998; Xu et al., 2003). According to these results, the aim of this study was to examine the overall performance, carcass parameters, abdominal fat and economic production of the addition of L-carnitine at different levels to broiler rations.

MATERIALS AND METHODS

This study was conducted at the Bakrajo Poultry Breeding Field, Animal Sciences Department, College of Agricultural Sciences, the Sulaimani University between March 8th 2017 and April 25th, 2017 to study the effect of dietary supplementation with different levels of L-carnitine (0, 100, 200, 400, 800 mg/kg) on the performance and carcass parameters of Ross 308 broiler chickens.

Two hundred and sixty one-day-old Ross 308 broiler chicks were obtained from Lawa Hatchery in Arbil Province and were randomly distributed into five treatment groups (52 chicks for each group) with four replicates (Table 1). Chicks were raised on floor cages (110×120×60 cm); and lighting was continuous (24 hours/day) at starter period (21 hours/day) at grower and (24 hours/day) at finisher periods.

Table 1. The Experimental treatments

Treatment	Feeding system
T1 (control)	Feed with 0 mg/kg L-carnitine
T2	Feed with 100 mg/kg L-carnitine
T3	Feed with 200 mg/kg L-carnitine
T4	Feed with 400 mg/kg L-carnitine
T5	Feed with 800 mg/kg L-carnitine

Temperature and humidity of the rooms were measured by electronic thermometers that were placed at different locations of the room about 50-60 cm above the floor level.

Feeding program

Feed and water were providing *ad libitum* during the experimental period. The diets were determined according to NRC (1994). The nutrition substances were as follows: Starter feed: (CP = 22.8% and ME = 3,079 kcal/ kg) between 1-11 days of age; Growth feed: (CP = 21.0% and ME = 3,139kcal/ kg) between 11-28 days of age; Finisher feed: (CP =19.1% and ME = 3,212 kcal/ kg) between 29-49 days.

Ingredients composition of commercial feed were soybean meal, wheat, yellow corn, sunflower seed oil, limestone, vitamin, minerals, salt (NaCl), and calcium phosphate (Table 2).

Production Traits

Live Body Weight

Birds weighted every week at day 1, 7, 14, 21, 28, 35, 42, 49 of broilers age by the following:

Body Weight = weight of the birds (g)/number of birds

Weight Gain

The average daily body weight gain was calculated by subtracting the average initial live weight of a certain period (which was usually weekly) from the average final live weight of the same period for each chick.

Feed Intake

Feed intake in each replicate was measured and recorded at the end of each week by subtracting feed residual from the total amount of feed supplied by the following formula:

Feed Intake Weekly = The feed intake (g/week)/(number of birds)

Feed Conversion Ratio

Feed Conversion Ratio is the amount of feed intake estimated to unit weight for each weight gain estimated in the same unit and calculated by the following formula:

Feed Conversion Ratio = Average of feed intake by one bird in a week (kg)/Average of weight gain by one bird in the same week (kg).

Table 2. Ingredient of the composition of commercial feed used in the experiment

Ingredients %	Period		
	Starter	Grower	Finisher
Yellow corn	32	32	35
Soybean meal	34	28	22.5
Protein conc.*	5	5	5
Wheat	24.3	30.2	32.5
Sunflower oil	3.5	3.5	3.7
Limestone**	1	1.2	1.2
Salt	0.2	0.1	0.1
Total	100	100	100
	Calculated composition***		
Protein	22.8	21	19.1
ME Kcal / Kg	3079	3139	3212
Calcium	0.76	0.82	0.81
Fiber	3.7	3.5	3.3
Lys.	1.34	1.19	1.04
Me.	0.89	0.83	0.77
Fat	5.6	5.6	6.0

* Protein concentrate used in the diets were produced in Holland (WAFI) which contains: 40% crude protein, 2100 Kcal ME / Kg, 5% crude fat, 2% crude fiber, 6.5% calcium, 2.50% phosphorus, 3.85% lysine, 3.70% methionine, and 4% cystine.
** Limestone:
*** The calculated composition of the diets was determined according to NRC (1994).

Mortality

Mortality is the ratio of number of died birds tototal number of birds of each treatment and calculated weekly by the following formula:

Mortality = [(number of died birds)/(total number of birds)] × 100

Carcass Traits

At the end of the experiment, 8 birds from each treatment (2 male and 2 female birds from each replicate) were randomly chosen for slaughter and evaluation carcass traits, dressing percentages educated with or without giblets and abdominal fat were determined as follows:

Dressing percentage with giblets = (Carcass weight with giblets/Live body weight) × 100

Dressing percentage without giblets = (Carcass weight without giblets/Live body weight) × 100

Abdominal fat percentage = (abdominal fat /live body weight) × 100

Economic Efficiency

Economic Efficiency of the experiment was calculated according to following equations:

Viability (%) = (number of live bird at final day/number of live bird at first day) × 100

European Production Efficiency Factor = (viability (%) × body weight (kg)/age (day) × feed conversion ratio) × 100

European Broiler Index = (viability (%) × average daily gain (g/check/day)/feed conversion ratio) × 10

Statistical Analysis

General Linear Model (GLM) within the statistical program XLSTAT (2004, version-7.5) was used to analyze treatments and periods affecting productive traits within the factorial Completely Randomized Design (CRD).

The significant differences between means of traits were determined using Duncan's multiple range test under the probability ($p<0.05$) (Duncan, 1955). The total variance was partitioned into main effects and their interaction according to the following model:

$Yij = \mu + Ti + Pj + TP\ ij + eij$

Where:

Yij= Observation of the performance traits.

μ = Overall mean.

Ti = Effect of treatments (T1 0%, T2 0.01%, T3 0.02%, T4 0.04%, T5 0.08%)

Tj = Effect of periods (day 1, 7, 14, 21, 28, 35 and 42 of age).

TDij = Interaction between treatments and periods.

eij= Random error assumed to be equal to zero and variance is $б^2e$ (N~ 0, б2e)

RESULTS AND DISCUSSIONS

The Effect of Treatments and Sex on Live Body Weight, Carcass, Clear Carcass and Abdominal Fat at Day 49 old Broiler Chickens.

Effect of treatments and sex at final period of experiment day (49) on live body weight,

carcass (g and %) and clear carcass (g and %) was not significant (Table 3). While abdominal fat (g and %) was significantly ($p<0.05$) affected by treatments. Females in T3 had significantly ($p<0.05$) higher abdominal fat (43.75g) when compared with other males and females in same or other treatments except for males and females in T4. On the other hand, females in T2 and T3 had significantly ($p<0.05$) higher abdominal fat (g and %) than males in same treatments. While abdominal fat (%) of females in T3 was significantly ($p<0.05$) higher than all females and males in other treatments. In general, the results revealed that abdominal fat (g and %) of males and females in T5 were significantly ($p<0.05$) or numerically lower than males and females in other treatments. Those results were similar to my results (Lien and Horng, 2001; Celik and Ozturkcan, 2003). All have shown that carcass weight and carcass yield of broilers was not affected by diet supplementation. Sarica et al., (2005) reportednon-significant effect of dietary L-carnitine on carcass weight in Japanese quail fed diet, contained 200 mg LC/kg. Barker and Sell (1994) showed that L-carnitine supplementation (0, 50 and 100 mg/kg) had no effect on performance and carcass composition of broilers and young turkeys fed with low- and high-fat diets. Zhang et al. (2010) and Michalczuk et al., (2012) reported non-significant increase in carcass yield by dietary supplementation of L-carnitine. Bozkurt et al., (2008) indicated that adding % 5 animal or vegetable fat in broiler breeder hens and males diet had no significant effect on performance at 22, 34, 46 and 58 weeks of age. Some studies have shown that supplemental L-carnitine had a significant effect to reduce abdominal fat content of broilers (Lettner et al., 1992; Markwell et al., 1973; Marquis and Fritz, 1965). L-carnitine supplementary to the diet had a positive effect to decrease the abdominal fat of carcasses on males (Rabie et al., 1997a; Rabie et al., 1997b; Rabie and Szilagyi, 1998; Xu et al., 2003). Burtle and Liu (1994) indicated that L-carnitine supplementation to diets increases fat metabolism and decreases abdominal fat. Parsaeimehr et al. (2014) showed that supplementing L-carnitine (300 mg/kg) significantly decreased the abdominal fat percentage of broiler chickens.

The Effect of Treatments on Weight Gain, Feed Intake and Feed Conversion Ratio From 1-49 Day-Old

Table 4 indicates that there was not any significant effect of treatments on weight gain, feed intake and feed conversion ratio at day 49. Nevertheless, feed conversion ratio was numerically better in T4 followed by T2, which had better weight gain compared toother treatments. These results are in agreement with those reported by other authors for broiler chickens (Buyse et al., 2001; Barker and Sell, 1994; Cartwright, 1986; Leibetseder, 1995). Lien and Horng (2001) showed that growth performance of broilers, in terms of body weight and feed intake, were not affected from diet supplemented feeding with 0.05% L-carnitine from 5 to 7 weeks of age. Buyse et al. (2001) and Rezaei et al. (2007), found that L-carnitine supplemented to chickens had no effect on feed conversion, feed intake, and weight gain.

Murali et al. (2015) showed that dietary L-carnitine (900 mg/kg diet) supplementation had no effect on feed consumption in broilers during growing period (0-6 wks.). Xu et al. (2003) observed that dietary supplementation of L-carnitine to commercial male broilers at 0, 25, 50, 75, or 100 ppm had no significant effect on daily body gain or feed conversion. Corduk et al. (2007), Sarica et al. (2007) and Daşkiran et al. (2009) revealed that various levels of L-carnitine did not affect body weight gain and feed intake of quails. Deng et al. (2006) found that short-term supplementation of L-carnitine at levels of 0 (control), 100 or 1000 ppm for chickens after hatching for 4 weeks did not have any effect on growth rates, feed intake or feed utilization efficiency. Yalçin et al. (2008) revealed that L-carnitine supplementation at 100 mg/kg had no significant effect on feed intake and feed conversion ratio. Sarica et al. (2005) showed that supplementation of L-carnitine (25-100 mg/kg) had no significant effect on daily body gain from commercial male broilers. Arslan et al. (2003 and 2004) reported that L-carnitine administration via drinking water 100 mg/l to Turkish native geese and 200 mg/l to Turkish native duck had no significant effect on growth performance on ducks and geese.

Table 3. The effect of treatments and sex on Live Body Weight, Carcass (g and %), Clear Carcass (g and %) and Abdominal Fat (g and %) at day 49 of age of broiler chickens

		Traits						
Treatments	Gender	Live BW	Carcass, g	Carcass, %	Clear Carcass, g	Clear Carcass, %	Abdominal Fat, g	Abdominal Fat, %
T1(0 mg/kg)	Male	2412.50±51.53	2018.75±46.79	85.91 ±0.52	1633.7 ±48.19	67.67 ±0.59	32.50b ±1.44	1.35b ±0.06
	Female	2400.00±61.23	1967.50±89.33	84.00±1.72	1275.00 ±428.61	52.35±17.46	32.50b±1.44	1.36b ±0.06
T2(100 mg/kg)	Male	2487.50±89.84	2056.25±80.42	82.64 ±0.72	1695.00 ±67.11	68.13 ±0.85	22.50c ±2.50	0.90c ±0.09
	Female	2375.00±101.3	1967.50±92.59	82.89 ±0.36	1601.25 ±88.70	67.32 ±0.84	33.75b ±2.39	1.42b ±0.04
T3(200 mg/kg)	Male	2462.50±96.55	2040.00±76.45	82.86 ±0.53	1686.25 ±80.24	68.41 ±0.80	30.00bc ±4.08	1.22bc ±0.17
	Female	2300.00±67.70	1873.75±64.07	81.44 ±0.79	1543.75 ±69.11	67.03 ±0.98	43.75a ±3.14	1.90a ±0.13
T4(400 mg/kg)	Male	2550.00±95.74	2151.25±113.42	84.28 ±2.17	1790.00 ±107.14	70.10 ±2.45	36.25ab ±3.75	1.42b ±0.14
	Female	2462.50±134.43	1867.50±293.55	75.09 ±9.56	1725.00 ±92.28	70.07 ±0.73	37.50ab ±3.22	1.52b ±0.08
T5(800 mg/kg)	Male	2525.00±110.88	2127.50±127.64	84.09 ±1.72	1780.00 ±120.98	70.29 ±2.03	22.50c ±1.44	0.89c ±0.04
	Female	2425.00±118.14	1961.25±155.33	81.66 ±7.03	1646.25 ±97.11	68.58 ±6.04	22.50c ±4.78	0.92c ±0.18

[a-j] Means followed by different letters are statistically different.

Table 4. The effect of treatments on weight Gain, feed intake and feed conversion ratio from day 1-49

Treatments	Feed intake (g)	Weight gain (g)	FCR
T1 (0)	4004.96±49.62	2358.75±36.99	1.70±0.02
T2 (100 mg)	4016.79±42.95	2384.50±91.38	1.69±0.07
T3 (200 mg)	4123.34±100.87	2335.75±52.16	1.77±0.04
T4 (400 mg)	4056.74±58.45	2459.50±82.52	1.66±0.07
T5 (800 mg)	4189.48±112.47	2430.00±93.72	1.74±0.11

The Effect of Treatments on Economic Efficiency

Table 5 revealed that there was no significant effect of treatments on economic efficiency (European Production Efficiency Factor and European Broiler Index), while T4 at (EPEF and EBI) had a higher number compared to control and other treatments but there was no significant ($P<0.05$) effect on treatments.

Table 5. The effect of treatments on Economic Efficiency

Treatment	European Production Efficiency Factor	European Broiler Index
T1 (0 mg)	250.51±13.27	245.56±13.01
T2 (100 mg)	286.50±30.89	281.02±30.44
T3 (200 mg)	254.43±15.71	249.57±15.45
T4 (400 mg)	301.54±29.84	295.97±29.53
T5 (800 mg)	282.22±36.20	277.16±35.75

The Effect of Interaction Between Treatments (Different Levels of L-Carnitine) and Periods on Body Weight

Effect of interactions between treatments and periods on body weight was shown in Table 6. The body weight increased with the increase of age periods, whereat effect of all treatments on body weight at P8 was significantly ($p<0.05$) higher than the same treatment prior to the period. Moreover, the effect of treatments was significant ($p<0.05$) at P6 and P7. At P6 significantly ($p<0.05$) higher body weight was obtained by birds in T4 followed by T5 compared to T3 and T1, respectively. Additionally, birds in T2 had significantly ($p<0.05$) higher body weight compared to birds in T1 at P6. While birds at P6 in T4 revealed significantly higher body weight compared to all other treatments except T5. There were no significant differences between treatments at other periods, although numerically higher body weight was obtained from birds in T4 followed by T5 at all other periods except P1 and P2. Hrnčár et al. (2015) also reported significant effect of L. carnitine on body weight at day 28, 35 and 42 compared to control. Rabie and Szilagyi (1998) and Buyse et al. (2001) also reported that supplementation of L-carnitine had a significant effect on body weight of chickens at the end of fattening period. While, Hrnčár et al. (2015) showed that supplementation of L-carnitine did not affect body weight at P1, P2, P3, and P4 periods. Buyse et al. (2001) reported a non- significant increase in average body weight of chickens receiving L-carnitine at 14, 21 and 28 days of rearing. Rabie et al. (1997a) reported that L-carnitine supplementary to diets had no significant impact on body weight of broilers at the end of the experimental period.

Table 6. Effect of interaction between treatments and periods on body weight

Periods (Days)	Treatments (different levels of L- carnitine)				
	T1 (0 mg)	T2 (100 mg)	T3 (200 mg)	T4 (400 mg)	T5 (800 mg)
P1 (1)	47.50^{j}±1.55	46.75^{j}±1.03	45.50^{j}±0.86	46.75^{j}±0.94	45.00^{j}±1.00
P2 (7)	104.14^{ij}±2.27	107.31^{ij}±1.03	100.96^{ij}±1.55	107.98^{ij}±3.44	107.79^{ij}±3.18
P3 (14)	205.00^{i}±5.66	216.71^{i}±5.77	199.60^{i}±7.40	221.11^{i}±8.55	221.44^{i}±3.09
P4 (21)	389.63^{h}±5.64	428.39^{h}±14.28	412.13^{h}±7.13	444.00^{h}±14.23	441.75^{h}±13.92
P5 (28)	695.31^{g}±14.74	728.12^{g}±32.32	687.50^{g}±25.25	781.25^{g}±34.04	768.75^{g}±25.25
P6 (35)	1125.00^{f}±40.50	1300.00^{de}±81.17	1215.63^{ef}±60.89	1371.88^{d}±59.37	1346.88^{d}±43.41
P7 (42)	1734.38^{c}±51.12	$1859.38b^{c}$±106.23	$1762.50b^{c}$±55.66	1890.63^{b}±55.05	1862.50^{bc}±61.66
P8 (49)	2406.20^{a}±37.32	2431.25^{a}±92.06	2381.25^{a}±52.41	2506.25^{a}±81.88	2475.00^{a}±93.54

$^{a-j}$ Means followed by different letters are statistically different at $p<0.05$

The Effect of Interactions Between Treatments (Different Levels of L-Carnitine) and Periods on Feed Intake.

Table 7 summarizes the significant ($p<0.05$) effect of interactions between treatments and periods on feed intake. Effect of treatments on feed intake at P6, P7, and P8 was significant ($p<0.05$), where significantly ($p<0.05$) higher feed intake were observed on birds in T5 at P6 which was also significantly ($p<0.05$) higher than other treatments in the same period. The first group was followed by birds in T3 and T5

at P7 which was significantly ($p<0.05$) higher than T1. The birds at P8 in T2 had significantly ($p<0.05$) lower feed intake than T3 and numerically lower intake when compared with other treatments in thesame period. Sayed et al. (2001) showed that supplementation of L-carnitine (50 mg/ kg) to diet containing 2 and 4% of sunflower oil increased feed intake, and Rabie et al. (1997a) demonstrated that L-carnitine supplementation (50, 100 and 150 mg/kg) had significant effects on feed intake.

Bayram et al. (1999) noticed there was a significant improvement in feed intake in quails fed ona diet supplemented with 500 mg LC/kg. However, the effect of treatments on feed intake at P2, P3, P4 and P5 were not significant. Rezaei et al. (2010) also found supplementation of L-carnitine had no significant effect on feed intake in the broiler chickens. Xu et al. (2003) reported that the supplementation of dietary L-carnitine had no significant effect on feed intake of broiler chickens and young turkeys. Barker and Sell (1994); Leibetseder (1995) and Buyse et al. (2001) reported that the supplementation of dietary L-carnitine did not affect feed intake. Lien and Horng (2001) Sarica et al. (2005) reported that L-carnitine supplement on diet had no significant effect on feed intake of Quail. Yalçin et al. (2008) indicated that L-carnitine supplementation at 100 mg/kg did not affect feed intake.

Table 7. Effect of interaction between treatments and period on feed intake

Periods (Days)	Treatments (different levels of L- carnitine)				
	T1 (0 mg)	T2 (100 mg)	T3 (200 mg)	T4 (400 mg)	T5 (800 mg)
P2 (7)	92.98^{i}±1.17	90.48^{i}±1.88	92.69^{i}±4.33	96.92^{i}±1.81	97.12^{i}±2.77
P3 (14)	184.62^{h}±2.91	195.14^{h}±4.30	186.54^{h}±3.09	194.90^{h}±2.52	196.34^{h}±4.61
P4 (21)	292.64^{g}±11.74	337.07^{g}±4.89	335.48^{g}±2.88	343.00^{g}±9.68	339.62^{g}±10.91
P5 (28)	613.99^{f}±6.87	657.76^{f}±18.63	647.33^{f}±15.69	671.55^{f}±13.75	645.48^{f}±10.76
P6 (35)	911.21^{cde}±19.75	902.97^{cde}±9.03	912.69^{cde}±12.45	908.26^{cde}±13.25	1020.38^{a}±51.95
P7 (42)	916.23^{cde}±34.34	959.01^{abc}±16.24	1006.29^{a}±41.40	960.70^{abc}±19.04	988.31^{ab}±37.86
P8 (49)	914.77^{cde}±34.07	874.36^{e}±20.04	942.31^{bcd}±36.82	881.41^{de}±20.35	902.24^{cde}±36.57

[a-j] Means followed by different letters are statistically different at $p<0.05$

The Effect of İnteraction Between Treatments (Different Levels of L-Carnitine) and Periods of Weight Gain

Influence of interaction between treatments and periods on weight gain was significant ($p<0.05$) as described in Table 8. Weight gain at P8 was significantly ($p<0.05$) higher compared to other periods followed by P7, P6, P5, P4, P3, and P2 of all treatments, respectively. Moreover, the highest weight gain was obtained by birds in T1 at P8 which significantly ($p<0.05$) differed from T2 at thesame period. While, at P7 birds in T1 had significantly ($p<0.05$) higher weight gain compared to T5 and numerically higher weight when compared with other treatments, although birds at P6 in T1 had significantly ($p<0.05$) lower weight gain when compared with all other treatments (Table 8). A number of studies hasshown that supplemental L-carnitine improved body weight gains of broilers (Lettner et al., 1992; Gropp et al., 1994; Rabie et al., 1997a). According to Parsaeimehr et al (2014), diet with L-carnitine significantly ($P<0.01$) increased the body weight gain of broiler chicks during the period between 28 to 42 days of age. Taklimi et al. (2015) reported that supplementation of 600 to 800 mg/kg L-carnitine in diet had significant increases on weight gain for broiler chickens. Abdel-Fattah et al. (2014) noticed that supplementation of L-carnitine (200-400 mg/kg) in Japanese quail diet significantly increased body weight gains, while, theeffect of treatment on weight gain at P2, P3, P4, and P5 were not significant. Parsaeimehr et al. (2014) reported that experimental diets with L-Carnitine had no effect on body weight gain in theperiodbetween 1-21days of age. Corduk et al. (2007), Sarica et al. (2007) and Daşkiran et al. (2009) reported that various levels of L-carnitine did not affect body weight gain over 28 days of the experimental period. Xu et al. (2003) indicated that dietary supplementation of L-carnitine to broilers had no significant effect on daily body weight gain. Barker and Sell (1994) also reported thenon-significant effect of l-carnitine on body weight gain from their study.

Table 8. Effect of interaction between treatments and periods on weight gain

Periods (Days)	Treatments (different levels of L- carnitine)				
	T1 (0 mg)	T2 (100 mg)	T3 (200 mg)	T4 (400 mg)	T5 (800 mg)
P2 (7)	59.81^{k}±2.51	57.39^{k}±1.39	55.46^{k}±0.93	61.23^{k}±3.65	62.79^{k}±2.82
P3 (14)	97.69^{jk}±5.73	112.57^{jk}±5.28	98.64^{jk}±6.24	113.13^{jk}±6.67	113.65^{jk}±4.72
P4 (21)	184.63^{ij}±5.30	211.61^{hi}±12.04	212.53^{hi}±7.37	222.89^{ghi}±5.87	220.31^{ghi}±12.62
P5 (28)	305.69^{fg}±14.47	299.81^{fgh}±21.01	275.38^{fgh}±18.84	337.25^{f}±20.66	327.00^{f}±12.69
P6 (35)	429.69^{e}±54.44	571.88^{bcd}±50.80	528.13^{bcd}±38.31	590.63^{abcd}±27.18	578.125^{bcd}±36.57
P7 (42)	609.38^{abc}±19.34	559.38^{bcd}±27.18	546.88^{bcd}±7.86	518.75^{cd}±40.34	515.625^{d}±26.70
P8 (49)	671.88^{a}±43.11	571.88^{bcd}±51.12	618.75^{ab}±44.04	615.63^{ab}±30.77	612.50^{ab}±72.52

[a-j] Means followed by different letters are statistically different at p<0.05

The Effect of Interaction between Treatments (Different Levels of L- Carnitine) and Periods on Feed Conversion Ratio.

The effects of interaction between treatments and periods on feed conversion ratio were summarized in Table 9. There was no significant difference between all treatments at all periods except at P5 and P6. Whereat, birds in T3 had significantly (p<0.05) lower feed conversion ratio compared to T5.

While birds at P6 in T1 and T3 had significantly (p<0.05) lower feed conversion ratio when compared with all other treatments in the same period. A better and significant (p<0.05) feed conversion ratio was obtained at P8 of all treatments followed by below periods. Significantly (p<0.05) better-feed conversion ratios was obtained on birds in T1 at P8.

While lower feed intake levels were obtained by birds in T3 at P5.Parsaeimehr et al (2013) and Schuhmacher et al. (1993) showed that diet with L-carnitine had a significant effect on feed conversion ratio. Barker and Sell, (1994) and Xu et al. (2003) reporteda diet with levels of animal fat + 300 mg/kg L-carnitine, which had a significant (P<0.05) effect on feed conversion ratio.

Bayram et al. (1999) reported significant decreases in feed efficiency in quails supplemented with 500 mg/kg diet L-carnitine. Parsaeimehr et al. (2014) reported that a dietary L-carnitine supplementation (200-300 mg/kg) had asignificant effect in improving feed conversion. While Buyse et al. (2001) and Rezaei et al. (2007) found that L-carnitine had no effect on feed conversion to chickens.

Effect of L-carnitine on feed conversion efficiency in geese at P1, P2, P3, P4, P8 were not significant (Arslan et al., 2004).

Leibetseder (1995) investigated the broilers fed with diets supplemented with 0 or 50 g fat/kg. and he found that feed conversion of broilers was not influenced by dietary carnitine (L or DL form) at a dosage of 200 mg/kg diet.

Table 9. The effect of interaction between treatments and periods on Feed Conversion Ratio

Periods (Days)	Treatments (different levels of L- carnitine)				
	T1 (0 mg)	T2 (100 mg)	T3 (200 mg)	T4 (400 mg)	T5 (800 mg)
P2 (7)	1.56^{efgh}± 0.05	1.58^{efgh}± 0.05	1.67^{cdefgh}± 0.05	1.60^{defgh}± 0.07	1.55^{fgh}± 0.02
P3 (14)	$1.91b^{cdef}$± 0.11	1.74^{cdefgh}± 0.07	1.92^{bcdef}± 0.14	1.740^{cdefgh}± 0.10	1.73^{cdefgh}± 0.03
P4 (21)	1.59^{efgh}± 0.07	1.61^{cdefgh}± 0.11	1.58^{efgh}± 0.04	1.54^{fgh}± 0.01	1.55^{fgh}± 0.04
P5 (28)	2.02^{abc}±0.10	2.23^{ab}±0.18	2.39^{a}±0.17	2.01^{abcd}±0.13	1.99^{bcde}±0.10
P6 (35)	2.23^{ab}±0.28	1.62^{cdefgh}±0.14	1.76^{cdefgh}±0.14	1.55^{fgh}±0.09	1.79^{cdefgh}±0.16
P7 (42)	1.54^{fgh}±0.07	1.73^{cdefgh}±0.11	1.84^{bcdefg}±0.05	1.90^{bcdef}±0.20	1.93^{bcdef}±0.11
P8 (49)	1.37^{h}±0.05	1.57^{efgh}±0.13	1.54^{fgh}±0.08	1.45^{gh}±0.10	1.56^{fgh}±0.23

[a-j] Means followed by different letters are statistically different p<0.05

CONCLUSIONS

The results of the present study showed that dietary supplementation with different levels of L-carnitine had significant effect on body weight at 6^{th} and 7^{th} period, there was a significant effect on weight gain at 6^{th} and 8^{th} period, on feed intake at 6^{th}, 7^{th} and 8^{th} period and L-carnitine had significant effect on feed conversion ratio at 5^{th} and 6^{th} periods. L-carnitine had a significant effect to reduce abdominal fat but there was no significant effect on thecarcass with giblet and without giblet. L-carnitine had no significant effect on body weight, feed intake, weight gain and feed conversion ratio at the final of the experimental

period.Using 0.08% (T5) L-Carnitine group seemed to have a beneficial effect on most of the performance traits (live body weight, feed intake, feed conversion ratio, weight gains and abdominal fat).

RECOMMENDATIONS

For the better production performance, we recommend the use of 0.04% L-carnitine on (T4) and 0.08% on (T5) and wait until 8 weeks of age to obtain the better production in broiler chicken.

REFERENCE

Abdel-Fattah S.A., El-Daly E.F., Ali N.G., 2014. Growth performance, immune response, serum metabolites and digestive enzyme activities of Japanese quail fed supplemental L-carnitine. Global Veterinaria, 12, 277-286.

Arslan C., Citil M., Saatci M., 2003. Effects of L-carnitine administration on growth performance, carcass traits, blood serum parameters and abdominal fatty acid composition of ducks. Archives of Animal Nutrition, 57(5), 381-388.

Arslan C., Citil M., Saatci M., 2004. Effect of L-carnitine administration on growth performance, carcass traits, serum lipids and abdominal fatty acid compositions of geese. Revue de médecine vétérinaire, 155, 315-320.

Barker D.L., Sell J.L., 1994. Dietary carnitine did not influence performance and carcass composition of broiler chickens and young turkeys fed low-or high-fat diets. Poult. Sci., 73, 281-287.

Bayram İ., Akıncı Z., Uysal, H., 1999. The effects of L-carnitine and vitamin C supplementation to quail rations on performance and egg production (in Turkish, with English abstract). Y. Y. Ü. Vet. Fak. Derg. 10, 32–37.

Bozkurt M., Cabuk M., Alcicek A., 2008. Effect of dietary fat type on broiler breeder performance and hatching egg characteristics. The Journal of Applied Poultry Research, 17(1), 47-53.

Brooks G.A., 1998. Mammalian fuel utilization during sustained exercise. Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology, 120, 89–107.

Burtle G.J., Liu Q.H., 1994. Dietary carnitine and lysine af-fect channel catfish lipid and protein composition. J. World Aquaculture Soc., 25, 169-174

Buyse J., Janssens G.P., Decuypere J., 2001. The effects of dietary L-carnitine supplementation on the performance, organ weights and circulating hormone and metabolite concentrations of broiler chickens reared under a normal or low temperature schedule. In British Poultry Science, 42, 230–241.

Carter A.L., Abney T.O., Lapp D.F., 1995. Biosynthesis and metabolism of carnitine. Journal of Child Neurology, 10, 3-7.

Cartwright A.L., 1986. Effect of carnitine and dietary energy concentration on body weight and body lipid of growing broilers. Poult. Sci., 65(1), 21.

Celik E., Ozturkcan O., 2003. Effects of dietary supplemental L-carnitine and ascorbic acid on performance, carcass composition and plasma L-carnitine concentration of broiler chicks reared under different temperature. In Archives of Animal Nutrition, 57, 27–38.

Corduk M., Ceylan N., Ildiz, F., 2007. Effects of dietary energy density and L- carnitine supplementation on growth performance, carcass traits and blood parameters of broiler chickens. In South African Journal of Animal Science, 37(2), 65–73.

Daşkiran M., 1996. Dose effects of L-carnitine on performance, carcass characteristics and body fat composition of 19, 35, and 49-day old broiler chickens (Doctoral dissertation, Oklahoma State University).

Daşkıran M., Önol A.G., Cengiz Ö., Tatlı O., Sarı M., 2009. Effects of dietary methionine levels and L-carnitine supplementation on performance and egg quality parameters of layers. Journal of Animal and Feed Sciences, 18(4), 650-661.

Deng K., Wong C.W., Nolan J.V., 2006. Long-term effects of early-life dietary Lcarnitine on lymphoid organs and immune responses in leghorn- type chickens. J. Anim. Phys. Anim. Nutr., 90, 81-86.

Gropp J.M., Schumacher A., Schweigert F.J., 1994. Recent research in vitamin nutrition with special emphasis to vitamin A, β-carotene and L-carnitine. In Proc., Meeting Arkansas Nutr. Conf., Fayetteville, A. R: Arkansas Poultry Federation, 124-134.

Hrnčár C., Verguliaková S., Svorad P., Weis J., Aprášová H., Mindek S., Fik M., Bujko J., 2015. Effect of L-carnitine supplementation on fattening and carcass parameters of broiler chickens. Actafytotechnica et zootechnica, 18(1), 15-19.

Laudadio V., Dambrosio A., Normanno G., Khan R.U., Naz S., Rowghani E., Tufarelli V., 2012. Effect of reducing dietary protein level on performance responses and some microbiological aspects of broiler chickens under summer environmental conditions. Avian Biology Research, 5, 88-92.

Leibetseder J., 1995. Untersuchungenüber die wirkungen von L Carnitin bei mhuhn. Arch. Anim. Nutr., 48, 97-108.

Lettner F., Zollitsch W., Halbmayer E., 1992. Use of L-carnitine in the broiler ration. Bodenkultur, 43(2), 161-167.

Lien T.F., Horng Y.M., 2001. The effect of supplementary dietary L-carnitine on the growth performance, serum components, carcass traits and enzyme activities in relation to fatty acid β-oxidation of broiler chickens. Br. Poult. Sci., 42, 92-95.

Lin C.Y., Frias G.W., Moran E.J., 1980. Genetic and environmental aspects of obesity in broilers. World Poult. Sci. J., 36, 103-111.

Markwell M.A.K., McGroarty E.J., Bieber L.L., Tolbert N.E., 1973. The subcellular distribution of carnitine acyltransferases in mammalian liver and kidney a new peroxisomal enzyme. Journal of Biological Chemistry, 248(10), 3426-3432.

Marquis N.R., Fritz I.B., 1965. The distribution of carnitine, acetylcarnitine, and carnitineacetyl-transferase in rat tissues. Journal of Biological Chemistry, 240(5), 2193-2196.

Matalliotakis I., Koumantaki Y., Evageliou A., Matalliotakis G., Goumenou A., Koumantakis E., 2000. L-carnitine levels in the seminal plasma of fertile and infertile men: correlation with sperm quality. In International Journal of Fertility, 45, 236–240.

Murali P., George S.K., Ally K., Dipu M.T., 2015. Effect of L-carnitine supplementation on growth performance, nutrient utilization, and nitrogen balance of broilers fed with animal fat. Vet. World, 8(4), 482-486.

Michalczuk M., Lukasiewicz M., Niemiec J., Wnuk A., Sosnowska N.M., 2012. Effect of L-carnitine on performance and dressing percentage of broiler chickens. Anim. Sci., 51, 89–99.

Novotny B.J., 1998. L-carnitine. What difference does it make? Symp. Proc. 15th Dec., Leuven, Belgium.

Parsaeimehr K., Afrouziyeh M., Hoseinzadeh S., 2014. The effects of L-carnitine and different levels of animal fat on performance, carcass characteristics, some blood parameters and immune response in broiler chicks. Iranian Journal of Applied Animal Science, 4(3), 561-566.

Rabie M.H., Szilagyi M., Gippert T., Votisky E. and Gerendai D., 1997b. Influence of dietary L-carnitine on performance and carcass quality of broiler chickens. ActaBiologicaHungarica, 48, 241-252.

Rabie M.H., Szilagyi M., 1998. Effects of L-carnitine supplementation of diets differing in energy levels on performance, abdominal fat content, and yield and composition of edible meat of broilers. In British Journal of Nutrition, 80, 391–400.

Rabie M.H., SzilÁgyi, M., Gippert, T., 1997a. Effects of dietary L-carnitine supplementation and protein level on performance and degree of meatness and fatness of broilers. Acta Biologica Hung. 48, 221-239.

Rezaei M., Hajati H., 2010. Effect of diet dilution at early age on performance, carcass characteristics and blood parameters of broiler chicks. Italian Journal of Animal Science, 9(1), 19.

Rezaei M., Attar A., Ghodratnama A., Kermanshahi H., 2007. Study the effects of different levels of fat and L-carnitine on performance, carcass characteristics and serum composition of broiler chicks. Pakistan journal of biological sciences: PJBS, 10(12), 1970-1976.

Sarica S., Corduk M., Kilinc K., 2005. The effect of dietary L-carnitine supplementation on growth performance, carcass traits, and composition of edible meat in Japanese quail (*Coturnix coturnix japonica*). Journal of applied poultry research, 14(4), 709.

Sarica S., Corduk M., Ensoy U., Basmacıoğlu H., Karataş U., 2007. Effects of dietary supplementation of L-carnitine on performance, carcass and meat characteristics of quails. South African Journal of Animal Science, 37(3), 189-201.

Sayed A.N., Shoeib H.K., Abdel-Raheem H.A., 2001. Effect of dietary L-carnitine on performance of broiler chickens fed on different levels of fat. Assiut Vet. Med. J, 45(89), 37-47.

Schuhmacher A., Eissner C., Gropp J.M., 1993. Carnitine in fish, piglets and quail. Vitamine und weitere Zusatzstoffebei mensch und Tier (Vitamins and other supplements for humans and animals), 407-412.

Taklimi S.M., Ghazvinian K., Kasgari M.R.A., 2015. Effect of L-carnitine on performance and carcass quality of broiler chickens. Acad. J. Sci. Res., 3, 50-54.

Xu Z.R., Wang M.Q. Mao H.X. Zhan X.A., Hu C.H., 2003. Effects of L-carnitine on growth performance, carcass composition, and metabolism of lipids in male broilers. Poultry Science, 82, 408–413.

Yalçin S., Ozsoy B., Cengiz O., Bulbul T., 2008. Effects of dietary L-carnitine supplementation on growth performance and some biochemical parameters in Japanese quails (*Coturnix coturnix japonica*). Rev. Med. Vet, 159, 502-507.

Zhang Y., Ma Q., Bai X., Zhao L., Wang Q., Ji C., Liu L., Yin H., 2010. Effects of dietary Acetyl-L-carnitine on meat quality and lipid metabolism in arbor acres' broilers. Asian-Australasian Journal of Animal Sciences, 23(12), 1639-1644.

Scientific Papers. Series D. Animal Science. Vol. LXI, Number 1, 2018
ISSN 2285-5750; ISSN CD-ROM 2285-5769; ISSN Online 2393-2260; ISSN-L 2285-5750

THE EFFECT OF ADDITION MANGOSTEEN PEEL MEAL (*Garcinia mangostana* L.) IN THE RATION ON THE PROTEIN EFFICIENCY RATIO OF SENTUL CHICKEN

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Abstract

Sentul chicken is a specific local chicken from Ciamis region in West Java and a dual- purpose type that can utilized for eggs and meat production. In other way, these birds are very good for chicken meat species, because they have a compact body and white skin colour. One of the alternatives to improve performance is by giving the ration added with a mangosteen peel meal (Garcinia mangostana). Mangosteen peel meal (MPM) is one of the medicinal plants used as a herbal medicine containing xanthone compounds as antioxidants, and antimicrobials. The research aimed is to find out of protein efficiency ratio of Sentul chicken fed diets containing mangosteen peel meal (Garcinia mangostana). This experiment used 100 day old chicks of Sentul chicken that were raised in cages until 10 weeks old. It was used completely randomized design, with four level of mangosteen peel meal (ration without MPM (R_0), and ration that added 2.5% (R_1), 5% (R_2), and 7.5% (R_3), of MPM, and five replications, each replicated consisted of five Sentul chickens. Protein consumption, body weight gain and protein efficiency ratio were parameters observed. The results that using mangosteen peel meal until 7.5%, gave no significant effect on feed and protein consumption, but the use of ration with the addition of mangosteen peel meal at 5% and 7.5% gave a significant effect on body weight gain and protein efficiency ratio. This research indicated that the addition of mangosteen peel meal (MPM) in the ration until 7.5 % gave the best protein efficiency ratio of Sentul chicken.

Key words*: mangosteen peel meal, Sentul chicken, protein efficiency ratio.*

INTRODUCTION

Sentul chicken is a specific local chicken from Ciamis region in West Java and a dual- purpose type that can utilized for eggs and meat production. The growth rate of Sentul chicken is high, if maintained intensive so that it can be slaughter at the age of 10 weeks (Kurnia, 2011). To obtain maximum performance there must be balanced with the provision of quality rations, balanced and in accordance with the needs. Feed additives added to the ration are intended to improve the feed consumption, digestibility, and endurance of chicken livestock. To increase growth is by using antibiotics to increase productivity, but the continuous use of synthetic antibiotics will cause resistant and residual in chicken carcasses, which is harmful to human consumption. An alternative substitute for natural synthetic feed additive one of them is mangosteen peel. Mangosteen peel contains xanthone compounds as antioxidants, and antimicrobials (Mardawati et al*.,* 2008). The nutrient content contained in the skin of mangosteen fruit is 6.45% crude fat, 3.02% protein, ash 2.17%, total sugar 2.10%, and carbohydrates 82.50% (Permana, 2010). Mangosteen skin also contains xanthone compounds that function as antioxidants, antiviral, antifungal and antimicrobial, and are not found in other fruits. Xanthone compounds consist of mangostin, mangostenol A, mangostinone A, mangostinon B, trapezi-folixanthone, tovophyllin B, alpha mangostin, beta mangostin, garcinon B, mangostanol, flavonoid epicatechin and gartanin (Qosim, 2007). Xanthone compounds contained in the skin of mangosteen can improve the structures of intestinal villi in the process of nutrient absorption. Antibacterial herbs are able to suppress the growth of pathogenic bacteria in the intestine (Velmurugan and Citarasu, 2010), so the higher body weight growth of chickens.
Antioxidant compounds (xanthones) contained in mangosteen peel can also prevent or neutralize free radicals due to air pollution in the environment. An increase in ambient temperature over a comfortable temperature zone range causes oxidative stress, leading to the occurrence of free radical attack on the cell membrane. Free radicals are an atom, a

molecule, or a compound in which it contains one or more unpaired electrons, making it highly reactive (Andayani, 2008). The need for antioxidants in rations is considered based on the content of polyunsaturated fatty acids, each 1% polyunsaturated fatty acid required 30 IU/kg vitamin E ration as antioxidant or 30 ppm in the form (DL-α-Tocopheryl acetate) (Leeson and Summers, 2001). Based on the calculation of antioxidant requirement in research ration equal to vitamin E (DL-α-Tocopheryl acetate) about 80 ppm, assuming the highest xanthone content is found in mangosteen skin, ie 107.76 mg per 100 g of fruit peel (Iswari, 2011), the need of mangosteen peel meal in chicken ration is about 7.5% per kg of ration.

The using of mangostee peel meal (MPM) in chicken rations should be limited, because of the presence of tannins known as anti-nutrients, tannins may affect carbohydrate degradation. The content tannin contained in mangosteen peel is 16.8% (Ngamsaeng, 2004). Protein is essential organic substances and essential for growth and production (Leeson and Summers, 2001). The quality of the ration will certainly affect the growth rate of Sentul chicken. To determine the biological evaluation of protein is needed to see its effect on poultry. One of the measures of protein quality is the Protein Efficiency Ratio (PER), which is simply the weight gain of animal divided by protein intake (Leeson and Summers, 2001). The Protein Efficiency Ratio determines the efficiency level of poultry in converting each gram of protein into some weight gain. Sentul chicken is prospective in supply of animal protein of poultry, so we need research toward quality protein ration that will give resulting affect the best body weight.

MATERIALS AND METHODS

Livestock experiments. The study used 100 day old chicks of Sentul chicken witch the average of body weight was 34.00 gram (coefficient of variation 8.09%). The Sentul chicken kept in cage until the age of 10 weeks.

Cage. 20 cages were used and were measured 90 cm long, 90 cm wide and 60 cm high, each cage consisted of 5 chickens

Trial rations. The feed ingredients of ration comprised of yellow corn meal (56.00%), soy-bean meal (12.00%), rice bran (21.50%), fish meal (9.25%), $CaCo_3$ (0.50%) and bone meal (0.75%). Rations were prepared based on protein and metabolic energy requirement for Sentul chicken growth phase, ie. 17% protein and metabolic energy 2850 kcal/kg (Widjastuti, 1996). The treatment consisted of the use of mangosteen peel meal (MPM) ie: P0 = 0% MPM, P1 = 2.5% MPM, P2 = 5.0% MPM and P3= 7.5% MPM.

Experimental design. Experiments were conducted experimentally using Completely Randomized Design, consisting of 4 treatment and 5 replications. Data were analyzed using Variance Analysis and differences between treatments using Duncan Multiple Test. The parameters were protein consumption, body weight gain and Protein Efficiency Ratio (PER).

RESULTS AND DISCUSSIONS

The average protein consumption, body weight gain and Protein Efficiency Ratio (PER) of Sentul chicken from each treatment are showed in Table 1.

Table 1. The protein consumption, body weight gain and Protein Efficiency Ratio of Sentul Chickens

Variable	Treatment			
	P0	P1	P2	P3
Protein Consumption (g)	284.70[a]	263.34[a]	266.80[a]	268.25[a]
Body Weight Gain (g)	387.79[a]	420.62[ab]	431.13[b]	438.10[b]
Protein Efficiency Ratio	1.36[a]	1.60[b]	1.62[b]	1.63[b]

Note: P0 = 0 % MPM/kg ration, P1 = 2.5% MPM/kg ration, P2 = 5% MPM/kg ration; [a-b] Mean values within a row having different superscripts are significantly different by least significant difference test.

Protein Consumption

Table 1 shows that protein consumption is obtained by calculating the amount of feed consumed multiplied by the protein content of the ration. The average protein consumption was 263.34 – 284.70 g. The result of variance analysis showed that the addition of MPM in

ration had no significant effect (P>0.05) on protein consumption. These results illustrate that feed consumption in each treatment is in the same range, so the addition of MPM in rations up to the level of 7.5% did not give a negative effect on feed consumption, and will have an impact on the protein consumed. The ration containing MPM from 2.5% until 7.5% did not influence palatability and chicken appetite. According to Pond and Church (1995), the palatability of rations is an important factor that determines the level of feed consumption and palatability depending on the smell, taste, color and texture of the ration.

Mangosteen peel meal contains tannin which is a limiting factor and anti-nutrients. According Ngamsaeng (2004), the mangosteen peel meal (MPM) contains a fairly high tannin and can affect the palatability rate of the ration, but before used, MPM was dried in the sun first, so the tannin content decreased and it will reduce the bitter taste and smell typical of mangosteen peel, consequently no effect on the feed and protein consumption.

Body Weight Gain

The body weight gain of each treatment is showed in Table 1. The average of body weight gain was 387.79 - 438.10 g. The results of variance analysis showed that the treatments P0 and P1 did not give a significant effect on the body weight gain, but the treatments P2 and P3 were significantly higher (P<0.05) than P0 treatment. The treatments P1, P2, and P3 did not show any significant effect on the body weight gain. Its mean that MPM from 2.5% up until 7.5% in ration did not influence palatability and appetite, so the body weight gain was increased. This is because mangosteen peel meal contains xanthone compounds as antioxidants, anti-viral, anti-fungus and anti-microbial, that can improve the structure of intestinal villi in the absorption of nutrients, and can suppress the growth of pathogenic bacteria in the intestine, thus increasing weight gain (Velmurugan and Citarasu, 2010). Xanthone compounds are also able to suppress oxidative stress that affects the growth rate of better chickens (Lannang et al., 2006).

The content of tannin in mangosteen peel meal does not give negative effect on the body weight gain. According to the research of Rateh (2014), the content of tannins in basal ration with the addition of mangosteen peel meal must be 1.5% or 0.36 g/kg. The calculated tannins contained in rations with mangosteen peel meal in each treatment were still below the tolerance limit, ie P1 = 0.6 g/kg, P2 = 1.2 g/kg, and P3 = 1.8 g/kg. According Kumar (2005), the limit of tannins use in the ration is 2.6 g/kg. Because of that, the body weight gain gave better, than the control treatment without MPM.

Protein Efficiency Ratio (PER)

In Table 1 can be seen that the value of protein efficiency ratio on Sentul chicken were variation from the lowest R0 = 1.36 to the highest P3 =1.63. PER value achieved by each treatment can be seen in Figure 1. The results of the variance analysis show that the treatment with addition of MPM in the ration affected significant the PER.

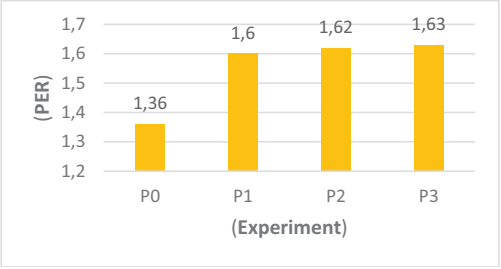

Figure 1. Protein Efficiency Ratio

The efficiency of protein ratio in the treatments P1, P2 and P3 was significant higher (P<0.05) than P0 treatment to the Sentul chicken. PER value of the treatment P1, P2 and P3 did not show any significant effect. This means that use of MPM until 7.5% in the ration produces better PER value than the control ration. This is because the addition of MPM in the ration does not affect the consumption of protein, but increased the body weight of Sentul chicken. Mangosteen peel meal contains xanthone compounds and xanthone compounds found in the peel of mangosteen can improve the structure of the intestinal villi and thus give effect the process of nutrient uptake. Optimal absorption of nutrients will affect the increase in body weight and will consequently have a positive impact on the value of PER. Leeson and Summers (2001) states that the protein

efficiency ratio in the ration related directly to the biological value of protein ration itself.

CONCLUSIONS

It can be concluded that the addition of mangosteen peel meal (MPM) in the ration until 7.5% gave the best effect on the Protein Efficiency Ratio of Sentul chicken and mangosteen peel meal can be an alternative source of feed additive from herbal.

ACKNOWLEDGEMENTS

The research work has been conducted in the Grand Research Academic Leadership project. Sources of funds were from Padjadjaran University, through the Directorate of Research, Community Service and Innovation Padjadjaran University, Indonesia.

REFERENCES

Andayani R., 2008. Penentuan aktivitas antioksidan kadar fenolat total likopen pada buah tomay (*Solanium lycopersicum* L). Jurnal sains dan teknologi farmasi 13(1).

Iswari K., 2011. Kulit Manggis Berkhasiat Tinggi. Madya Centradifa. Jakarta.

Kumar V., Elangovan A.V., Mandal A.B., 2005. Utilization of reconstitued high tannin sorgumin the diets of broiler chicken. J.Anim. Sci., 18(4), 538-544.

Kurnia Y., 2011. Morfometrik Ayam Sentul, Kampung dan Kedu pada Fase Pertumbuhan dari Umur 1-12 Minggu. Skripsi. Program Alih Jenis. Departemen Produksi dan TeknologiPeternakan, Fakultas Peternakan, Institut Pertanian Bogor. Bogor.

Lannang A.M., Komguem J., Ngninzeko F.N., Tangmouo J.G., Lontsir D., Ajaz A., Choudhary M.I., Sondengam B.L., Ur-Rahman A., 2006. Antioxidant benzophenones and xathones from the root bark of *Garcini smeathmannii*. Bull. Chem. Soc.

Leeson A., Summers E., 2001. Nutrition of the Chicken. 4th ed. Department of Animal and Poultry Science University of Guelph, Canada.

Mardawati E, Cucu Achyar S., Herlina Marta, 2008. Kajian Aktivitas Antioksidan dan Ekstrak ulit Manggis (*Garcinia mangostana* L.) dalam Rangka Pemanfaatan Limbah Kulit Manggis di Kecamatan Puspahirang Kabupaten Tasikmalaya. Laporan Akhir Penelitian. Lembaga Penelitian UNPAD. Bandung.

Ngamsaeng A., 2004. Effects of mangosteen peel (*Garcinia mangostana* L.) supplementation on rumen ecology, microbial protein synthesis, digestibility and voluntary feed intake in beef steer. Tropical Feed Resources Research and Development Center, Department of Animal Science, Thailand.

Permana A.W., 2010. Kulit Buah Manggis Dapat Menjadi Minuman Instan Kaya Antioksidan.WartaPeneltian dan Pengembangan Pertanian Vol. 32, No.2, BP2TP, Badan Litbang. Kementan RI, Indonesia.

Pound W.G., Churc D.C., Pond K.R., 1995. Basic Animal Nutrition and Feeding. Edke-4, New York, John Wiley & Sons.

Qasim Warid Ali, 2007. Kulit Buah Manggis Sebagai Antioksidan. Artikel Tim Ahli Divisi TTG Lembaga Pengabdian Masyarakat (LPM) Universitas Padjadjaran, Bandung. 35.

Rateh Wulandari., Irfan Djunaidi H., Edhy Sudjarwo, 2014. Pengaruh penambahan tepung kulit manggis (*Garcinia mangostana* L.) sebagai feed addive terhadap penempilan produksi burung puyuh. Artikel fapet.ub.ac.id.

Vemurugan S., Citarasu T., 2010. Effect of herbal antibacterial extracts on the gut floral changes in indian white shrimp *Fenneropenaeus indicus*. Rom. Biotech. Lett., 5709-5717

Widjastuti T., 1996. Penentuan Efisiensi Penggunaan Protein, Kebutuhan Protein dan Energi untuk Pertumbuhan dan Produksi Telur Ayam Sentul pada Kandang Sistem Cage dan Sistem Litter. Disertasi. Program Pascasarjana Unpad. Bandung.

Scientific Papers. Series D. Animal Science. Vol. LXI, Number 1, 2018
ISSN 2285-5750; ISSN CD-ROM 2285-5769; ISSN Online 2393-2260; ISSN-L 2285-5750

THE USE OF SOME ENERGETICS SYRUPS ON BEES DEPRIVED ON NATURAL PICKING AND ITS EFFECTS ON SOME MORPHOLOGICAL AND PRODUCTIVE PARAMETERS

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Abstract

Many research about use of energy syrups in bee nourishment show advantages and disadvantages of each ingredient. The aim of this study was to analyze the influence of three types of such energy supplements (sugar syrup 2:1, corn syrup and enzyme inverted sugar) on some parameters of bee families deprived by natural picking. These parameters (the number of bees, the amount of food supplies and the number of brood cells) were determined for 9 weeks, and the recorded values were processed and analyzed statistically for comparison with the results of other bee families maintained in the field. Values obtained from bees with access to natural picking were superior to those obtained from bees deprived on it. The colonies fed with enzymatic invert sugar syrup registered higher values of the monitored parameters and the lowest values were recorded in those fed with sugar syrup 2:1. Smaller values obtained from bees deprived by natural picking may also be caused by quality of food sources and the stress caused by the restriction of their flight.

Key words*: bee nourishment, brood, corn, sugar.*

INTRODUCTION

Is true that bees are very important for maintaining wild plants biodiversity and for increasing crop production (Double, 2014), but we also have to know that at present bees are disturbed by many stress factors, among which chemical substances from agriculture, diseases and pests, to which we can also add inappropriate supplementary food recipes (Alaux et al., 2010).In agriculture, are not only dangerous chemicals that are sprayed on flowering plants, but also those that are used in the treatment of seeds (Rolke, 2016).

The additional feed of bees influences directly and obviously not only the level of apiculture production, but also the reproduction, the health status and implicitly the processes of development of bee colonies (Pop, 2006).

By feeding point of view, bees are independent of man because they collect and prepare their own food. In the years that do not provide optimal conditions for the development of bee colonies (Pătruică, 2013) beekeepers must compensate the lack of energy (manna, nectar) and protein (pollen) from nature by feeding bees; this process is also necessary in the event of insufficient flight surface, sometimes caused by too many bee colonies in that area (Sammataro and Weiss, 2013).

The most commonly used energy syrups are those of sugar, prepared by beekeepers in different concentrations or those of enzymatic inverted sugar and corn hydrolysed syrup. The use of sugar has been the subject of numerous studies that have highlighted the stimulating effects of this product on the development of bees (Moraru, 2006); it was first used on feeding bees by Réaumur on 18th century.

It is well known that all energy syrups are enzymatically transformed by bees into honey (Hausmann, 2005) and therefore they must contain ingredients to facilitate this process. Due to the disadvantages of its use (risk of crystallization, fermentation, working time, storage space), sugar syrup is successfully replaced in many areas of the world by hydrolyzed corn syrup, especially to provide the necessary food supplies during cold season (Ruiz-Matute et al., 2010).

However, studies show that some energy syrups contain toxic chemicals for bees (insecticides, neonicotinoids) that come from the raw material used in the manufacturing

process (Kessler, 2015). In fact, the European Commission also presents information about the risk of transmitting such substances to syrups used in bee-keeping (Rondeau, 2014).
Corn hydrolysed syrup/high-fructose corn syrup (HFCS), produced since 1960 (Schorin, 2005), is an inexpensive source of carbohydrates for bee-feeding, and therefore its excessive use is found in apiculture, although various studies show the negative effects of honey from its processing to consumers (Ferder, 2010); nowadays the producing industry calls it corn syrup.
An alternative to sugar syrup that avoids the use of corn syrup is the enzymatic invert sugar syrup produced by specialized companies from market, but which is also not a proven safe source for the health of bees or people who consume resulting honey.
In view of these considerations, we can state that not all the advantages and disadvantages of the medium and long term use of these types of energy syrups in feeding bees have been elucidated and that's why the beekeepers are the ones who take the feeding option.
Beekeepers and researchers are further concerned with determining the quality of supplementary feed bee recipes and, of course, with determining their influence on the profitability of beekeeping, which is based on health, queens prolificity, production.
In this context, the purpose of this study is to analyze the impact of three types of energy syrups (2: 1 sugar syrup, hydrolysed corn syrup and enzymatic invert sugar syrup) on some morpho-productive parameters such as the number of bees, food supplies and number of brood cells, of some bee families isolated from natural food collecting (maintained in bee lofts) during 9 experimental weeks.

MATERIALS AND METHODS

The studied biological material was represented by adult queen less bees (*Apis mellifera*, Carpathian ecotype), collected in June 2017 from two bee families in Deleni (Vaslui, Romania) and then stored for 24 hours in a room (18ºC, dark, without food). The following day were placed in 12 wooden boxes (120-130 g bees/box) together with a paired queen bee, and then stored for 48-60 hours in a room (18°C, 55% U, dark).

After 60 h bee boxes were distributed as follows: 9 bee boxes were deprived by natural picking and 3 bee families had access to the nature (natural food sources).
The depriving by natural picking involved the introduction of beehives in lofts (1,5 x 1 x 2 m) made of metal mesh with rhombic holes (3 mm), equipped with 3 cylindrical plastic bottles (150 ml capacity), for energy syrup, water and pollen powder (Table 1).

Table.1 Experimental research scheme

Specification	Lots of experience *Apis mellifera carpatica*			
	A0	A1	A2	A3
Operating system	With access to natural picking	No access to natural picking (bee lofts - 3 m³)		
Food used	Natural food (nectar, pollen)	Pollen powder, water at all + A1- sugar syrup 2: 1 A2- corn syrup A3- Enzymatic invert sugar syrup -2 times feeding x 150ml / week		
Follow-up indicators	- the number of bees			
	- the amount of food supplies			
	- brood cells number			

Determination of these bee quality assessment indicators was done by specific methods, namely counting of brood cells and cells with food supplies and periodic weighing of individuals from colonies; knowing one bee average weight (100 mg) we determined the total bees number of each colony (nr. of bees= total bees weigh/100) and knowing one honey cell average weight (0.25 g) we determined the total food supplies (total honey = number of honey cells x 0.25).
The experiment consisted in the organizing of 4 lots (A0, A1, A2, A3) of 3 bee colony each, with 1200-1300 individuals (working bees, drones), maintained in beehives (232 x 175 x 165 mm) with 5 wax frames (10 x 10 cm); after growing by bees, the interior of each frame had 1 dm² surface, meaning around 400 cells on one face/ around 800 cells on both faces.
The bees in the A0 group had access to the natural picking and the bees from the other lots (A1, A2, A3) had the flight restricted by the volume of lofts into which they were introduced (3 m³) and were fed with pollen

powder (*ad libitum*), water (*ad libitum*) and various energy syrups (150 ml x 2 times a week/bee family): group A1 with 2: 1 sugar syrup, corn hydrolyzate syrup on lot A2 and group A3 with enzymatic invert sugar syrup. Simultaneously with the development of colonies, they were additionally added 2 beehive boxes, with 5 frames each (assembled with wax honeycombs).

This research was made over a 63 days period and required weekly counts of the number of bees in each family, the number of brood cells and the amount of food supplies.

The recorded data was statistically processed by calculating the estimators (arithmetic mean, standard deviation of mean and coefficient of variation.)

RESULTS AND DISCUSSIONS

Regarding the quantity of food supplies, we can see in Table 2 that the small values of this parameter oscillated with the large ones throughout the experiment and this was due to the different moments when colony required nutrients for wax production of young bees (which are the wax-secreting) or for feeding and warming brood from frames we've added.

This indicator recorded the smallest values in the group fed with 2:1 sugar syrup at all times of control and this because the bees of this lot had a higher irascibility condition caused by the smell of sugar syrup. Significant differences between the control and experimental groups on this parameter were recorded in week 2 when the A0 group had 33.25 ± 6.16 g of honey compared to 86.58 ± 9.38 g of honey in the group fed with sugar syrup, 109.33 ± 9.68 g to corn hydrolyzate syrup and 113.83 ± 12.85 g to the enzymatic invert sugar syrup. These differences were due to the lack of natural picking in nature from that period of lot A0 (Table 2).

Table 2. Amount of honey reserves (grams) in bee colonies

Specification	n	A0	A1	A2	A3	Compared groups	Significance
Week 1	3	68.66±9.89	25.58±5.35	40.33±8.78	44±7.85	A0 vs A1 A0 vs A2 A0 vs.A3	* (p<0.05) ns (p>0.05) ns (p>0.05)
Week 2	3	33.25±6.16	86.58±9.38	109.33±9.68	113.83±12.85	A0 vs A1 A0 vs A2 A0 vs.A3	** (p<0.01) ** (p<0.01) ** (p<0.01)
Week 3	3	254.16±15.15	226.66±15.57	272.83±15.79	286.60±15.87	A0 vs A1 A0 vs A2 A0 vs.A3	ns (p>0.05) ns (p>0.05) ns (p>0.05)
Week 4	3	192±9.38	93.75±12.33	133.33±11.41	164.16±13.88	A0 vs A1 A0 vs A2 A0 vs.A3	** (p<0.01) * (p<0.05) ns (p>0.05)
Week 5	3	129.16±8.95	14.75±5.06	26.00±3.40	36.58±8.52	A0 vs A1 A0 vs A2 A0 vs.A3	ns (p>0.05) ns (p>0.05) ** (p<0.01)
Week 6	3	362.33±17.65	388.66±19.78	447.33±22.97	484.83±21.45	A0 vs A1 A0 vs A2 A0 vs.A3	ns (p>0.05) * (p<0.05) * (p<0.05)
Week 7	3	434.33±20.72	95.91±9.69	125.66±9.88	149.33±14.54	A0 vs A1 A0 vs A2 A0 vs.A3	*** (p<0.001) *** (p<0.001) *** (p<0.001)
Week 8	3	651.83±33.22	24.33±7.22	51.66±8.51	69.25±15.63	A0 vs A1 A0 vs A2 A0 vs.A3	*** (p<0.001) *** (p<0.001) *** (p<0.001)
Week 9	3	1254.17±87.44	424.83±27.15	470.16±21.60	504.83±20.29	A0 vs A1 A0 vs A2 A0 vs.A3	*** (p<0.001) *** (p<0.001) ** (p<0.01)

Very significant differences were recorded in the last 3 weeks of control when the amount of food supplies was higher in the A0 lot than in the experimental lots. At the last check there were very significant differences between the groups A0 and A1 and A2, respectively, so the

group that had access to natural picking had 1254.17 ± 87.44 g of honey reserves versus 424.83 ± 27.15 g in the group fed with sugar syrup 2:1, 470.16 ± 21.60 g to corn syrup; significant differences were between A0 and A4 (1254.17 vs 504.83 ± 20.29 g of honey). Additional feeding of bees plays an important role in the number of brood cells and implicitly in the general development of the bee colony (Brodschneider and Craislheim, 2010).

The number of brood cells recorded higher values during the entire study period in the group that benefited from natural harvesting and this was due to the quality of the natural food sources, superior to those used in the groups maintained on lofts.

The smallest values of this indicator were recorded in the group fed with sugar syrup at all control moments. In the first week there were no significant differences between the 4 values, thus recording 1153 ± 40,25 brood cells at A0, 952 ± 85,11 at A1, 1069.7 ± 65.64 for A3, respectively 1119 ± 56.78 of brood cells to A4 (Table 3).

Table 3. The number of brood cells from bee colonies

Specification	n	A0	A1	A2	A3	Compared groups	Significance
Week 1	3	1153±40.25	952±85.11	1069.7±65.64	1119±56.78	A0 vs A1 A0 vs A2 A0 vs.A3	ns (p>0.05) ns (p>0.05) ns (p>0.05)
Week 2	3	2242.70±35.47	1994.70±74.19	2272.70±41.70	2373.7±46.05	A0 vs A1 A0 vs A2 A0 vs.A3	* (p<0.05) ns (p>0.05) ns (p>0.05)
Week 3	3	2972.70±59.08	2251±86.50	2611±58.89	2832.70±38.55	A0 vs A1 A0 vs A2 A0 vs.A3	** (p<0.01) * (p<0.05) ns (p>0.05)
Week 4	3	2755.70±66.21	1999.70±128.86	2459.70±78.17	2574.30±69.26	A0 vs A1 A0 vs A2 A0 vs.A3	** (p<0.01) * (p<0.05) ns (p>0.05)
Week 5	3	3698.67±51.10	3455.33±37.95	3450.33±32.37	3543±83.82	A0 vs A1 A0 vs A2 A0 vs.A3	* (p<0.05) * (p<0.05) ns (p>0.05)
Week 6	3	4559.33±78.30	4203.33±95.91	4223±100.85	4289.33±71.88	A0 vs A1 A0 vs A2 A0 vs.A3	* (p<0.05) ns (p>0.05) ns (p>0.05)
Week 7	3	5873±55.19	5372.66±48.50	5463±44.10	5715±55.24	A0 vs A1 A0 vs A2 A0 vs.A3	** (p<0.01) ** (p<0.01) ns (p>0.05)
Week 8	3	6279.33±60.88	5538±82.61	5887±98.14	6146.33±106.76	A0 vs A1 A0 vs A2 A0 vs.A3	** (p<0.01) * (p<0.05) ns (p>0.05)
Week 9	3	6147.66±95.88	5083±92.08	5538±117.93	5813.33±109.15	A0 vs A1 A0 vs A2 A0 vs.A3	** (p<0.01) * (p<0.05) ns (p>0.05)

In the seventh week we observed significant differences between the control group (5873 ± 55.19) and the group fed with sugar syrup (5372.66 ± 48.50) as well as one fed with corn syrup (5463 ± 44.10), while insignificant differences (5873 ± 55.19 vs 5715 ± 55.24) of the target indicator were recorded between the control group and the one fed with enzymatic sugar syrup. In fact, the values recorded in lot A4 were the closest to the values recorded at A0 throughout the experiment.

The number of bees in the 12 colonies of the experiment recorded close values taken in the control weeks, but also in view of this indicator, we observed the superiority of the group that had access to the natural picking to the groups maintained in bee lofts; this was generally due to stress caused by the restriction of the flight of the bees.

In the last week, there was a greater difference between the results of the A0 lot and the other three lots, of which the lot A4 (enzymatic inverted sugar syrup) came closest to the control group from the point of view of this parameter (number of bees) (Figure 1).

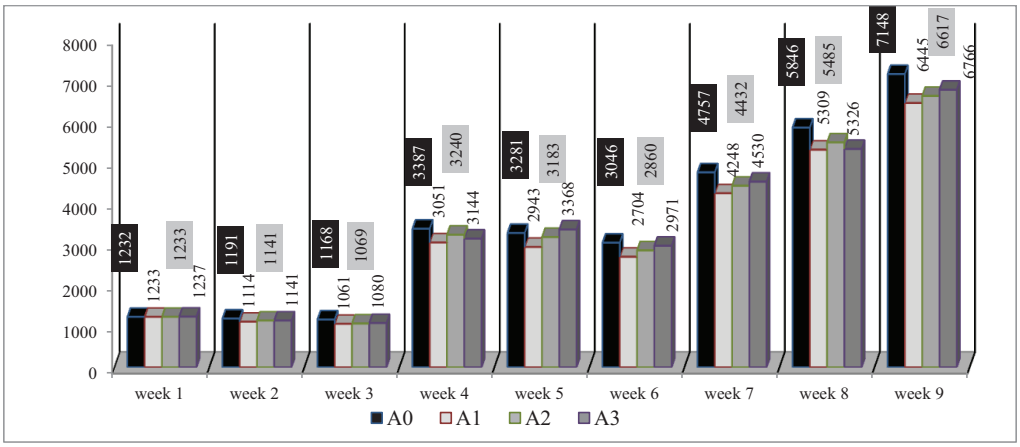

Figure 1. Number of bees from colonies

CONCLUSIONS

This research study examined the influence of energy sources (2:1 sugar syrup, corn syrup and enzyme invert sugar) on 3 quality indicators (number of bees, number of brood cells and the weight of the food supplies) of bees without access to natural food and we hope interpreting the results of it will bring more information to beekeepers about the wear of the prepared sugar syrup or of the commercial syrups on the bees who process it.

Regarding the quantity of food reserves, we noticed that the lowest values of this indicator were recorded in the group fed with sugar syrup, and the highest values in the group that benefited from the natural picking, in all control periods, except for the weeks 2 and 6 when the weather conditions were not favorable for the natural food collecting of the bees in the field; the superiority of nature was better remarked on the last week of control, when there were very significant differences between the group from field and those fed with sugar syrup 2:1 and corn syrup.

The number of brood cells is an indicator of appreciation of the quality of bees that reflects concretely the development status of the colony. Considering to it, we have noticed that the highest values were recorded in lot A0 and the lowest in lot A1 during the entire period. The group fed with enzymatic inverted sugar syrup was the one that had the closest values to those of the group with access to the natural picking, with insignificant differences between them during all the control periods; for example, at the last control, when bee families were already developed on 3 hive boxes, the A0 group had 6147.66 ± 95.88 brood cells and the A4 group had 5813.33 ± 109.15.

The number of bees recorded close values at all 12 bee families throughout the experiment, with the exception of the last control, when there were larger differences between the field group and those 3 groups from bee lofts.

The evolution of all the monitored indicators was favorable to the control group compared to the experimental lots and this was especially due to general behavior of the bees from experimental groups, who tried to escape from the lofts all the time and thus created a state of continuous agitation in those spaces.

It was also significant the more irascibility state of the bees from lofts fed with sugar syrup prepared by us, caused by the smell of this artificial food recipe.

Besides, apiculture practice as well as literature suggests that sugar syrup is very attractive to bees and determines the honey theft of bees during additional feeding.

Generally, the three morpho-productive parameters observed were higher in the group that had access to natural picking (A0) and lower values in the group that was maintained on bee lofts and fed with sugar syrup 2:1 (A1). Closer values to those of the control group were recorded on the group fed with enzymatic invert sugar syrup (A4), while the group fed with corn syrup (A3) recorded values between those of groups A1 and A4.

The use of energy syrups in bee nourishment is not yet fully elucidated with regard to the long-term health consequences of bees and humans, and therefore this domain of research continues to be an interesting subject for beekeepers and specialists around the world.

REFERENCES

Alaux C., Ducloz F., Crauser D., Le Conte Y., 2010. Diet effects on honeybee immunocompetence; Biol. Lett., 6(4), 562-565.

Brodschneider R., Craislheim K., 2010. Nutrition and health in honey bees. Apidologie, 41, 278-294.

Doublet V., Labarussias M., de Miranda J.R., Morit R.F.A., Paxton R.J., 2014. Bees under stress: sub lethal doses of a neonicotinoid pesticide and pathogens interact to elevate honey bee mortality across the life cycle. Environmental Microbiology, 10.1111/1462-2920.12426.

Ferder L., Ferder M.D., Inserra F., 2010. The role of high-fructose corn syrup in metabolic syndrome and hypertension. Current Hypertension Reports, 12, 105–112.

Hausmann C., Wackers F.L., Dorn S., 2005. Sugar convertibility in the parasitoid *Cotesia glomerata* (Hymenoptera:Braconidae). Archives of Insect Biochemistry and Physiology, 60,223–229.

Kessler S., Tiedeken E.J., Simcock K.L., Derveau S., Mitchell J., Softley S., Stout J.C., Wright G.A., 2015. Bees prefer foods containing neonicotinoid pesticides. Nature, 521(7550), 74-76.

Moraru P., 2006. Nutriția și alimentația albinelor. București, România.

Pătruică S., Huțu I., 2013.Economic benefits of using prebiotic and probiotic products as supplements in stimulation feeds administered to bee colonies.Turk. J. Vet. Med. & Anim. Sci., 37(3), 259-263.

Pop I.M., Halga P., Avarvarei T., 2006. Nutriția și alimentația animalelor. Ed. Tipo Moldova, Iași, România.

Rolke D., St Fuchs S., Grünewald B., Gao Z., Blenau W., 2016. Large-scale monitoring of effects of clothianidin-dressed oilseed rape seeds on pollinating insects in Northern Germany: effects on honey bees (*Apis mellifera*). Ecotoxicology, 25(9), 1648-1665.

Rondeau G., Sánchez-Bayo F., Tennekes H.A., Decourtye A., Ramírez-Romero R., Desneux N., 2014. Delayed and time-cumulative toxicity of imidacloprid in bees, ants and termites. Scientific Reports4, Article number: 5566.

Ruiz-Matute A., Weiss M., Sammataro D., Finley J., Sanz M.L., 2010. Carbohydrate composition of High-Fructose Corn Syrups (HFCS) used for bee feeding: effect on honey composition. Journal of Agriculture and Food Chemistry, 58(12), 7317–7322.

Sammataro D., Weiss M., 2013. Comparison of productivity of colonies of honey bees, *Apis mellifera*, supplemented with sucrose or high fructose corn syrup. J. Insect Sci., 13(19), 1–13.

Schorin M.D., 2005. High Fructose Corn Syrups: Composition, consumption and metabolism. Nutrition Today, 40, 248–252.

Scientific Papers. Series D. Animal Science. Vol. LXI, Number 1, 2018
ISSN 2285-5750; ISSN CD-ROM 2285-5769; ISSN Online 2393-2260; ISSN-L 2285-5750

THE INFLUENCE OF THE ZEOLITES USE ON BLOOD PARAMETERS OF COWS

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Abstract

Dairy cows' nutrition should be balanced because important quantities of mineral salts, especially calcium and phosphorus, but also magnesium, potassium, sodium, chlorine etc. are eliminated through milk. Also, mineral substances have a training role in the body, participating in a high proportion in the structure of supporting tissues, as well as a functional role by maintaining the main physiological functions. The use of zeolites in the form of volcanic tuff can influence the mineral and hematological parameters in the blood of cows in the calving period, as well as lactating cows. A lower variation in calcium, phosphorus and magnesium in blood is observed when using the volcanic tuff in the cows' feed during the pre-calving period and the first 2 weeks after calving. In the pre-calving and the first 2 weeks after the calcification, the values of erythrocytes, leukocytes and hemoglobin have lower values but are not statistically provided. During the lactation period, the three hematological parameters of the cows were physiologically normal, not being influenced by the administration of zeolite to the ration of dairy cows.

Key words*: cows, volcanic tuff, mineral elements, biochemical and hematological blood parameters.*

INTRODUCTION

Natural zeolites are porous materials characterized by their ability to remove or absorb water through a reversible process, allowing them to adsorb molecules and exchange constitutive cations (Mumpton, 1999). By being based on these characteristics, specialists have begun to use zeolites with good results in many domains, including husbandry.

The use of zeolites may have an effect in the prevention or treatment of certain diseases of animals grown in farms and for which small mineral deficiencies may appear relatively easy (Katsoulos et al., 2005).

In this respect, zeolites can provide the necessary coconut minerals and microelements for cows, thus stimulating their production and improving their health state (Karatzia et al., 2016).

Zeolites can be a valuable source of minerals, such as iron, calcium, phosphorus, magnesium, sodium, zinc, and manganese. As a result, the volcanic tuff, which is a source of natural zeolites, may be a source of minerals for cows, the proportion of use in food varying according to the physiological state, ration structure, gastrointestinal pH (Bosi et al., 2002).

Considering the fact that zeolites can produce ionic changes favorable to the cow's organism (Katsoulos et al., 2005), the aim of the present paper is to test the influence of the addition of volcanic tuff introduced into the ration of cows during the calving period, as well as of lactating cows blood content in macroelements and blood parameters by carrying out blood hematological and biochemical examinations.

MATERIALS AND METHODS

The biological material used was represented by 40 Holstein Fries cows that were divided into two homogeneous batches in terms of weight and physiological status. The analysis period began 2 weeks before calving and continued until the end of the first month of lactation.

During the experimental period all the cows were given the same mixture of compound feed, specific to the physiological condition, and the administration was done *ad libitum*. The difference between the batches was represented by the proportion of volcanic tuff added to the cows' diet, respectively the ratio of the control batch was not supplemented with the tuff, and the experimental batch received in

the food 350 g tuff/head/day (Table 1). This amount of volcanic tuff has previously been tested in other experiments that have taken place during the research, proving to be the optimal amount of zeolite for the lactating cows which were analyzed (Drăgotoiu et al., 2017).

Table 1. Experimental scheme

Batch	n	Treatment	Objectives
Control batch (C)	15	Total Mixed Ratio (TMR)	evaluating the effects of the administration of rations with the addition of natural zeolites over the blood parameters by conducting hematological and biochemical examinations
Experimental batch (E2)	15	TMR + 350 g tuff/head/day	

To assess the bioavailability of the mineral elements in the volcanic tuff structure, the blood calcium, magnesium phosphate levels in cows before 2 weeks of calving, 2 weeks post-calving, and lactating cows were measured.

The determination of the blood calcium and magnesium was done through the complexometric method, and phosphorus through the spectrophotometric method.

The hematological examination aimed to determine the number of leucocytes, erythrocytes and hemoglobin, using an automatic analyzer, where the operation was based on the principle of fluorescence flow cytometry, using semiconductor laser and hydrodynamic focusing.

RESULTS AND DISCUSSIONS

The blood levels of macroelements (calcium, phosphorus and magnesium) and the hematological parameters for the calving cows as well as the lactating cows are shown in Tables 2, 3, 4 and 5.

Table 2. The blood content of macroelements for pre-calving and post-calving cows

Period	Calcium (mg/dl)		Phosphorus (mg/dl)		Magnesium (mg/dl)	
	Control batch	Experimental batch	Control batch	Experimental batch	Control batch	Experimental batch
2 weeks before calving	7.29± 0.03	7.37± 0.03	4.88± 0.01	4.97± 0.02	2.02± 0.004	2.04± 0.006
1 week before calving	7.12± 0.05	7.22± 0.02	4.47± 0.02	4.47± 0.03	1.92± 0.003	1.94± 0.004
calving	7.14± 0.05	7.33± 0.03	3.98± 0.02	4.04± 0.04	1.78± 0.005	1.92± 0.005
1 week after calving	7.69± 0.07	8.08± 0.05	4.38± 0.01	4.78± 0.03	1.91± 0.006	1.99± 0.005
2 weeks after calving	8.05± 0.06	8.26± 0.05	4.67± 0.02	4.98± 0.02	2.06± 0.004	2.14± 0.006

Table 3. The blood content of macroelements for lactating cows

Month	Calcium (mg/dl)		Phosphorus (mg/dl)		Magnesium (mg/dl)	
	Control batch	Experimental batch	Control batch	Experimental batch	Control batch	Experimental batch
March	8.12± 0.02	8.08± 0.03	5.12± 0.02	4.88± 0.05	2.14± 0.006	2.05± 0.008
April	8.34± 0.04	8.29± 0.01	4.37± 0.01	4.47± 0.07	2.47± 0.008	2.51± 0.009
May	7.25± 0.07	7.43± 0.04	4.21± 0.03	3.88± 0.06	2.22± 0.009	2.18± 0.006
June	8.27± 0.09	8.58± 0.08	4.68± 0.04	4. 81± 0.07	1.78± 0.006	1.82± 0.008
July	9.05± 0.07	9.85± 0.07	5.53± 0.02	6.14± 0.09	1.92± 0.004	1.98± 0.009
August	8.52± 0.09	9.34± 0.05	6.01± 0.03	6.32± 0.05	2.11± 0.005	2.03± 0.006
September	8.12± 0.10	9.04± 0.05	5.27± 0.02	5.76± 0.08	2.57± 0.008	2.42± 0.008
October	8.01± 0.04	9.12± 0.07	4.88± 0.01	5.84± 0.02	2.45± 0.007	2.61± 0.007

Table 4. Hematological parameters for cows during the calving period

Period	Leukocytes (x 10^3/mm^3)		Erythrocytes (x 10^6/mm^3)		Hemoglobin (g/dl)	
	Control batch	Experimental batch	Control batch	Experimental batch	Control batch	Experimental batch
2 weeks before calving	10.32±0.04	10.28±0.03	5.72±0.02	5.79±0.03	10.12±0.04	10.05±0.05
1 week before calving	10.26±0.03	10.02±0.05	5.67±0.04	5.98±0.02	10.03±0.03	10.07±0.04
calving	9.63±0.06	9.87±0.05	5.10±0.03	5.21±0.03	9.17±0.06	9.15±0.05
1 week after calving	9.89±0.04	10.14±0.02	5.35±0.02	5.50±0.05	9.54±0.03	9.70±0.03
2 weeks after calving	10.04±0.03	10.20±0.04	5.60±0.01	5.81±0.03	9.98±0.05	10.07±0.04

Table 5. Hematological parameters for cows during the lactation period

Month	Leukocytes (x 10^3/mm^3)		Erythrocytes (x 10^6/mm^3)		Hemoglobin (g/dl)	
	Control batch	Experimental batch	Control batch	Experimental batch	Control batch	Experimental batch
March	10.27±0.03	10.17±0.05	5.68±0.04	5.79±0.04	10.09±0.05	10.21±0.04
April	10.44±0.04	10.31±0.04	5.82±0.03	5.98±0.03	10.17±0.06	10.01±0.06
May	10.75±0.04	10.65±0.07	5.91±0.02	5.98±0.07	9.97±0.08	10.15±0.05
June	11.77±0.07	11.01±0.05	5.48±0.02	5.51±0.07	9.82±0.04	9.87±0.03
July	11.82±0.06	11.77±0.05	5.23±0.05	5.18±0.05	9.76±0.04	9.68±0.06
August	11.62±0.05	11.89±0.08	5.01±0.04	5.09±0.02	9.55±0.07	9.45±0.04
September	11.11±0.06	11.15±0.04	5.87±0.06	5.62±0.05	9.95±0.03	9.78±0.03
October	10.31±0.06	10.41±0.05	6.08±0.01	5.99±0.04	10.11±0.07	10.07±0.08

In order to assess the bioavailability of the mineral elements in the volcanic tuff structure, the blood calcium levels were measured, with a decrease in the blood collected from the cows one week before calving and those on the day of calving, after which the level gradually increased, approaching to the one from the end of March (8.12 mg/dl, Table 3).

A similar tendency is observed for phosphorus, respectively for the cows in the first 2 weeks of lactation, where it is observed a recovery of the blood phosphorus level.

For the experimental batch, in the food of which the volcanic tuff was used, it is observed a lower variation of the calcium and phosphorus values before and after the calving, the differences being not statistically assured, respectively, the tuff is a source of macroelements. On the contrary, the control batch shows significant differences between the two periods, especially in the first week after calving.

In the case of magnesium no differences were observed in the pre and post-calving periods.

In the first 3 months of lactation, the values were close, and from the 4th month of administration was increased the value of this index for the experimental batch, the differences being significant from August, respectively after 6 months of natural zeolite administration.

In terms of phosphorus, the values followed the same behavior as of calcium, respectively they increased for the cows belonging to the experimental batch due to the synergism between the two macroelements, the administration of the volcanic tuff favoring the absorption of this mineral element.

The analyzes which were done in order to determine the magnesium in the cow's blood showed that the values were at normal physiological values for both batches, suggesting that taurines eliminated the excess magnesium brought through the volcanic tuff.

Thilsing-Hansen et al. (2002; 2003) appreciated that zeolite-calcium ratios below 5 did not effectively prevent parturient hypocalcaemia, whereas ratios of 10 to 20 proved very efficient in preventing hypocalcaemia. Feeding zeolite in the dry period significantly decreased plasma phosphate before as well as after calving. The phosphate level was normalized within one

week after calving. Plasma magnesium was significantly lower among the experimental cows on the day of calving, but stayed within the normal range of plasma magnesium.
Thilsing-Hansen and Jorgensen (2001) demonstrated that serum calcium analysis revealed a greater calcium concentration in zeolite-treated cows.
The hematological examination (Tables 4 and 5) aimed to determine the number of leucocytes and erythrocytes, hemoglobin (Hb) from the blood collected from pre, post-calving and lactating cows.
In the case of blood taken from the cows from the control batch during the pre-calving period, there was a significant decrease ($P<0.05$) in the values of leucocytes, erythrocytes and hemoglobin, after which the values gradually returned to normal within the next 2 weeks.
In contrast, for the experimental batch the differences in values of the three hematological parameters during the pre and the first 2 weeks, the post-calving phase shows slight decreases but which are statistically insignificant (Table 4).
In the lactation period (Table 5), the number of leukocytes ranged between the normal physiological limits, and was not influenced by the administration of the zeolite to the ration of dairy cows, with an increase in values in the summer months due to the increased cortisol secretion and the increased viscosity of blood.
The number of erythrocytes respected the physiological specificity of the species, with no significant differences between the two batches. In the summer months, there was noticed a decrease in the number of erythrocytes in both batches due to the increased water consumption and the reduced oxygen intake that causes the body to maintain its thermal balance at high temperatures.
The hemoglobin level did not show any variation between the two batches, being observed a decrease while increasing the temperature and with a decrease in the number of erythrocytes.
Quiroz-Rocha et al. (2009) has noted the fact that the hematological analyses were not significantly different between the pre-calving and post-calving batches, compared to the biochemical analytes (calcium, and phosphorus), at which the differences were statistically assured.
The obtained results by Găvan et al. (2010) revealed that the values of red cell count, hemoglobin concentration and hematocrit decreased after parturition and them increased again in early to mid-lactation.

CONCLUSIONS

The use of the volcanic tuff in the cows' feed during the pre-calving period and the first 2 weeks after calving determined a lower variation of the calcium and phosphorus values in blood.
In the case of magnesium from the blood samples taken from cows there were no differences noted in the pre- and post-calving periods.
For the experimental batch, the differences in values of the three hematological parameters, erythrocytes, leucocytes and hemoglobin, during the pre-calving and the first 2 weeks post-calving, show slight diminishes but which are not statistically provided.
The number of white blood cells, erythrocytes, and hemoglobin ranged from normal physiological limits in the lactation period, and was not influenced by the administration of zeolite in the ration of dairy cows.
As a consequence, it is necessary to balance the nutrition of dairy cows, being useful to take into account the level at which the mineral elements are not toxic to the organism, the requirements of this category of bulls, as well as the mineral content of the feed structure of the rations.

ACKNOWLEDGEMENTS

This research work was financed from Project PN III Bridge nr.11/2016 „Innovative technologies of using natural zeolites in dairy cows feed with positive impact upon environment and production efficiency".

REFERENCES

Bosi P., Creston D., Casini Luisa, 2002. Production performance of dairy cows after the dietary addition of clinoptilolite. Italian Journal of Animal Science, 1(3), 187-195.

Drăgotoiu D., Marin Monica, Pogurschi Elena, Drăgotoiu Tomița, 2017. The efficiency of using zeolites in dairy farms. Conference Proceedings, vol. Ecology and environmental protection, 17th International Multidisciplinary Scientific Geo Conference SGEM 2017, 17(61), 1129-1136.

Găvan C., Retea C., Motorga V., 2010. Changes in the hematological profile of Holstein primiparous in periparturient period and in early to mid-lactation. Scientific Papers: Animal Sciences and Biotechnologie, 43(2), 244-246.

Karatzia M.A., Katsoulos P.D., Karatzias H., Zeyner A, 2016. Blood selenium, copper, and zinc in dairy heifers during the transition period and effects of clinoptilolite administration. Czech Journal of Animal Science, 3, 133-139.

Katsoulos P.D., Roubies N., Panousis N., Karatzias H., 2005. Effects of long-term feeding dairy cows on a diet supplemented with clinoptilolite on certain serum trace elements. Biol.TraceElem.Res., 108(1-3), 137-145.

Mumpton F.A., 1999. La rocamagica: Uses of natural zeolites in agriculture and industry. Pro.Nat.Acad. Sci. USA, 96(7), 3463-3470.

Quiroz-Rocha G.F., LeBlanc S.J., Duffiled T.F., Wood D., Leslie K.E., Jacobs R.M., 2009. Reference limits for biochemical and hematological analytes of dairy cows one week before and one week after parturition. Ca.Vet.J., 50(4), 383-388.

Thilsing-Hansen T., Jorgensen R.J., 2001. Hot topic: prevention of parturient paresis and subclinical hypocalcemia in dairy cows by zeolite A administration in the dry period. Journal of Dairy Science, 84(3), 691-693.

Thilsing-Hansen T., Jorgensen R.J., Enemark J.M., Larsen T., 2002. The effect of zeolite A supplementation in the dry period on periparturient calcium, phosphorus, and magnesium homeostasis. Journal of Dairy Science, 85(7), 1855-1862.

Thilsing-Hansen T., Jorgensen R.J., Enemark J.M., Zelvyte R., Sederevicius A., 2003. The effect of zeolite A supplementation in the dry period on blood mineral status around calving. ActaVet.Scand.Suppl., 97, 87-95.

Scientific Papers. Series D. Animal Science. Vol. LXI, Number 1, 2018
ISSN 2285-5750; ISSN CD-ROM 2285-5769; ISSN Online 2393-2260; ISSN-L 2285-5750

ANALYSIS OF FODDER PLANTS FROM A VEGETAL FARM FOR INCREASING THE RENTABILITY

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Abstract

Following the research executed in a vegetal farm, the analysis of several types of plants assortments (sunflower, autumn barley, corn and wheat), with regards to the agricultural technologies applied, as well as their economic impact, with the purpose of determining improvement measures regarding structure of cultures and economic-financial performances within future years. The yields per unit area were determined by the varieties and hybrids used in each crop, by the applied crop technology, by the quality of the agricultural work carried out and by the pedological and climatic conditions. The average yield on crops was as follows: 3,500 kg/ha for wheat, 5,000 kg/ha for winter barley, 2,500 kg/ha for sunflower, 6,000 kg/ha for maize. The total crop yield was conditioned by the production capacity per unit area and cultivated area. Thus, total crop yields were as follows: 2,975 t wheat, 750 t barley, 1,375 t sunflower, 600 t corn. The obtained products were fully utilized on the market, with the exception of wheat. Of the total production of 2,975 t of wheat, 950 t were allocated to the landowners in the company, each providing an in-kind rent of 500 kg of wheat per hectare. Therefore, only 2,025 t of wheat became the commodity production, meaning 68.06% of the total production obtained in this crop.

Key words*: assortment, technology, fodder plants.*

INTRODUCTION

Feed technology develops the main elements of technology in maintaining the permanent and temporary meadows, for the cultivation of annual and perennial fodder plants, and helping to provide sufficient quantities of good quality feed (Ignat, 2000).

Regarding from a viewpoint of economic structure in the rural area, agricultural actions maintain generally the majority of land, agriculture being considered consequently as the mainstay of the rural economy.

The rural area is, from an occupational standpoint, prevalently a manufacture surface, where the activities of the primary districts hold a fairly high value on the economic ladder.

The agricultural-victual fields (field culture, grass plots, vegetable farming, viticulture, fruit plantations, animal breeding, forestry and exploit) requires understanding that (and with future comprehension too) a plot of 0.5-10 ha is enough for a family just to support itself, by traditional agricultural methods, albeit not enough to ensure that the entire population is fed (Ionel, 2003).

The matter can be explained by improper technical endowment, decreased efficiency per ha, and the ineptitude of the management.

MATERIALS AND METHODS

The seed represents the base of the growth of the harvest. The efficiency of all technological links that apply in vegetal production depend on the quality of the variety or the hybrid with which it is worked (Onisie and Jităreanu, 2000).

There are the varieties and hybrids used:

- for wheat cultivation the varieties were used: Crina, Alex, Dropia;
- for corn cultivation the hybrids were used: Florencia, Furio, Raissa;
- for fall barley cultivation the varieties were used: Madalin, Orizont, Precoce, Productiv;
- for sunflower cultivation hybrids were used: Favorit, Festiv, Rigasol, Performer;
- for sowing wheat the seed was treated with Vitavax 200 in a dose of 2 1/t per seed.

After canola cultivation, sowing the wheat was done at the beginning of October, and after

corn and sunflower the study was continued until the second round of 10 days of October.
The used density was of 500 kg/m^2 resulting quantity of seed used 230-260 kg/ha according to the indices of quality of the seed.
In spring after the snow melted the fertilization with ammonium nitrate worked started.
The combat against the weeds started towards the end of April with the herbicide Mustang in doses of 0.5 l/ha.
The harvesting began halfway through June and it continued until the beginning of July due to small breaks for hoarding production and price fluctuations on the market.
The harvesters SEMA 110 existing in the unit as well as a borrowed CLAAS were used.

RESULTS AND DISCUSSIONS

In terms of profit per hectare, the most efficient crops are in order: autumn barley with 734.88 lei/ha, sunflower with 710.97 lei/ha, corn with 698.27 lei/ha and on the last place autumn wheat with 266.68 lei/ha.
Taking as a point of reference the profit per kilogram of production, the hierarchy of crops is the following: sunflower, barley, corn, and winter wheat.
Depending on the rate of profitability, first was barley, then corn, sunflower, and last place wheat.
In order to establish the crop hierarchy by several criteria reflecting profitability, namely: income per ha, profit per hectare, profit per kilogram, rate of profitability, there was used the point method. Thus, each culture was awarded a number of points based on the performance achieved by these criteria.
The average production was the following: 3,500 kg/ha wheat, 5,000 kg/ha fall barley, 2,500 kg/ha sunflower, 6,000 kg/ha corn. The total production obtained by cultivation has been conditioned by the yield in the unit of surface and cultivated surface. Thereby, the total productions were the following: 2,975 t wheat, 750 t barley, 1,375 sunflower, 600 t corn (Table 1, Figure 1, Figure 2).

Table 1. The average and total production

Production	Wheat	Barley	Sunflower	Corn
Average (kg/ha)	3,500	5,000	2,500	6,000
Total (t)	2,975	750	1,375	600

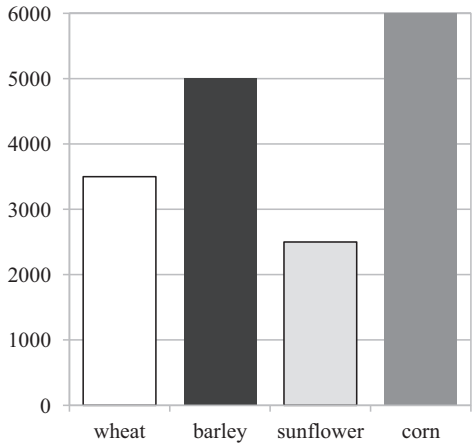

Figure 1. Average productions (kg/ha)

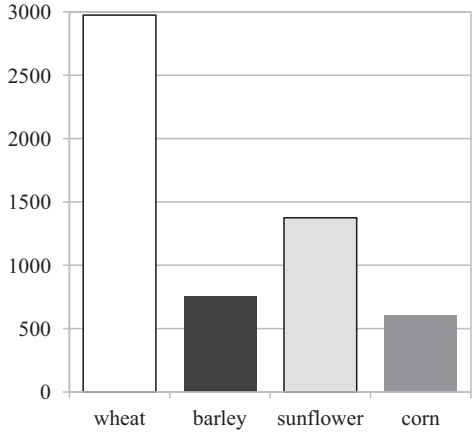

Figure 2. Total productions (t)

According to the hectare profit prism, the most efficient cultivations were in order: the fall barley with 734.88 lei/ha, sunflower with 710.97 lei/ha, corn with 698.27 lei/ha and fall wheat with 266.68 lei/ha (Figure 3).
The analysis of the total costs of production: For achieving the mentioned cultivations, the vegetal farm spent 585.21 thousand lei, total which was spent determined by the cultivation surface and cost of each hectare.
The percentage of each cultivation was: 47.84% wheat, 30.97% sunflower, 6.07% fall barley and 5.51% corn (Figure 4).

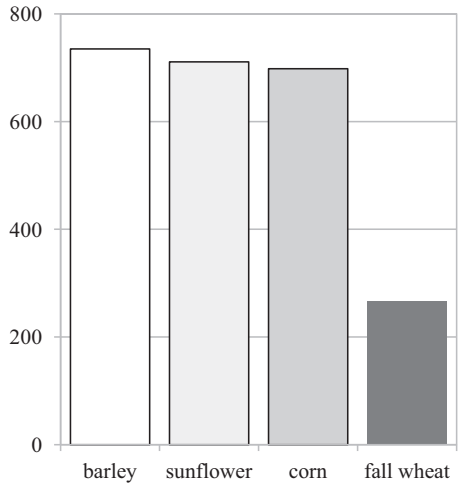

Figure 3. Profitability of the cultures

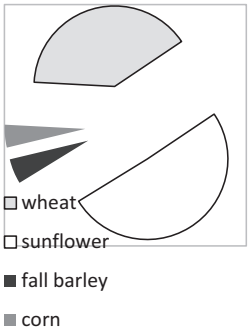

Figure 4. Total production costs

CONCLUSIONS

The analyzed vegetal farm has met the following difficulties in vegetal production:
- the aggressive climatic conditions, which affected the production and costs;
- the increased price of the material has an impact on the level of spending with mechanical work, lack of necessary diesel, even in the sowing company;
- increased price of fertilizers restricted the cultivators from applying the recommended doses of modern technology, with unwanted effect towards the performances in production and decreasing in fertilizing the soil;
- the absence of a price of acquisition guaranteed influenced the profit level, even though the cultivations were rentable, the increased offer on some products led to the decrease of the acquisition price on the market;
- the increased price of the pesticide and herbicide, of the chemical substances used against diseases and pests determined the farmers to use more reduced doses;
- due to the high rates on the credits by the bank the loans became unappealing and the financial resources of the economical agents in agriculture had to restrict to the profit which wasn't enough for the production.

Taking into account the economic aspects of using the cultural assortment and specific technology, this study lead to the conclusion that the farm must diversify its cultural structure:
- call upon varieties and well-performing hybrids, resistant to drought, illness and pests;
- sign farm contracts on prices bartered with the input providers;
- sign advantageous commercial contracts with the beneficiaries in order to have safe sales and profit.

Taking into consideration the economic efficiency on cultures, analyzed through the prism of income per hectare, profit per kilogram of product and return rate the hierarchy of the cultures was, in decreasing order: barley, sunflower, corn, wheat.

Taking this into account, in the future we must focus on a better strategy to settle culture structure by increasing the culture area for the plants that ensure a higher rentability.

REFERENCES

Onisie T., Jităreanu G., 2000. Agrotehnica, Editura Ion Ionescu de la Brad, Iaşi.
Ionel A., 2003. Cultura pajiştilor şi a plantelor furajere. Editura A'92, Iaşi.
Ignat A., 2000. Plante furajere. Editura Didactica si Pedagogica, Bucuresti.

Scientific Papers. Series D. Animal Science. Vol. LXI, Number 1, 2018
ISSN 2285-5750; ISSN CD-ROM 2285-5769; ISSN Online 2393-2260; ISSN-L 2285-5750

MATHEMATICAL MODEL OF OPTIMIZATION ENERGY METABOLISM AND PROTEIN QUALITY TO SWINE

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Abstract

The new systems which assess the nutritive value and determine the nutrient requirement triggered changes in the manner of formulation and development of optimized pig diets. The purpose of this paper is to present a viewpoint on a possible solution for diet optimization. We considered the determination of a possible „common denominator" between the nutritional requirements, mathematical algorithms that can be applied to the stated problem and the economic aspects assimilated as purpose functions in formulating the mathematical models that are used.

Key words*: mathematical modelling, energy metabolism, protein metabolism, pig nutrition.*

INTRODUCTION

One of the characteristics by which we can estimate the stage of development of a certain discipline is its degree of mathematization. Thus, Galileo Galilei said that „The great book of nature can be read only by the one who knows language in which this book was written and this language is the mathematics".
Truly important is the effective contribution and not the sophistication or elegance of the used mathematical instrument.
A relatively simple mathematical idea ca have an unexpected effect if used with skill. On the other hand, very elegant mathematical considerations may be of no use for the actual problems of that particular discipline.

MATERIALS AND METHODS

One can distinguish a number of 4 stages of the contribution of mathematics to the development of a scientific discipline:
Stage 1: Data collection, analysis and interpretation.

Stage 2: Quantitative formulation on scientifically principles and empirical laws.

Stage 3: Regarding the mathematical development of a model we can perceive two trends concerning the very operation of modelling. A first trend is the exaggerated simplification of the concrete situation, which has the advantage of increased possibilities for effective mathematical developments allowing the use of the model in a large range of different contexts.
A second trend, more and more present, due to the development of the computing capacity, is to include in the model as many as possible characteristics of the concrete situation and to use numeric methods to obtain the results.

Stage 4: Utilization of the mathematical models for the progress of the scientific knowledge
A mathematical model once developed and its predictions inferred by reasoning, we can ask ourselves can we use this material past its simple adequacy. In many cases the model and its predictions can lead us either to discovering and rendering evident unknown aspects or to clarifying others partially known.

RESULTS AND DISCUSSIONS

A mathematical model of the energy and proteic metabolism applied to pigs

NORMS FOR GROWING AND FATTENING PIGS
(1) Body weight and chemical composition assessment

The body weight (kg), function of age, is calculated with a Gompertz equation:

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

where:

A = body weight at maturity
B = growth coefficient
t = age in days
t^x = inflexion point, the time in days when the gain peaks

The net weight *Gn* may be assessed with the formula:

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

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

The values Pr, were calculated with the formula

$$Pr = B \times Pt \times \ln\left(\frac{Pt}{P\hat{t}}\right) \qquad [kg] \qquad (3)$$

where Pt, kg is given by the relation:

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

values B and $P\hat{t}$ are given in the gable below, which also shows the maximal values of Pr and of the minimal ratio $\frac{Lr}{Pr}$

The daily gain of lipids Lr was calculated with the ratio Lr/Pr:

$$Lr = \propto \times Pr \qquad [kg] \qquad (5)$$

where $\propto$ was calculated differentiated by males, females and castrated pigs according to the age (Burlacu et al., 1996):

For young boars:

$$\propto = e^{-2{,}633+0{,}08t-0{,}0014t^2+0{,}0000154t^3-0{,}0000000793t^4+0{,}0000000001513t^5} \qquad [kg] \qquad (6)$$

For young sows:

$$\propto = e^{-2{,}633+0{,}08t-0{,}0014t^2+0{,}0000154t^3-0{,}0000000793t^4+0{,}0000000001513t^5} \qquad [kg] \qquad (7)$$

For castrated pigs:

$$\propto = e^{-2.074+0.6364t-0{,}001317t^2+0{,}0000168t^3-0{,}0000000977t^4+0{,}0000000000206t^5} \qquad [kg] \qquad (8)$$

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

$$\mathrm{Ar} + \mathrm{Cenr} = \beta \times \mathrm{Pr} \qquad [kg] \qquad (9)$$

where β has the following values :

For young boars and castrated pigs:

$$\beta = e^{2.739-0.0434t+0{,}000421t^2-0{,}000001325t^3} \qquad [kg] \qquad (10)$$

For young sows:

$$\beta = 37.423 \times e^{-\frac{t}{10.703}} + 4.154 \times e^{-\frac{t}{744.84}}$$

The net weight $Gn\,([kg]\ at\ age\ t\ +\ 1) = Gn + \Delta Gn\ (at\ moment\ t)$, where *t* initial = 35 days, and Gn initial = $9.5\ kg$ for all sexes and categories

(2) Assessment of *EM* norms

$$\mathrm{EM} = \mathrm{EMm} + \mathrm{EPr} + \mathrm{ELr} \qquad [MJ/day] \qquad (12)$$

for the calculation of EM

$EMm =$ requirement of metabolisable energy for maintenance [MJ/day] $[kg]$ (11)
$EPr =$ requirement of metabolisable energy for body protein synthesis [MJ/day]
$ELr =$ requirement of metabolisable energy for body fat synthesis [MJ/day]
the following formulas are to be used:

$$EMm = 1.75 \times Pt^{0.75}\ [MJ/day] \qquad (13)$$

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

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

where:

$$Lr\ (lipid\ gain, in\ kg.) = 1{,}1 \times Pt^{0.07} \times Pr$$

(3) Assessment of the norms of available protein and limiting amino acids

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

where:

Pm (net protein for maintenance)

$$Pm = 0.04 \times Pt \quad [kg] \quad (17)$$

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

lysine requirement = $PA \times 70\ [g]$

met. + cys. requirement = $PA \times 40\ [g]$

tryptophan requirement = $PA \times 15\ [g]$

threonine requirement = $PA \times 45[g]$

PARTICULAR CASES OF DIET CALCULATION (RESTRICTED FEEDING)

In the practice, we are often confronted with situations when feeding is restricted. In this situation (restricted feeding), the manner of calculating changes. Therefore, we are presenting subsequently the manner of calculation of the requirement of energy and amino acids:

Inputs: Body weight: *G* [kg]

Average daily body weight: ΔG [kg]

Age: *t* [days]

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

Stage I. Calculation of the requirement of metabolisable energy and protein corresponding to the minimal $\frac{Lr}{Pr}$ ratio

The value of minimal $\frac{Lr}{Pr}$ ratio was calculated using the experimental data:

$$\left(\frac{Lr}{Pr}\right)_{min} = a + \frac{b}{1 + e^{-\frac{t-c}{d}}} \quad [kg] \quad (18)$$

where for the commercial castrated type we used the following values:

$a = 0.677;\ b = 1.95;$

$c = 148;\ d = 23.63$

The value of the retained protein is given in this case by:

$$Pr = \frac{\Delta G}{1.05\left(1 + \left(\frac{Lr}{Pr}\right)_{min} + \beta\right)} \quad [kg] \quad (19)$$

where: ΔG average daily gain [kg]

For $\beta, Pt, Pm, PA, EMm, EPr, Lr, ELr, EM, LizD, M + CD, TRID, TREONINAD$ the formulas from the above relations are to be used.

OBSERVATION 1: The dimensions important in determining the requirement of energy and protein according to this system of calculation are the metabolisable energy EM and the available protein PA.

The two calculation stages presented above show a striking fact, at first sight, but perfectly justified physiologically: for a restricted feeding and lower weight gains than the maximal values, there are variable values of the ratio $\frac{Lr}{Pr}$

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

involves the existence of norms that belong to some intervals:

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

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

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

Observation 1 is shown graphically in Figure 1.

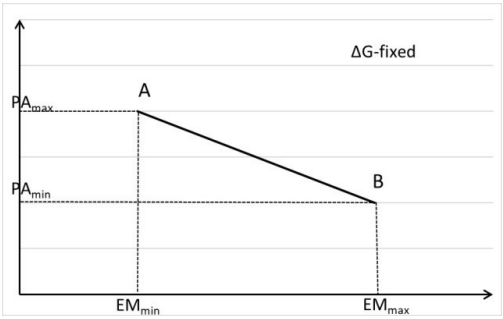

Figure 1. Observation 1

Any pair *EM, PA* of segment *AB* represents pertinent values to obtain the set weight gain. Obviously, each time there will be a different quality indicator as given by $\frac{Lr}{Pr}$ ratio.

***OBSERVATION 2**:* It can be immediately observed that the protein norms assessed with the system presented here eliminate the value of the digestible protein. Yet, diet optimisation also involves the essential use of an equation in *PBD.*

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

$$VB = \frac{PA}{PBD} \Longrightarrow PBD = \frac{PA}{VB}; 0 < VB < 1$$

As *VB* cannot be known beforehand it results that the norm of *PBD* depends on the nature and structure of the dietary raw materials, since it is no longer unique the value of *PBD* can no longer be used traditionally in the "norm tables" even though diet optimisation is done using the digestible nutrients.

Stage II. The calculation of the requirement of metabolisabe energy and protein corresponding to the maximal ratio $\frac{Lr}{Pr}$.

We calculate the maximally ingested metabolisable energy:

$$EM_{max} = 44\left(1 - e^{-0.0204^{G}}\right), \qquad [MJ]$$

We calculate the maximal value of the retained protein with the formula:

$$PR_{max} = B \times Pt \times \ln\frac{P\hat{t}}{Pt}$$

With the formula

$$EPr = 54.6 \times Pr, \qquad [MJ/day]$$

we calculate the energy required to retain the protein corresponding to *Pr* max.

We calculate the energy required to retain lipids:

$$Elr = EM_{max} - EM_m - EPr - Q' \qquad [MJ]$$

Which gives the maximal value for the retained lipids:

$$Lr_{max} = \frac{ELr}{53.3} \qquad [kg]$$

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

$$\left(\frac{Lr}{Pr}\right)_{max} = \frac{Lr_{max}}{Pr_{min}}$$

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

The dependence of *PBD* requirement function of the biological value of the diet is shown in Figure 2.

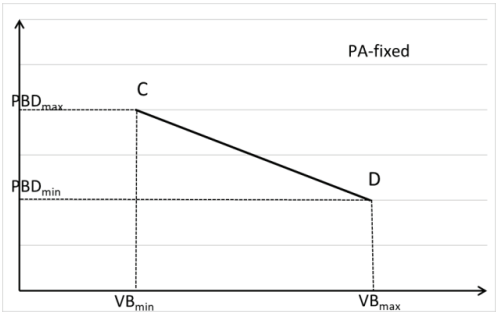

Figure 2. The dependence of *PBD* requirement function of the biological value of the diet

The dependence of *PBD* requirement function of the available protein is shown in Figure 3.

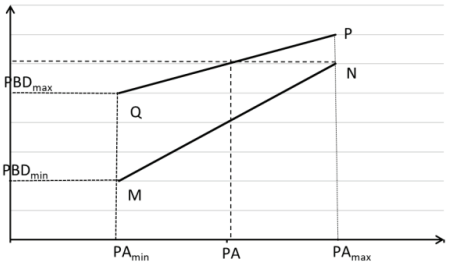

Figure 3. The dependence of *PBD* requirement function of the available protein

Figure 4 shows graphically the connection between *EM* and *PBD*.

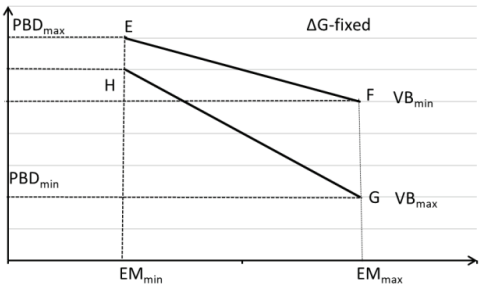

Figure 4. Connection between EM and *PBD*

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

The existence of an area *EFGH* for the requirement of *EM* and *PBD* is due to the two parameters

$$\frac{Lr}{Pr} \in \left[\left(\frac{Lr}{Pr}\right)_{min}; \left(\frac{Lr}{Pr}\right)_{max}\right] \text{ and}$$

$$VB \in [VB_{min}; VB_{max}].$$

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

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

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

CONCLUSIONS

Mathematical modelling can contribute to the agricultural scientific knowledge in many ways, some of which are presented below:

(1) Biological hypothesis expressed in mathematical language can offer a description on quantitative understanding of the biologic problems.

(2) Stimulation of the mathematical modeling can prove a conceptual frame which can contribute to the discovery of unknown domains, and can stimulate the appearance of new ideas and of experimental approaches on more rigorous basis. The scientific researcher can waste the resources solving false problems, and the specialist in development can transmit these information to the producers, who might even use them.

(3) A mathematical model can provide a way through which the data accumulated by research can be put to use by the farmers in a readily accessible form.

(4) The practical advantages of the methods produced by search can be studied with a model, stimulating thus the use of improved methods.

(5) The mathematical modelling determines the appearance of experiments which are less ad-hoc, because with the help of is easier to design experiments which to answer certain research requirements, of to dissociate between alternative mechanisms.

(6) Within a system with more components, the model offers a manner of gathering the knowledge on the component parts in order to give them a correct image on the behavior of the whole system. Thus, the information regarding the energy content of a concentrate is useless in the absence of the data on prices of the end products.

(7) Modelling can contribute to securing a strategical and tactical backing for a research program, justifying the activity of the scientists and promoting collaboration.

(8) A model can be an efficient means to summarize data, and a prudent method of interpolating and extrapolating.

(9) The data becoming more and more precise, but more and more expensive, a model can use data more efficiently, sometimes using them better.

(10) The power of prediction of an efficient model can be used in many ways: research, development, managing, planning priorities. For example, a model can offer answers to questions such as: What if …?; Which would be the consequences of cutting the concentrate intake in cattle on the milk yield and forage requirement? Which would be the effect of increasing the weaning weight of calves on the subsequent meat production? etc.

In connection to the concrete activity in biomathematics we note there main directions: in genetics, physiology and biotechnology.

The differences existing between the three domains can also be found in the mathematical models mostly used. In genetics and breeding the population studies require probabilist methods while in the other two domains the determinist mathematical disciplines seem to be more adequated.

As concerns the problems connected to the interdisciplinary team we must say that any attempts of interdisciplinary study are useful. For the mathematicians the presence of another specialist for dialogue is essential. The quality of the model depends on the quality of the dialogue, and the quality of the dialogue depends on the quality of men and on the working atmosphere.

REFERENCES

Burlacu R., 2003. Mathematical models of applied biology of some metabolic processes, Progress in research on energy and protein metabolism, EAAP publication, Wageningen Academic Publishers, 139-146.

Burlacu Gh., Burlacu R., Cavache A., 2002. Potenţialul productiv al nutreţurilor şi utilizarea lor. Bucureşti: Editura CERES.

Danfær A., 1990. A dynamic model of nutrient digestion and metabolism in lactating dairy cows, 671 Report from the National Institute of Animal Science, Denmark.

Dent J.B., Blackie M.J., 1974. Systems simulation in agriculture, Applied Science Publishers LTD, London.

Doucet P., Sloep P.B., 1992. Mathematical modelling in the Life Science, Ed. Ellis Horwood, New York London Toronto Syddey Tokyo Singapore.
Emmans G.C., 1992. Animal growth and feed intake, A collection of papers 1981-1990, SAC, Edinburgh.
France J., Thornley J.H.M., 1986. Mathematical models in agriculture, Butterworths, London Boston Durban Singapore Sydney Toronto Wellington.
Parks J.R., 1982. A theory of feeding and growth of animals, Springer-Verlag, Berlin Heidelberg New York.
Kyriazakis J., Emmans G.C., 1992. The effects of varying protein and energy intakes on the growth and body composition of pigs, British Journal of Nutrition, 68, 03, 603-615.
Kyriazakis J., Emmans G.C., 2002. The effects of varying protein and energy intakes on the growth and body composition of pigs. British J. Nutr., 68(03), 615-625.
Whittemore C., 1993. The Science and Practice of Pig Productions, Longmane Scientific and Technical, London.
Whittemore C., 2003. The Science and Practice of Pig Productions. London: Longmane Scientific and Technical.

Scientific Papers. Series D. Animal Science. Vol. LXI, Number 1, 2018
ISSN 2285-5750; ISSN CD-ROM 2285-5769; ISSN Online 2393-2260; ISSN-L 2285-5750

THE COMBINED ADD EFFECTS OF FODDER ADDITIVES (YEA-SACC 1026+ACTIGEN) ON SOME PRODUCTION AND CONSUMPTION INDICES AND ON HEALTH STATUS IN CHICKEN BROILERS

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Abstract

The study followed the effects of combined add of fodder additives (YEA-SACC 1026+Actigen) on production and consumption indices and on health status in broiler chickens. The experiments were done on 50 Ross-308 broiler chickens, grouped in two lots, with 25 capita/lot and during 42 days. In the experimental lot, in the mixed fodder were added YEA-SACC 1026+Actigen in same proportion of 0.1%+0.08% in starter phase 1 (1-14 days), in production phase 2 (15-35 days) and in finishing phase 3 (36-42 days). For the control lot was used only mixed fodder. During the experiments were followed the average body weight, daily body gain and fodder consumption and conversion index, and for the health status were effected blood analyses.

The combined use of probiotic YEA-SACC 1026+prebiotic Actigen in experimental lot determined the increase of body weight with 20.45%, daily body gain with 13.78% and the decrease of fodder conversion index with 7.32% given to control lot. The blood index analysis for glutation peroxidase (GPx) was better in experimental lot with 100.76 U/ml HT given to control one with 74.42 U/ml Ht.

Key words*: meat broilers, YEA-SACC 1026+Actigen, blood indices, production performances.*

INTRODUCTION

Having in view that starting with January 2006, European Union interdicted the use of promoters, basis on antibiotics in farm animal fodder, it became necessary to find some alternatives (EC Regulation No. 1831 2003; De Jong et al., 2012).

Such promoter is the probiotic YEA-SACC 1026 with important role in increasing the fodder assimilation degree and its efficiency.

The living culture of *Saccharomyces cerevisiae* raised on yellow maize, malt and molasses, has subsequent composition: minimum 28% of pure protein, minimum 6% of pure fat, maximum 14% of pure fiber, maximum 8% of dry substance and contains minimum 5 millions of cells/gram. The increased concentration degree of product leads to a reduced daily administration dose, thus in meat chickens and generally in poultry, the administration dose is 1 kg/ton (Mohnl, 2011).

An extremely efficient prebiotic, basis on oligosaccharides, is represented by Actigen product of Alltech Company® from Kentucky (USA), obtained from the cellular walls of *Saccharomyces cerevisiae* yeast, cultured on a complex mixture of sugars. The effected studies on its action manner marked that prebiotic blocks the pathogen bacteria and has immune stimulating characteristics determining the reactivity increase of intestinal lymphocytes as transformation consequence of lymphoblasts and increasing phagocytosis capacity of blood white cells (Nawaz et al., 2016; Patterson and Burkholder, 2003).

Actigen is the second generation of unique bioactive fraction, derived from external cellular wall of a specific yeast strain, *Saccharomyces cerevisiae*, selected by Alltech Company®. Depending on targeted species, their age and selected protocol, the Actigen is included to a rate of 200 to 800 g/ton of poultry fodder.

Concerning the performances of this product, some researches (Paryad and Mahmoudi, 2008) observed that administration of probiotic YEA-SACC 1026 in different doses in meat chickens registered the best results in experimental lots as concerns the their body weight, fodder

conversion index and health status given to control lot.

Gheisari and Kholeghipour (2010), pursuant to living yeast administration in meat chickens (dose of 0.1%), obtained the best results for body weight (2780.87 g given to 2762.179 g), for average daily gain (55.93 g given to 55.57 g) and for fodder conversion index (1.82 given to 1.87) comparatively with the control lot and other experimental ones, in which the living yeast was administered in dose of 0.2% and 0.3%.

Another researchers as Gao et al. (2008), administrating living *Saccharomices cerevisiae* yeast in meat chickens (dose of 2.5%) obtained the best result concerning the body weight (2459 g given to 2378 g in control lot), average daily gain (57.5 g given to 55.6 g in control lot) and fodder conversion index (1.95 given to 2.03 in control lot).

The aim of our study was to follow the effects of combined add of some fodder additives (YEA-SACC 1026+Actigen) on production and consumption indices and on health status expressed by blood indices in chicken broilers.

MATERIALS AND METHODS

The researches was effected during September and October 2016 in the Biobasis of Poultry Farming Discipline from Department 2-Technological Sciences, Faculty of Animal Science and Biotechnologies, UASVM Cluj-Napoca, on a number of 50 meat chickens of (Ross-308 hybrids). Ross-308 is a tetralinear hybrid, created by Ross Breeders Company® from United Kingdom (www.aviagen.com), it is a pretentious hybrid as concerns the fodder quality, but is less pretentious for microclimate conditions (Van et al., 2010). The chickens were distributed in two lots of 25 capita/lot during a time period of 42 days. For the control lot L(M) was administered simple combined fodder having the same protein level as for experimental lot L1(E). The administered combined fodder provided 3030 kcal ME/kg in the first phase, 3134.8 kcal ME/kg in the second phase and 3144 kcal ME/kg in the finishing one. As concerns the level of crude protein, it was of 22.76% in the first phase, 21.31% in the second phase and 19.94% in the finishing one, providing a good biological value according to fodder energetic level.

During 42 days, in lot L1(E) was added in combined fodder the probiotic **YEA-SACC 1026+Actigen** in proportion of 0.1%+0.08% in starter phase 1 (1-14 days), in proportion of 0.1%+0.08% in production phase 2 (15-35 days) and 0.1%+0.08% in finishing phase 3 (36-42 days). The fodder and water were *ad libitum*.

For the control lot L(M) was used only mixed fodder. The chicken broilers benefited of the same breeding system and identical microclimate and feeding conditions. The experiment was done having into consideration all attendance and feeding rules specific for Ross-308 hybrid (www.aviagen.com).

During the experiment period, no vaccines or medication were done.

The chickens of two lots were weighed at the experiment start and further weekly, having in view the body weight, daily gain, fodder consumption and fodder conversion index.

After 42 days, from each lot were collected 5 blood samples for health status parameters' analysis. The blood analyses were effected with Spectrofotometer UV-VIS Screen Master Touch (Medical Lab of Veterinary Medicine Faculty). The experimental data were statistically analyzed with Student test by GraphPad InStat ver.3.10 program.

RESULTS AND DISCUSSIONS

The average values and variability of ***body weight*** in meat broilers on ***starter phase 1*** (1-14 days) are presented in Table 1.

Table 1. Average values and variability of body weight in broiler chickens on starter phase 1 (1-14 days) (g/capita)

Age (days)	L(M) n=25		L1(E) n=25 YEA-SACC 1026 0.1% +Actigen 0.08%	
	$X \pm S_x$	V%	$X \pm S_x$	V%
1 day	44.64±0.58	6.58	44.52±0.58	6.60
7 days	152.20±3.27	9.55	165.40**±3.54	10.13
14 days	363.60±12.82	11.62	473.32***±12.98	13.71

X=average; s_x=standard error of average; V%=variation coefficient;
*p<0.05 significant differences;
**p<0.01 distinct significant differences;
***p<0.001 very significant differences

Analyzing the data presented in Table 1 comes out that there are not significant differences for body weight between the lots at age of 1 day old of broiler chickens. At 7 day from the beginning, were observed distinct significant values (165.40±3.54 g given to 152.20±3.27 g),

but at 14 days between broiler lots appear very significant differences (473.32±12.98 g given to 363.60±12.82 g). Sarangi et al. (2016) reported at 14 days a body average weight of 422.43±4.46 g in Vencobb broiler chickens fed with basal diet and probiotic 100g/ton and 404.79±5.30 g in Vencobb broiler chickens fed with basal diet and prebiotic 400 g/ton.

The ***body weight gain*** realized in ***starter phase 1*** (1-14 days) is presented in Table 2.

Table 2. Evolution of average gain on starter phase 1 (1-14 days)

Age (days)		UM	L(M) n=25	L1(E) n=25 YEA-SACC 1026 0.1% +Actigen 0.08%
Phase 1 (1-14 days)	At	g	107.56	120.88
	7 days	%	100	112,38
	At	g	211.40	269.04
	14 days	%	100	127.26

The average gain difference, realized during 14 days, was greater in experimental lot given to control one, in L1(E) being observed after the phase 1 a difference of 9.18% given to L(M). These obtained values were greater than those of Saiyed et al. (2015), which reported at 7 days only 110.10±3.62 g in experimental lots fed with basal diet and probiotic 100g/ton and only 103.74±3.06 g in broilers fed with basal diet and probiotic 50 g/ton+prebiotic 250 g/ton.

Related to ***body weight average values and variability*** in broiler chickens, obtained on ***production phase 2*** (15-35 days), there are presented in Table 3.

Table 3. Body weight average values and variability in broiler chickens during production phase 2 (15-35 days) (g/capita)

Age (days)	L(M) n=25		L1(E) n=25 YEA-SACC 1026 0.1% +Actigen 0.08%	
	$X \pm S_x$	V%	$X \pm S_x$	V%
At 14 days	363.60±12.82	11.62	473.32***±12.98	13.71
At 21 days	644.80±17.62	13.66	787.96***±23.84	15.12
At 28 days	1039.64±23.29	11.20	1255.60***±36.53	14.54
At 35 days	1483.84±65.17	14.62	1882.72***±44.91	11.92

X=average; s_x=standard error of average; V%=variation coefficient; *p<0.05 significant differences; **p<0.01 distinct significant differences; ***p<0.001 very significant differences

From this table data, it can be observed that at 28 days there are distinct significant differences between lots L1(E) and L(M).

Comparing with our data, Sarangi et al. (2016) obtained at 21 days only 698.86±8.98 g in Vencobb broilers fed with basal diet and probiotic 100g/ton that means with 11.30% more reduced values.

The ***average body weight gain*** realized in this production phase is presented in Table 4.

Table 4. Evolution of average gain on production phase 2

Age (days)		UM	L(M) n=25	L1(E) n=25 YEA-SACC 1026 0.1%+Actigen 0.08%
Phase 2 (15-35 days)	At	g	281.2	307.92
	21 days	%	100	109.50
	At	g	394.84	467.64
	28 days	%	100	118.43
	At	g	444.2	627.12
	35 days	%	100	141.17

From these data can be observed that in lot L1(E) was an increase of average body weight gain until 28 days with +18.43%, and at 35 days with +41.17% given to lot L(M).

Saiyed et al. (2015) obtained at 35 days only 515.35±7.23 g in broiler chickens fed with basal diet and probiotic 100 g/ton+prebiotic 500 g/ton.

In the ***finishing phase 3***, from 36 days to 42 days, the evolution of ***average body weight*** is presented in Table 5.

Table 5. Body weight average values and variability in broiler chickens on finishing phase 3 (36-42 days)

Age (days)	L(M) n=25		L1(E) n=25 YEA-SACC 1026 0.1% +Actigen 0.08%	
	$X \pm S_x$	V%	$X \pm S_x$	V%
At 35 days	1483.84±65.17	14.62	1882.72***±44.91	11.92
At 42 days	2070.84±30.47	7.35	2494.4***±22.41	4.49

X=average; s_x=standard error of average; V%=variation coefficient; *p<0.05 significant differences; **p<0.01 distinct significant differences; ***p<0.001 very significant differences

From the presented data, there are observed that differences values are very significant between the experimental and control lots, and these values are greater than those reported by Sarangi et al. (2016) at 42 days of 1726.30±25.46 g in broilers fed with basal diet and probiotic 50 g/ton.

The very significant differences during finishing phase 3 could have as cause that from fodder composition was eliminated "the coccidiostatic" and replaced with the probiotic and prebiotic additives.

As concerns the ***body weight gain on finishing phase 3***, the obtained data are presented in Table 6.

Table 6. Evolution of average gain on finishing phase 3 (35-42 days)

Age (days)		UM	L(M) n=25	L1(E) n=25 YEA-SACC 1026 0.1% +Actigen 0.08%
Phase 3 (35-42 days)	At 35 days	g	444.2	627.12
		%	100	141.17
	At 42 days	g	587	611.68
		%	100	104.20

From the table data can be observed that during finishing phase 3 the average body weight gain in experimental lot L1(E) was greater with +4.20% given to control lot L(M). Saiyed et al. (2015) reported at 42 days values of 499.40±21.42 g when the broilers were fed with basal diet and probiotic 100 g/ton +prebiotic 500g/ton and 535.58±4.00 g when the broilers were fed with basal diet and prebiotic 500 g/ton.

As concerns the ***average fodder consumption*** during the experiment, the data are presented in Table 7.

Table 7. Evolution of fodder consumption in broiler chickens' lots during the study (1-42 days)

Age (days)		UM		L(M) n=25	L1(E) n=25 YEA-SACC 1026 0.1%+Actigen 0.08%
Starter phase 1 (1-14 days)	At 7 days	g		34.03	36.28
	At 14 days	g		36.05	37.77
	Average consumption phase 1	g		35.04	37.25
Production phase 2 (15-35 days)	At 21 days	g		63.15	68.96
	At 28 days	g		116.22	122.14
	At 35 days	g		159.55	149.75
	Average consumption phase 2	g		112.97	113.61
Finishing phase 3 (36-42 days)	At 42 days	g		188.71	199.62
Average daily consumption on entire period	Average consumption/ day/capita	g	X±	99.61±	102.42±
			s_x	24.37	26.92
			s	59.43	67.25

X=average; s_x=standard error of average; s=standard deviation

From the presented data can be observed that even the average fodder consumption in experimental lot was greater than control one, there not existed statistical differences between the two lots. Compared to our data, Saiyed et al. (2015) reported more reduces values on entire period of 83.80±1.21 g/bird/day in broiler lots fed with basal diet and probiotic 100 g/ton+prebiotic 500 g/ton.

The ***fodder conversion index*** data during the experimental period are presented in Table 8.

Table 8. Evolution of fodder conversion index during entire experimental period

Age (days)	UM	L(M) n=25	L1(E) n=25 YEA-SACC 1026 0.1% +Actigen 0.08%
Phase 1 (1-14 days)	Kg/Kg	1.53	1.33
		100	86.92
Phase 2 (15-35 days)	Kg/Kg	2.11	1.70
		100	80.56
Phase 3 (36-42 days)	Kg/Kg	2.25	2.28
		100	101.33
Final average on entire period	Kg/Kg	1.96	1.77
	%	100	90.30

The fodder conversion index was reduced with –9.70% in experimental lot L1(E) given to control one L(M). The analysis of these fodder conversion indices explain the efficiency of probiotic **YEA-SACC 1026** combined with prebiotic ***Actigen***, resulting that they reduced the index value in experimental lot given to control one. Sarangi et al. (2016) reported on entire period (0-42 days) an average value of 1.72±0.02 in broiler lots fed with basal diet and probiotic 100/50 g/ton, and Gheisari and Kholeghipour (2010) reported an average value of 1.87 in broilers fed with basal diet and prebiotic 0.3% powder of *Saccharomices cerevisiae*.

As concerns the ***health status***, expressed by blood indices GPx (glutation peroxidase), Hct (hematocrit), Hb (hemoglobin) and Ery (erythrocytes), the obtained results were different for the two studied lots.

The obtained ***GPx*** values are presented in Table 9.

Table 9. Values of GPx U/ml Ht (glutation peroxidase)

Samples	L(M) n=5	L3(E) n=5 YEA-SACC 1026 0.1% +Actigen 0.08%
1	72.4	96.7
2	70.9	100.00
3	75.61	90.3
4	75.61	112.1
5	77.6	104.7
$X±S_x$	74.42±1.21	100.76±3.67
S	2.71	8.22
V(%)	3.64	8.15

X=average; s_x=standard error of average; s=standard deviation; V%=variation coefficient.

For this index, were not observed statistical differences between the two lots, but analyzing these values (GPx) comparing with those ones obtained by other authors (Anderson et al., 1978; Ammerman et al., 1980) cited by

Ashayerizadeh et al. (2009), there were proper in lot L1(E) (Yea Sacc 1026) with 100.76 U/ml Ht (maximum value permitted of 144.74 U/ml Ht) and reduced in control lot L(M) with values of 74.42 U/ml Ht.

In this way, comes out that prebiotic and probiotic had positive influence on health status of broiler chickens.

The values of ***blood count*** (Hct, Hb, Ery) are presented in Table 10.

Table 10. Values of blood count (Hct-%, Hb-g/dl, Ery-mill/mm^3)

Specification	UM	Parameters	L(M)	L1(E)
		n	5	5
Hct	%	$X \pm S_x$	28.88±0.52	29.8±0.72
		S	1.16	1.62
		V%	4.01	5.43
Hb	g/dl	$X \pm S_x$	9.93±0.24	9.93±0.17
		S	0.55	0.17
		V%	5.53	1.71
Ery	millions/mm^3	$X \pm S_x$	2.42±0.11	2.21±0.16
		S	0.26	0.23
		V%	10.74	10.40

X=average; s_x=standard error of average; s=standard deviation; V%=variation coefficient.

Citing the specialty literature, in meat broiler chickens the normal blood count values are: Hct-% is between 25.7 and 31.5%; Hb-g/dl is between 7.65 and 10.6 g/dl; Ery-mill/mm^3 is between 1.7 and 2.7 mill/mm^3 (Ashayerizadeh et al., 2009). From our data comes out that these values are inside the normal limit parameters.

CONCLUSIONS

1. After we presented the obtained data comes out that the use of probiotic YEA-SACC 1026 combined with Actigen in fodder of broiler chickens determined a substantial improvement of production and consumption indices.
2. The body weight at 42 days in experimental lot L1(E) was superior with 20.45% given to control lot L(M).
3. The average daily gain on entire experimental period was superior in experimental lot L1(E) with 18.82% given to control lot L(M).
4. Even the fodder consumption was greater with 2.81% in experimental lot L1(E) given to control one L(M), the fodder conversion index was more reduced with 9.70% in experimental lot L1(E) than in control one L(M).
5. The health status was positively influenced in experimental lot and the values of blood count were in normal parameters.
6. The use of probiotic YEA-SACC 1026 combined with prebiotic Actigen in broiler chickens' fodder with the two mentioned doses is recommended because they have positive effects on all production indices and also on health status.

REFERENCES

Ashayerizadeh A., Dabiri N., Ashayerizadeh O., Mirzadeh K.H., Roshanfekr H. and Mamooee M., 2009. Effect of dietary antibiotic, probiotic and prebiotic as growth promoters, on growth performance, carcass characteristics and hematological indices of broiler chickens. Pak. J. Biol. Sci., 12, 52-57.

De Jong I., Berg C., Butterworth A., Estevéz I., 2012. Scientific report updating the EFSA opinions on the welfare of broilers and broiler breeders. Supporting Publications 2012: EN-295. Report for the European Food Safety Authority (EFSA). www.efsa.europa.eu/publications.htm

Gao J. et al., 2008. Effects of yeast culture in broiler diets on performance and immunomodulatory functions. Poultry Science 87, 7, 1377-1384.

Gheisari A.A., B. Kholeghipour, 2010. Effect of dietary inclusion of live yeast (*Saccharomyces cerevisiae*) on growth performance, immune responses and blood parameter of broiler chickens. Poultry Science Association Inc. https://www.researchgate.net/publication/228349372

Mohnl M., 2011. Poultry production: How probiotics can play a role. Poultry Health Featured Articles, The Poultry Site. Available from: http://www.thepoultrysite.com/articles/2256/ Accessed on 03-10-2011.

Nawaz H., Abbas Irshad M., Mubarak Ali, 2016. Effect of probiotics on growth performance, nutrient digestibility and carcass characteristics in broilers. JAPS: Journal of Animal & Plant Sciences, 26(3), 599-604.

Paryad A., Mahmoudi M., 2008. Effect of different levels of supplemental yeast (*Saccharomyces cerevisiae*) on performance, blood constituents and carcass characteristics of broiler chicks. African Journal of Agricultural Research, 3, 12, 835-842.

Patterson H.A., Burkholder K.M., 2003. Application of prebiotic and prebiotics in poultry production. Poultry Science, 82, 627-631.

Saiyed M.A., Joshi R.S., Savaliya F.P., Patel A.B., Mishra R.K., Bhagora N.J., 2015. Study on inclusion of probiotic, prebiotic and its combination in broiler diet and their effect on carcass characteristics and economics of commercial broilers. Veterinary World 8(2), 225-231.

Sarangi N.R., Babu L.K., Kumar A., Pradhan C.R., Pati P.K., Mishra J.P., 2016. Effect of dietary

supplementation of prebiotic, probiotic, and symbiotic on growth performance and carcass characteristics of broiler chickens. Veterinary World, 9(3), 313-319.
Van I. et al., 2010. Cresterea si industrializarea puilor de carne. Ed. Total Publishing, Bucuresti, ISBN 978-606-8003-10-8.
EC Regulation No. 1831/2003 of European Parliament and the Council of 22 Sept 2003 on additives for use in animal nutrition. Off. J. Eur. Union. Page L268/36 in OJEU of 10/18/2003. Brussels, 0020 Belgium.
Ross-308 Broiler Performance Objectives, Home page address: www.aviagen.com.

Scientific Papers. Series D. Animal Science. Vol. LXI, Number 1, 2018
ISSN 2285-5750; ISSN CD-ROM 2285-5769; ISSN Online 2393-2260; ISSN-L 2285-5750

INFLUENCE OF THE AREA AND LACTATION ON PHYSICO-CHEMICAL PARAMETERS AND THE CONTENT OF HEAVY METALS IN THE DONKEY MILK

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Abstract

Donkey milk is considered a substitute for breast milk. Because of its nutritional and compositional properties it is very close to breast milk. Equine asinus can be considered suitable for feeding newborns, against cow's milk, which can cause intolerance. We determined the physico-chemical parameters of donkey milk with CombiFoss FT. The total germ count (TNG) was analyzed by BactoScan and the total number of somatic cells (TNS) by Fossomatic FC. Determination of heavy metals in milk was performed by mass spectrometry to identify Pb, Cd, Cu and Zn. Heavy metals are present in the environment but also in food and can cause serious health problems to the consumer. For the consumer, the main source of cadmium exposure is the food they consume, water and smoking. Cd contamination of dairy milk may also be due to the use of Cd-based chemicals, fertilizers, feed and waste water.

Key words*: donkey, heavy metals, lactation, milk, protein.*

INTRODUCTION

The donkey milk is a complex aliment, due to its chemical composition (protein, lizozim, fat acids, vitamins) and it represents an important nutritive contribution to the human organism (Shibamoto et al., 2009). Milk is a beneficial aliment for the human organism, present in daily nourishment, but it requires increased attention, due to toxic chemical compounds that may be present (microbiologic contaminants, total number of germs, total number of somatic cells). Among others, steel production, as well as burning coal and waste can increase the concentration of zinc in the environment. It can be inhaled along with dust and smoke, taken in through alimentation, or through exposure to various work places. The quantity that protrudes through the skin is relatively small. Zinc can be stored in the entire organism (ATSDR, 2007; Dash et al., 2013; Mukhopadhyay et al., 2005). All these factors (pollution, industrial activities, consumption of contaminated aliments, energy production) influence the human body over a prolonged period of time and may cause a vast array of health issues (Reimann et al., 2005; Concha et al., 1998; Aposhian et al., 2004). It can be said that heavy metal particles are causing a large number of drastic illnesses. Heavy metals find their way into the human organism through alimentation and cause concerning effects due to bioacumulation (Vinodhini et al., 2008; Coroian et al., 2017; Dash et al., 2013; Piver et al., 1989).

The presence of heavy metals like Cd in aliments is due to usage Cd based fertilizers and residual waters (EFSA, 2009), as well as packing various foods in metal cans. The symptoms of prolonged intoxication with relatively large quantities of Cd are mainly coughing, irritability and pain associated with chest burns.

Clinic analyses have highlighted the apparition of pneumonia, respiratory obstruction, affected pulmonary function as well as a decrease of the organisms vital capacity (EFSA, 2009). For acute intoxications we can observe: nausea, looseness of bowels and dizziness (Muzi et al., 2001; Vantroyen et al., 2004). Oral Cd intoxications lead to severe irritation of the gastro-intestinal epithelium accompanied by dizziness, nausea, excessive salivation, abdominal pains, cramps, looseness of bowels (Andersen et al., 1988). Pb intoxication cause symptoms such as abdominal pains,

constipation, cramps, nausea and anorexia (Rosenman et al., 2003). The purpose of the research was to determine the physico-chemical composition, the TGN and SCN present in donkey milk under the influence of lactation and geographical location, as well as assessing the concentration of heavy metals present in donkey milk.

MATERIALS AND METHODS

Physico-chemical analysis and TGN (Total Germ Number) and SCN (Somatic Cell Number) analysis of donkey milk

Donkey milk samples have been gathered from traditional farms located in two areas: Sălaj and Cluj. A number of five samples have been taken for each lactation and each location. The milk has been harvested using traditional methods (manual) in an appropriate hygienic environment. The samples have been stored in sterile containers, the ones allocated for the physico-chemical and microbiological analysis being refrigerated, while the ones assigned for heavy metals analysis have been frozen. The equipment used to conduct the physico-chemical analysis was CombiFoss FT, BactoScan for the TGN and Fossomatic FC for the SCN.

The analysis of heavy metals in donkey milk

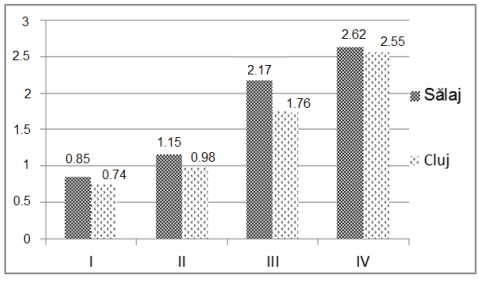
Heavy metals in donut milk were analyzed by inductively coupled plasma mass spectrometry or ICP-MS used to identify and quantify Pb, Cd, Cu, and Zn elements. The samples have been taken through the mineralization process as following: microwave digestion of 1 ml of milk, using 8 ml HNO_3 65% and 2 ml H_20_2 30%. The digestion process consisted of four stages, as following: stage 1 - temperature - 145^oC; pressure - 30 (bar); ramp time - 5 min; maintenance time - 15 min; stage 2 - temperature -180^oC; pressure - 30 (bar); ramp time - 1 min; maintenance time -10 min; stage 3 - temperature -120°C; pressure - 30 (bar); ramp time - 1 min; maintenance time - 15 min; stage 4 - temperature -100°C; pressure - 0 (bar); ramp time - 1 min; maintenance time - 10 min. The equipment used was a Berghoff MWS-3 (Eningen, Germany) microwave digestor. The sample was cooled to room temperature, dissolved in 25 ml of ultrapure water then filtered through 0.45 µm cellulose membrane. The concentration of heavy metals were determined using an ICP-MS ELAN DRC II Perkin-Elmer.

RESULTS AND DISCUSSIONS

The fat content of the donkey milk varies according to lactation and the harvesting area as follows: in lactation I, the fat content has the lowest average values (0.74-0.85%) (Figure 1. a). The highest values can be seen in lactation IV (2.55-2.62).

Similar results have been reported for fat (Coroian et al., 2016). The protein varies similarly to fat, showing the lowest mean values in lactation I (1.72-1.74%) and the highest values in lactation IV (1.87-1.90%) (Figure 1. b).

Lactose varies according to lactation as follows: 6.71% in lactation I in the Sălaj area and the highest values are in lactation IV 6.89% of Sălaj area (Figure 1. c). The water content ranges from 86.5% in lactation I, Sălaj and 89.81% in lactation IV (Figure 1. d). The results obtained for physicochemical parameters are in agreement with other studies such as those reported by Gastaldi et al., 2010, Husby et al., 2014, Cotte et al., 2003, El-Hatmi et al., 2015.



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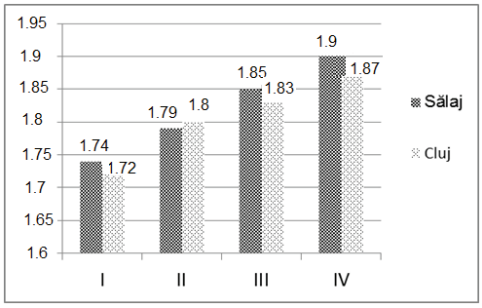

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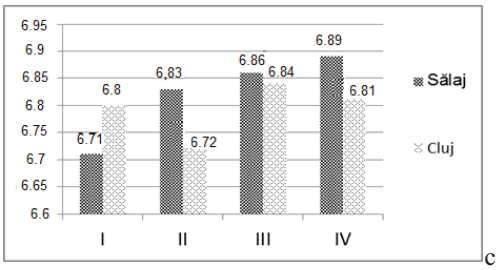

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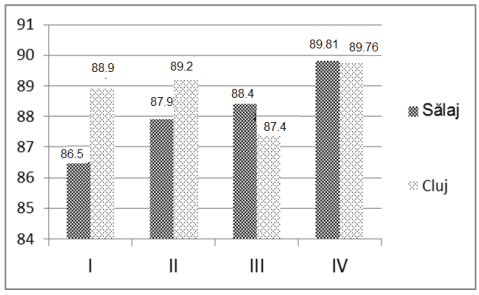
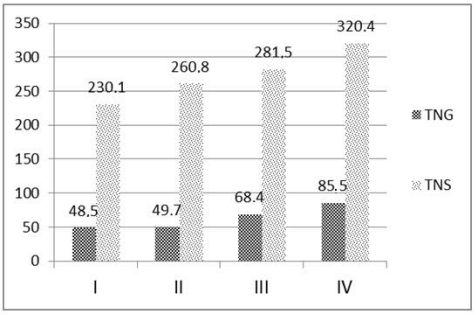


d

Figure 1. Fat (a), protein (b), lactose (c), water (d) content of lactating milk according to lactation (I-IV) and sampling area (Sălaj and Cluj)

TNG varied in the 48.5 range, the lowest values in lactation I, Sălaj area and 85.5, the highest values in lactation IV, Sălaj area. TNS varied in the range 230.1, lactation I, Sălaj and 320.9, lactation IV Cluj area (Figure 2. a, b).



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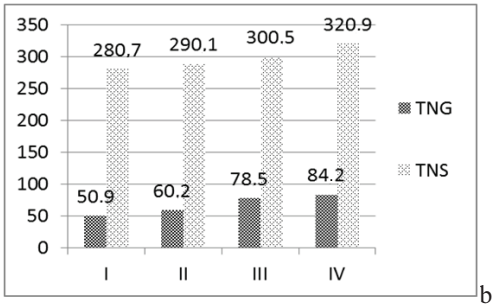
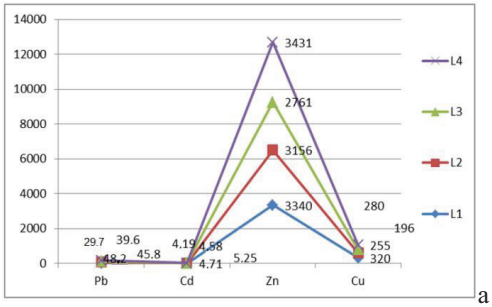


b

Figure 2. TNG and TNS from lactating milk according to lactation and area Sălaj (a) and Cluj (b)

The Pb ranged from 29.7 (µg/L) for lactation I, in the Sălaj area, showing the lowest values, and 48.2 (µg/L) for lactation IV. The level of Pb in donkey milk in the Cluj area varied in the range of 19.59 (µg/L), lactation II and 32.6 (µg/L) in lactation IV (Figure 3, a and b). The Cd ranged from 4.19 (µg/L), lactation I and 5.25 (µg/L), lactation 3, in the Sălaj area. The values for Cd are lower for donkey milk in Sălaj compared to the Cluj area. Cd in donkey milk in the Cluj area varies between 5.66 (µg/L), lactation I, and 6.89 (µg/L) in lactation IV. Zn in lactation III in the Sălaj area shows the lowest level, 2761 (µg/L), and highest in lactation IV, 3431 (µg/L). In the Cluj area, the lowest level for Zn is 2019 (µg/L), in lactation 3 and highest in lactation I, 2430 (µg/L). The level of Cu varied within the range, 196 (µg/L), lactation III, and 320 (µg/L), lactation I, Salaj area, and with lower values in the Cluj area, with variations of 127 (µg/L), lactation IV and 266 (µg/L), lactation I (Figure 3 a, b). These results are similar to those reported by Coroian et al., 2017, for cow's milk.



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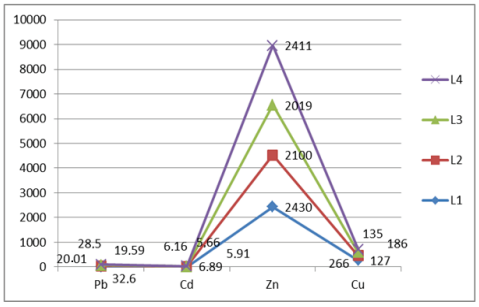

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Figure 3. The content of heavy metals (Pb, Cd, Zn and Cu) from lactation milk in the Sălaj area (a) and Cluj area (b)

Heavy metals studies have also been reported by Malag et al., 2005, Caggiano et al., 2005,

Coni et al., 1996, and observe high amounts depending on the area, feed, and the product analyzed. The elimination of lead in the body can be achieved through urine and faeces, and in very small amounts is in the hair, nails, saliva, human milk, sweat (Dale et al., 1994; Hsu et al., 1981; ATSDR, 2007). Regarding Cu, daily intake of food is up to 3 mg. With, it has an essential role in the body. The sources of contamination are mostly pesticides and copper treatments and copper-made machinery (Suharoschi, 2013). The highest exposures to Cd are from food and tobacco, tobacco leaves accumulate large amounts of Cd. Exposures can also occur through contaminated water consumption. Cd is able to bind to various sulfhydryl groups in proteins, being active in plasma albumin (Voulvoulis et al., 2010). Once food or water contaminated with cadmium is consumed, it is eliminated in the faeces, it is not absorbed in the body in the intestinal tract, but it can be absorbed in the skin, muscles, bones in the kidneys and the liver (Shaikh et al. 1980). Concerning the contamination with Cu, the legislation imposes the following values expressed in mg/kg: 0.5 mg/kg in milk; 1.5 mg/kg in smoked and salted stalks; 2 mg/kg in eggs; 3 mg/kg in cheeses, meat, canned and semiconserve of peas in tomato sauce or vinegar (Suharoschi, 2013). Plant products can accumulate much larger quantities of heavy metals. Plants can accumulate Cd in concentrations ten times higher than soil concentrations. Leaves of plants grown near industrial, polluted areas can contain heavy metals such as Cd that reach values higher than 570 mg/100 g (EFSA, 2009).

Cd poisoning causes anemia, hypoproteinemia, hypoalbuminemia (Jablonska et al., 1971; Solecki et al., 1991). Assessing the level of heavy metals in milk is important because of the risks they can cause to the human body. It is important to establish the level of heavy metals in the donut milk as it is used in the diet of susceptible persons and those suffering from food allergies.

CONCLUSIONS

The average values for heavy metals varied depending on the lactation and the milk sample area. Protein is a very important parameter, especially when talking about milk processing in dairy products. The highest protein values are for samples from lactation 3th and 4th lactation, and the lowest values are for lactation of lactation 1 and 2. The lactation and sample collection area also affects the fat and lactose content of the donkey.

Heavy metals showed the highest average values in donkey milk samples in the Sălaj area and the lowest are the Cluj area. The donkey milk in Sălaj area showed the highest content in Pb, Cu and Zn and the lowest in Cd. High levels of Zn and Cu in the donut milk were highlighted in all analyzed samples.

REFERENCES

Andersen O., Nielsen J.B., Svendsen P., 1988. Oral cadmium chloride intoxication in mice: effects of dose on tissue damage, intestinal absorption and relative organ distribution. Toxicology, 48, 225-236.

Aposhian H.V., Zakharyan R.A., Avram M.D., Sampayo-reyes A., Wollenberg M.L., 2004. A review of the enzymology of arsenic metabolism and a new potential role of hydrogen peroxide in the detoxification of the trivalent arsenic species. Toxicol. Appl. Pharmacol., 198, 327-335.

Caggiano R., Sabia S., D'Emilio M., Macchiato M., Anastasio A., Ragosta M., Paino S., 2005. Metal levels in fodder, milk, dairy products, and tissue sampled in ovine farms of Southern Italy. Enviromental Research, 99, 48-57.

Concha G., Vogler G., Lezcano D., Nermell B., Vahter M., 1998. Exposure to inorganic arsenic metabolites during early human development. Toxicol. Sci., 44, 185-190.

Coni E., Bocca A., Coppolelli P., Caroli S., Cavallucci C., Trabalza Marinucci M., 1996. Minor and trace elemet content in sheep and goat milk and dairy products. Food Chem., 57, 253-260.

Coroian A., 2014. Toxicologia alimentelor. Editura Bioflux.

Coroian A., Mireşan V., Cocan D., Răducu C., Longodor A.L., Pop A., Feher G., Andronie L., Marchiş Z., 2017. Physical-chemical parameters and the level of heavy metals in cow milk in the Baia Mare area. Banat's Journal of Biotechnology, VIII(16), 69.

Coroian A., Mireşan V., Odagiu A., Andronie L., Răducu C., Marchiş Z., Coroian C.O., 2016. Influence of Season on Physico-Chemical Composition of Donkey Milk from Primiparous and Multiparous. ProEnvironment, 9, 400 – 403.

Cotte F., Calabro A., Monsu G., 2003. Il latte di asina: alimento per il futuro. Il Progresso Veterinario, 2, 63-68.

Dale L.S., 1994. Percutaneous absorption of inorganic lead compounds. Sci. Total Environ., 145, 55-70.

Dash H.R., Mangwani N., Chakraborty J., Kumari S., Das S., 2013. Marine bacteria: potential candidates for

enhanced bioremediation. Appl. Microbiol. Biotechnol., 97, 561-571.
EFSA, 2009. Cadmium in food. Scientific opinion of the Panel on Contaminants in the Food Chain.
El-Hatmi H., Jrad Z., Salhi I., Aguibi A., Nadri A., Khorchani T., 2015. Comparison of composition and whey protein fractions of human, camel, donkey, goat and cow milk, Mljekarstvo, Gabes, 159-167.
Gastaldi D., Bertino E., Monti G., Baro C., Fabris C., Lezo A., Medana C., Baiocchi C., Mussap M., Galvano F., Conti A., 2010. Donkey's milk detailed lipid composition, Frontiers in Bioscience, 2, 537-546.
Hsu J.M., 1981. Lead toxicity as related to glutathione metabolism. J. Nutr., 111, 26-33.
Husby S., Olsson C., Ivarsson A., 2014. Celiac Disease and Risk Management of Gluten, Risk Management for Food Allergy, Odense, 129-152.
Malhat F., Hagag M., Saber A., Fayz A.E., 2012. Contamination of cow' s milk by heavy metal in Egypt.
Mukhopadhyay J., Gutzmer, J., Beukes, N.J., 2005. Organotemplate structures in sedimentary manganese carbonates of the Neoproterozoic Penganga Group, Adilabad, India. J. Ear. Syst. Sci., 114, 247-257.
Muzi G., Dell'Omo, M., Madeo, G., 2001. Arsenic poisoning caused by Indian ethnic remedies. J. Pediatr., 139(1), 169.
Perfus-Barbeoch L., Leonhardt N., Vavasseur A., Forestier C., 2002. Heavy metal toxicity: cadmium permeats through calcium channels and disturbs the plant water status. Plant J., 32, 539-548.
Piver J.L., 1989. Medical consequences of stratospheric ozone depletion. Can. Fam. Physician, 35, 2283-2284.
Reimann C., de Caritat, P., 2005. Distinguishing between natural and anthropogenic sources for elements in the enviroment: regional geochemical surveys versus enrichment factors. Sci. Total Environ., 337, 91-107.
Rosenman K.D., Sims A., Luo Z., 2003. Occurrence of lead-related symptoms below the current occupational safety and health act allowable blood lead levels. J. Occup. Environ. Med., 45(5), 546-555.
Shaikh Z.A., Smith J.C., 1980. Metabolism of orally ingested cadmiun in humans. Dev. Toxicol. Environ. Sci., 8, 569-574.
Shibamoto T., Bjeldanes L.F., 2009. Introduction to Food Toxicology.AcademicPress Inc., CA, USA.
Solecki R., Hothorn L., Holzweissig M., 1991. Computerized analysis of pathological findings in long term trials with phenylmercuric acetate in rats. Arch. Toxicol. Suppl., 14, 100-103.
Suharoschi R., 2013. Toxicologia alimentelor, Editura AcademicPres, Cluj-Napoca.
Timreck C., 2012. Modeling the climatic effects of large explosive volcanic eruptions. Wiley Interdiscip. Rev. Clim. Change, 3, 545-564.
Vantroyen B., Heilier J.F., Meulemans A., 2004. Survival after a lethal dose of arsenic trioxide. J. Toxicol. Clin. Toxicol., 42(6), 889-895.
Vantroyen B., Heilier J.F., Meulemans A., 2004. Survival after a lethal dose of arsenic trioxide. J. Toxicol. Clin. Toxicol., 42(6), 889-895.
Vinodhini R., Narayanan M., 2008. Bioaccumulation of heavy metals in organs of fresh water fish *Cyprinus carpio* (Common carp). Int. J. Environ. Sci. Tech., 5, 179-182.
WHO, 1997. Environmental Health Criteria196: Methanol. World Health Organization, Geneva.
ATSDR, 2007. Toxicological Profile for Lead. US Department of Health and Human Services, 1-582, Atlanta, Georgia.

REPRODUCTION, PHYSIOLOGY, ANATOMY

Scientific Papers. Series D. Animal Science. Vol. LXI, Number 1, 2018
ISSN 2285-5750; ISSN CD-ROM 2285-5769; ISSN Online 2393-2260; ISSN-L 2285-5750

DETERMINATION OF HERD MANAGEMENT LEVEL BY SOME REPRODUCTION AND MILK YIELD TRAITS OF SIMMENTAL COWS AT INTENSIVE CONDITIONS IN TURKEY

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Abstract

The aims of this study were to determine some fertility and milk yield traits of Simmental cows under intensive dairy farm conditions in Konya region of Turkey, and to investigate herd management level by these traits. A total of 120 cows constituting the material of the study were grouped by parity (second and third) and calving season (winter, spring, summer and autumn). Two milk yield groups were designed by taking arithmetic average of previous lactation milk yield. The means for number of services per conception (NSC) and service period (SP) were found to be 2.37±0.016 and 92.0±5.32 days, respectively. Also, average daily milk yield (ADMY), and first and second lactation milk yields were determined as 18.7±0.36 kg, 4756±59.41 kg and 5918.7±75.30 kg, respectively. While ADMY values in cows with 3th parity were higher than those with 2nd parity (P<0.01), NSC of cows calved in the spring were found to be lower (2.11±0.15) than those calved in summer (2.90±0.26) (P <0.05). In addition, the SP of cows calved in winter, autumn and spring was found higher than those calved in the summer (P<0.01). The ADMY values cows of calved in the autumn were higher than those calved in the winter and spring (P<0.001) and NSC means were found lower (2.11±0.14) in cows with lower milk yield than those with higher yield (2.79±0.19) in the subsequent insemination period (P<0.01). These results indicate that milk yield was adequate, NSC was high and SP was close to the upper threshold. Finally, it is suggested that herd management indicators should exhaustively be reconsidered by fertility in the investigated dairy farm.

Key words: *Simmental, fertility, management, milk yield.*

INTRODUCTION

In recent years, herd size and intensification process in dairy farms have increased rapidly (Uzmay et al., 2010). Therefore, herd management strategies have importance in terms of continuity of herd and sustainable production in the dairy enterprises produced in intensive conditions. Considering that milk production of the main source of income of these enterprises is also taken into consideration, it is essential to attain ideal milk production from each cattle and calves per year (Uygur, 2004; Yüceer and Özbeyaz, 2010; Erez and Göncü, 2012). Milk and fertility traits having economic value are mostly influenced by herd management (Ozcelik and Arpacık, 2000; Uygur, 2004). Thus, knowing the traits of fertility and milk yield, which are part of herd management, have been regarded as the important indicators for proper herd management (Bolacali and Öztürk, 2017). Also, some researchers informed that controlling the reproductive performance traits of cattle implies more calf and milk production throughout the life (Uygur, 2004; Tekerli and Gündoğan, 2005; Erdem et al., 2007; Bayrıl and Yılmaz, 2010).

The increase in the numerical values of the fertility traits such as service period (SP), number of services per conception (NSC), calving interval (CI), and first calving age (FCA) affect the production costs of enterprises in a negative way. Besides, these traits can also be affected by environmental factors such as parity, calving season and milk yield (Rafique et al., 1999; Ural, 2012). This circumstance demonstrates the importance of determination of fertility and milk yield traits for dairy enterprises. To this aim, Çilek and Tekin (2005) calculated 305-day lactation milk yield, SP and NSC of Simmental cows reared intensive conditions in Turkey as 4700±69.2kg, 93.9±2.03d and 1.76±0.04, respectively. Besides, positive correlations between 305-day milk yield and SP ($r=0.17$; P<0.001) or NSC ($r=0.09$; P<0.05) were estimated. In a similar study that conducted by Bolacali et al. (2017),

average SP and NSC were determined as 116.41±1.43d and 1.75±0.03, respectively. Morever, calving season of cows were found to be effective on SP (P<0.01) and NSC (P<0.05), while parity was only significant on NSC (P<0.001).

Until now, many studies have aimed to determine fertility and milk yield traits. Since these parameters are considered to be a consequence of herd management, determination of these elements in large herds is important for solving the problems in the current husbandry practices. Also, it is believed that herd management elements will be beneficial to maximize the genetic capacities of cows and to constitute profitable production infrastructures. The objectives of this study were to determine some reproductive and milk yield traits of Simmental cows reared under private enterprise conditions and to examine the herd management situation of the enterprise in terms of these traits.

MATERIALS AND METHODS

In this study, a total of 120 Simmental cows reared under intensive dairy farm conditions in Konya region of Turkey was investigated. While SP and NSC values of cows belonging to 2014-2017 years were chosen as the milk yield traits, average daily milk yield (ADMY) and lactation milk yield (LMY) of cows in this period were used as the milk parameters. Besides, the calving season and parity of cows in the dairy enterprise were also recorded. Relevant milk and fertility records were recorded with computer-aided herd management system in the enterprise. The cows chosen as the experimental material were grouped by parity (2nd and 3rd lactation) and calving season (autumn, winter, spring and summer). Also, a single value was obtained by taking the arithmetic mean of previous lactation milk yields (5918.7±75.30 kg) of the cows. Those with low values from the mean were classified as the first group (Group-1: 4502.9±42.65 kg) and the high values were taken to the second group (Group-2: 6666.0±104.48 kg). Milk yield, parity, and calving season were grouped as above and the effects of these factors were investigated by using variance analysis technique.

The following mathematical models were applied:

For SP and NSC: $Yij=\mu+ai+eij$

where, Yij: μ: population average, ai: i. effect of the milk yield groups, i= low yield (Group-1), high yield (Group-2), and eij: random residual term.

For SP, NSC and ADMY: $Yij=\mu+ai+bj+eijk$

where, Yijk: μ: population average, ai: i. effect of calving season (i= autumn, winter, spring and summer), bj: j. effect of parity (j=2, 3), and eijk: random residual term.

Effect of milk yield on NSC and SP, and effect of lactation number on SP, NSC, and ADMY were revealed by independent simple *t-test*. In addition, the effects of the calving season on SP, NSC and ADMY were determined by one-way ANOVA. In the all statistical analyses, SPSS 20.0 program was used.

RESULTS AND DISCUSSION

In this study, mean lactation milk yields of Simmental cows reared in intensive conditions were found as 5918.7±75.30 kg (Table 1). These results were found lower than the findings of Koc (2011); Erdem et al. (2015); Kucuk-Baykan and Ozcan (2017), and higher than the findings of Cilek and Tekin (2005); Ozkan and Gunes (2007) and Kocak et al. (2008). Differences in the obtained findings might be caused by the variation in the environmental factors in the rearing conditions, and various genetic potential levels of the animals.

Table 1. Lactation milk yield by groups

	n	Lactation Milk Yield (kg)
Group 1	71	4502.9±42.65
Group 2	49	6666.0±104.48
Mean	120	5918.7±75.30

The means (±SE) of NSC and SP values from the examined reproductive traits were found as 2.37±0.12 and 92.0±5.32d, respectively (Table 2). In the similar studies those conducted in Turkey conditions, Cilek and Tekin (2005), Erdem et al. (2015) and Bolacali et al., (2017) calculated the means of these paremeters to be 1.76±0.04/93.9±2.03d; 1.96±0.05/92.8±1.46d and 1.75±0.03/116.41±1.43d, respectively. As seen, the findings obtained here for NSC were found to be higher than the values reported by Cilek and Tekin (2005), Erdem et al. (2015)

and Bolacali et al. (2017). However, the values determined for the other reproductive performance SP were found lower than the findings of Bolacali et al. (2017), while agreement with the results of the studies conducted by Cilek and Tekin (2005) and Erdem et al. (2015). According to these results, it can be mentioned that SP values were close to the upper limit of the acceptable levels for the enterprise in which the study were conducted. However, the results obtained for NSC were found as higher than the normal values. In this sense, rechecking insemination and detection of estrus applications in the herd might especially be advised. In this study, LMY affected NSC (P<0.01) but not affected SP (Table 2). The average NSC of cows with high LMY (2.79±0.19) was found to be higher than those with low LMY (2.11±0.14). Cilek and Tekin (2005) reported the positive correlations between milk yield and NSC or SP values. Based on these findings, it can be said that high milk yield had a negative effect on some reproductive traits. This case might be attributed to the changes in the endocrine system of cows and reduction in the immunity due to physiological stress (Nebel, and McGilliard, 1993; Cilek and Tekin, 2005; Walsh et al., 2011).

Table 2. Means (±SE) of NSC and SP by LMY groups

	n	NSC**	n	SP
Group 1	65	2.11±0.14	47	87.9±6.57
Group 2	42	2.79±0.19	27	99.1±9.05
Mean	107	2.37±0.12	74	92.0±5.32

** P<0.01; NSC: number of services per conception; SP: service period; LMY: lactation milk yield

In this research, no significant effect of parity on NSC and SP was obtained (Table 3). These findings were in agreement with the results of Cilek and Tekin (2005) and Ural (2012). However, Bolacali et al. (2017) reported that effect of parity on SP was not statistically significant, but effective (P <0.001) on NFC.

As seen in Table 3, ADMY of cows in the second lactation (15.5±1.15 kg) were lower than those in the third lactation (19.1±0.35 kg). Based on these results, it is possible to point out that ADMY parallelly increases with advancing parity. As well known, the increase of LMY and ADMY of cows continues until reaching mature age (5-6 years). Really, the increase in the ADMY might be assumed as an expected result when parities of the cows in this study were regarded.

The reports of Ozcelik and Arpacik (2000), Akman et al. (2001) and Ozkan and Gunes (2007) also support this study.

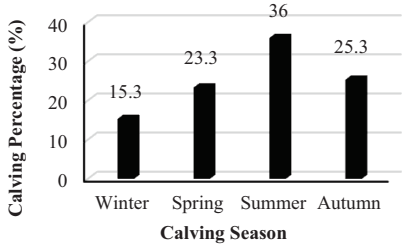

Figure 1. Change of calving percentage by seasons

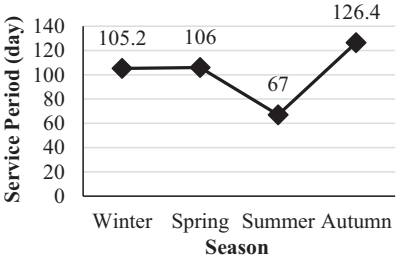

Figure 2. Changes of service period by seasons

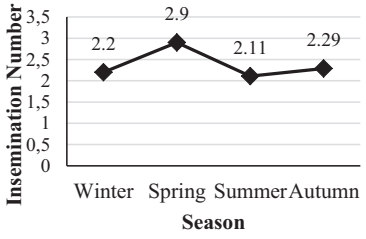

Figure 3. Changes of number of services per conception by seasons

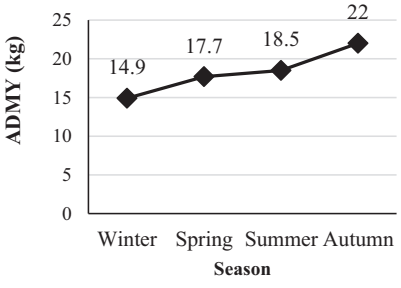

Figure 4. Changes of average ADMY (daily milk yield) by seasons

As seen in Figure 1, it was observed that the highest calving rate occurred in summer (36%) while the lowest percentage was obtained in winter (15.3%). In contrast, SP values of cows calved in summer were lower than those calved in the other seasons (P<0.01) (Figure 2). Findings obtained in this study are similar to results acquired by Bolacali et al. (2017). Contrary to these results, Cilek and Tekin (2005) found that calving season was only effective on NSC (P <0.05). However, while the lowest NSC (2.11±0.15) was found in cows calved in summer, the highest value (2.90±0.25 was obtained from cows calved in spring (Figure 3). Besides, it was determined that cows calving in the autumn months had higher (P<0.001). ADMY values than those of calving in the winter and spring (Table 3, Figure 4). Finally, it is possible to say that milk and fertility traits are markedly affected from calving season.

CONCLUSIONS

As a result, cows with higher lactation milk yields had higher NSC values than those with lower yields.

While parity was important for ADMY, calving season had a significant effect on both reproductive and milk yield traits.

The percentage of calving in the present enterprise was higher in summer than that determined in the other seasons.

When all results here are evaluated as a whole, it is considered that the milk yield level of Simmental cows reared in intensive conditions was adequate.

High NSC and close to the upper threshold of SP indicated that herd management strategy by reproductive traits in the investigated dairy enterprise should be rechecked.

Table 3. Means of NSC, SP and ADMY values by parity and calving season

Parity	n	NSC	n	SP	n	ADMY**
2	36	2.62±0.23	24	100.4±8.38	12	15.5±1.15
3	81	2.30±0.13	50	88.0±6.75	91	19.1±0.35
Mean	107	2.37±0.12	74	92.0±5.32	103	18.7±0.36
Calving Season		*		**		***
Winter	15	2.20±0.26 ^{ab}	15	105.2±15.75^{b}	8	14.9±1.18 ^{a}
Spring	31	2.90±0.25 ^{b}	22	106.0±9.19^{b}	29	17.7±0.57 ^{a}
Summer	47	2.11±0.15 ^{a}	30	67.0±4.78^{a}	46	18.5±0.44 ^{ab}
Autumn	14	2.29±0.34 ^{ab}	7	126.4±13.78^{b}	20	22.0±0.77 ^{b}

*P<0.05; ** P<0.01; *** P<0.001

NSC: number of services per conception; SP: service period; ADMY: average daily milk yield

REFERENCES

Akman N., Ulutaş Z., Efil H., Biçer S., 2001. Milk yield and reproductive traits of Holsteins raised at Gelemen state farm. J. Fac. Agric., 32(2), 173-179.

Bayril T., Yilmaz O., 2010. Reproductive Traits of Holstein Cows Raised in Kazova Vasfi Diren Agriculture Farm. Vet. J. YYU., 21(3), 163-167.

Bolacali M., Öztürk Y., 2017. Effect of non-genetıc factors on reproductıve traıts in Sımmental cows reared in subtropical climate condition. J. Anim.Plant Sci., 27(5), 1420-1425.

Cilek S., Tekin M.E., 2005. Environmental factors affecting milk yield and fertility traits of Simmental cows raised at the Kazova State Farm and phenotypic correlations between these traits. Turk. J. Vet. Anim. Sci., 29(4), 987-993.

Erdem H., Atasever S., Kul E., 2007. Milk yield and fertility traits of Holstein cows raised at Gokhoyuk state farm II. fertility traits. J. Fac. Agric., 22, 47-54.

Erdem H., Atasever S., Kul E., 2015. Relation of body condition score with milk yield and reproduction traits in Simmental cows. Large Anim. Rev., 21, 231-234.

Erez İ., Göncü S., 2012. The effects of early weaning practice at performances of Black And White calves. Çukurova Univ. J. Sci. Eng., 28-3.

Koc A., 2011. A study of the reproductive performance, milk yield, milk constituents, and somatic cell count of Holstein-Friesian and Montbeliarde cows. Turk. J. Vet. Anim. Sci., 35(5), 295-302.

Kocak S., Tekerli M., Ozbeyaz C., Demirhan I., 2008. Some Production Traits of Holstein, Brown-Swiss and Simmental Cattle Reared in Lalahan Livestock Research Institute. J. Lalahan Liv. Res. Ins., 48, 51-57.

Kucuk-Baykan Z., Ozcan M., 2017. Determination of Reproduction and Lactation Parameters in the First Production Year of Brown Swiss and Simmental Cows Imported From Austria. J. Fac. Vet. Med. Istanbul Univ., 43(2), 132-139.

Nebel RL., McGilliard ML., 1993. Interactions of high milk yield and reproductive performance in dairy cows. J. Dairy Sci., 76(10), 3257-3268.

Ozcelik M., Arpacik R., 2000. The effect of lactation number on milk production and reproduction in Holstein cows. Turk J Vet Anim Sci., 24, 39-44.

Özkan M., Güneş H., 2007. Researches on the milk production characteristics of Simmental Cattle in commercial farms in Kayseri. J. Fac. Vet. Med. Istanbul Univ., 33 (3), 17-30.

Rafique M., Chohan K.R, Amer M.A., 1999. Factors affecting calving interval and service period in Holstein-Friesian X Sahiwal crossbred cows. Pakistan Vet. J., 19(4), 173-175.

Tekerli M., Gündoğan M., 2005. Effect of certain factors on productive and reproductive efficiency traits and phenotypic relationships among these traits and repeatabilities in West Anatolian Holsteins. Turkish J. Vet. Anim. Sci., 29, 17-22.

Ural D.A., 2012. The analysis of milk yield and reproductive traits of Holstein cows raised at a private dairy farm in Bozdoğan county of Aydın, Turkey. Anim. Health Prod. Hyg., 1(2), 116-122.

Uygur A.M., 2004. Reproduction in herd management of dairy cattle. J. Anim. Prod., 45(2), 23-27.

Uzmay C., Kaya İ., Tömek B., 2010. Precision Dairy Herd Management Applications. J. Anim. Prod., 51(2), 50-58.

Walsh S.W., Williams E.J., Evans A.C.O., 2011. A review of the causes of poor fertility in high milk roducing dairy cows. Anim. Reprod. Sci., 123(3), 127-138.

Yüceer B., Özbeyaz C., 2010. Effect of immunity on growth, disease incidence and livability in calves after colostrum consuming. Vet. J. Ankara Univ., 57, 185-190.

Scientific Papers. Series D. Animal Science. Vol. LXI, Number 1, 2018
ISSN 2285-5750; ISSN CD-ROM 2285-5769; ISSN Online 2393-2260; ISSN-L 2285-5750

VARIATION IN MILK PRODUCTION OF BROWN SWISS COWS BY CALVING SEASON, STAGE OF LACTATION AND YEAR

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Abstract

The objective of the present investigation was to determine the change of milk production by calving season, stage of lactation and year in Brown Swiss cows. The research was carried out on Brown Swiss cows (n=30) reared at a private farm in Konya region of Turkey. Daily milk yield (DMY) values of the current lactation and lactation milk yield (LMY) values of initial two years (2015 and 2016) were assessed to be milk production parameters. To evaluate DMY, two stage of lactation (SL; 1=<157d and 2=≥157d) and calving season (CS; 1=from March to August and 2=from September to February) groups were designed. Group differences were tested by independent-samples t-test in SPSS. DMY means for SL1, SL2, CS1 and CS2 were calculated to be 20.25 kg, 17.79 kg, 17.85 kg and 19.69 kg, respectively. LMY means of two years were estimated to be 4975.53 kg and 5961.80 kg, respectively (P<0.001). It was concluded that milk yield level was positively affected by years in the evaluated dairy farm.

Key words*: Brown Swiss, environmental factor, dairy farm, milk yield.*

INTRODUCTION

As well known, animal production not only ensures essential foods, but also provides a great large scale occupation for human. At this point, high-grade livestock products such as milk, beef, egg or honey are highly demanded. Today, obtaining quality and quantity products carries equal importance by conscious suppliers.

Many cattle breeds have been raised throughout the world because of their different yields. In spite of Holstein is the most popular breed among the cattle breeds, Simmental, Jersey, Angus, Hereford or Brown Swiss may be classified as the other common ones. For example, Simmental and Brown Swiss are considered to be dual purposes (beef and milk). However, multi non-genetic factors, such as season, parity, stage of lactation or location may be effective on the productivity of cows. While Baul et al. (2010) calculated relatively lower milk yield in the first parity cows, Vijayakumar et al (2017) estimated the highest milk production from Korean Holstein cows in the early lactation period (55 to 90 d). Aksu and Atasever (2017) pointed out to seasonal changes of milk production of Holsteins in Turkey conditions.

Thusly, removing adverse factors influencing the production level of animals can be seen a great step to reach real genetic potentials of the cows.

The objective of this research was to determine the variation of milk production by calving season, stage of lactation and year in Brown Swiss cows in Turkey conditions.

MATERIALS AND METHODS

This study was conducted on Brown Swiss cows (n=30) reared at a private farm in Konya region of Turkey. All cows were clinically healthy and in the second or third parities. In the farm, the cows were milked two times in a day using automatic milking and kept the similar conditions by feeding and other practices during the research period. Daily milk yield (DMY) values of the recent lactation and lactation milk yield (LMY) values of initial two years (2015 and 2016) were evaluated as milk yield parameters. To examine DMY, two stage of lactation (SL; 1=<157d and 2=≥157d) and calving season (CS; 1=from March to August and 2=from September to February) groups were constructed. Differences between the groups were tested by independent-samples *t-test* in SPSS for windows packet program.

RESULTS AND DISCUSSIONS

In this study, means of DMY by CS and SL are given in Table 1. As seen, cows calved in the second period had about 1.84 kg more milk per cow when compared to those calved in the first period. This case indicates the positive effect of the cooler seasons on milk production of cows in the post-calving period. However, this difference was not found to be statistically significant. The findings here were not found to be parallel with the results of Rios-Utrera et al. (2013), who determined the DMY as significantly greater in cows that calved during the cold season than in those calved in the dry and rainy seasons. Similarly, Elahi Torshizi (2016) emphasized the higher level of milk production in cows which calved in autumn and winter.

Table 1. Means of DMY by two environmental factors

Factor	n	Mean (±*SE*)
CS		
1	11	17.85±0.77
2	19	19.69±0.97
SL		
1	15	20.25±1.05
2	15	17.79±0.79
General	30	19.02±0.69

CS: calving season (1= between March and August, 2= between September and February)
SL: stage of lactation (1=<157d, 2=≥157d)

It was also determined that cows in the first SL group had about 2.46 kg more milk production per cows with reference to the those of the second group (Table 1). As mentioned earlier, cows are exposed to peak milk production during the post-partum period. However, lactation persistency tended to gradually dropping after 60th day. In spite of the obtained variation between two DMY means by SL might be caused by this probability, this difference was not found to be significant, statistically. However, it was attractive in our study that the overall mean of DMY was calculated to be relatively low for Brown Swiss cows. Bergamaschi et al. (2015) emphasized in their study that Brown Swiss cows had more production capacity, according to applying management especially for the feeding program. At this point, redesigning the possible non-genetic factors related to feeding, husbandrial practices or barn conditions may be suggested for the responsible of the investigated farm in this research.

Effect of year on milk production was determined to be statistically significant (P<0.001). Change of LMY according to years is presented in Figure 1. Really, LMY means of chosen cows in 2016 (5961±151.04 kg) were 16.5% higher than those produced in 2015 (4975±122.43 kg).

This finding clearly revealed that husbandry conditions and herd management have tended to improve year by year.

This finding was found as harmonic with our opinions in the earlier paragraph in which the relationship of milk yield with herd management was pointed out.

Besides, calculated average of two years (5468.67±115.81 kg) was found to be higher than the results of some researchers (Dogan and Kaygisiz, 1999; Cak and Yilmaz, 2015), who carried out the studies on the same breed in Turkey conditions.

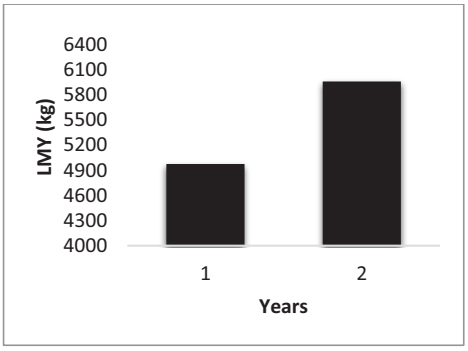

Figure 1. Change of LMY by years (LMY: lactation milk yield; Years: 1=2015 and 2= 2016)

CONCLUSIONS

In this study, Brown Swiss herd reared in Konya province of Turkey was investigated by three non-genetic factors affecting milk production.

It was concluded that milk production was positively influenced by year in the studied Brown Swiss herd.

To ensure more productive cows in the later times, exactly tracking the herd according to entire environmental factors may be advised to the herd owners.

REFERENCES

Aksu C., Atasever S., 2017. Changes of comfort parameters and test day milk yield in Holstein cows. Scientific Papers. Series D. Animal Science, Vol. LX, 145-147.

Baul S., Cziszter L.T., Acatincai S., Cismas T., Gavojdian D., Tripon I., Erina S., Buzamat G., 2013. Effect of Parity on Dynamics of Milk Yield and Composition during Normal Lactation in Dairy Cows. Sci. Papers: Anim. Sci. Biotechnol., 46(2), 285.

Bergamaschi M., Aprea E.,Betta E., Biasioli F., Cipolat-Gotet C., Cecchinato A., Bittante G., 2015. Effects of dairy system, herd within dairy system, and individual cow characteristics on the volatile organic compound profile of ripened model cheeses. J. Dairy Sci., 98, 2183-2196.

Cak B., Yilmaz O., 2015. Milk yield performances of Brown Swiss cows raised at Mus Alpaslan State Farm in Turkey. Bulg. J. Agric. Sci., 21 (2), 436–439.

Dogan M., Kaygisiz A., 1999. Relationships between milk yield traits and milk protein polymorphism in Brown Swiss Cattle in Turkey. Turk. J. Vet. Anim.Sci., 23 (Suppl. 1), 47-49.

Elahi Torshizi M., 2016. Effects of season and age at first calving on genetic and phenotypic characteristics of lactation curve parameters in Holstein cows. J. Anim. Sci. Technol., 58, 8.

Ríos-Utrera A., Calderón-Robles R.C., Galavíz-Rodríguez J.Y., Vega-Murillo V.E., Lagunes-Lagunes J., 2013. Effects of Breed, Calving Season and Parity on Milk Yield, Body Weight and Efficiency of Dairy Cows under Subtropical Conditions. Int. J. Anim. Vet. Adv., 5(6), 226-232.

Vijayakumar M., Park J.H., Ki S.K., Lim D.H., Kim S.B., Park S.M., Jeong H.Y., Park B.Y., Kim T., 2017.The effect of lactation number, stage, length, and milking frequency on milk yield in Korean Holstein dairy cows using automatic milking system Asian-Australas. J. Anim. Sci., 30(8), 1093-1098.

Scientific Papers. Series D. Animal Science. Vol. LXI, Number 1, 2018
ISSN 2285-5750; ISSN CD-ROM 2285-5769; ISSN Online 2393-2260; ISSN-L 2285-5750

THE INFLUENCE OF LIPID FACTOR ON THE MANIFESTATION OF ADAPTATION-COMPENSATORY REACTIONS OF GAMETE PLASMA MEMBRANES OF FARM ANIMALS

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Abstract

By the methods of spectrophotometric, chromatographic and morphological studies established that as a result of influence of the temperature factor takes place adaptation-compensatory reactions in the gamete plasma membranes of farm animals. As a result of the adaptive-compensatory reactions in the plasma membranes of bull and boar gametes to the influence of temperature factor occur a shift of the phase transition of lipids to a lower temperature. In plasma membranes the amount of phospholipids predominates over cholesterol. During the stages of cryopreservation of cock semen there is a decrease in the amount of cerebrosides of 1, 5, 6, and 8 fraction, which compensates by increasing of quantity of cerebrosides third faction. In a similar direction changes the phospholipid-cholesterol ratio, which tends to unity, that is, in the direction of eliminating the phase transitions of lipids. The observed processes may be a consequence of a change in the activity of the phospholipase system, which is a gamete protective reaction in response to the action of extreme cryopreservation factors. The obtained results of the studies of fatty acid composition of plasma membranes testify to its change under the action of cryogenic factors. The accumulated material allows us to note the manifestation of specific reactions characteristic of individual fatty acids during freezing and thawing of biological objects. The change in the content of phospholipids and the saturation of fatty acids of plasma membranes testifies a complex adaptive-compensatory reactions aimed at preserving the viability of gametes in the new conditions of their stay.

Key words*: gametes, temperature factors, adaptation reactions.*

INTRODUCTION

Anthropogenic impact on the environment has become so significant that it has become impossible to cease it, it remains to mitigate this action or adapt to new conditions. In the formation of regulation of compensation mechanisms, in response to the action of extreme factors of cryopreservation on spermatozoa, lipids are assigned one of the leading roles. In this regard, the great interests are adaptive-compensatory reactions of plasma membranes under the action of temperature factor on them. Due to the strictly coordinated work of membrane mechanisms, cellular homeostasis is maintained, fine regulation of functional activity is performed in response to the influence of environmental factors and changes within the cell (Vie et al., 2000).

All cell membranes, including plasma membranes, are mobile, fluid formations that have common structural features: they are ensembles of lipid and protein molecules held together by weak non-covalent intermolecular interactions. Hydrophobic interactions of long nonpolar chains of fatty acids with the remains of hydrophobic amino acids lying on a surface of these proteins play a key role in stabilization of the immersed position of membrane proteins (Белоус et al.,1982). When changing individual parameters of the medium, and, above all, temperature, there may be a transition of system in a solid crystalline state or in a liquid state, devoid of a certain structure. This much-needed state of the lipid phase membranes for the cell due to a strictly defined chemical composition of lipids and can easily be disturbed at changing the environment conditions. Such disturbance causes a number of adaptive-compensatory changes in the composition of biological membranes. These changes are very important for preserving their structural and functional activity of gametes.

In this regard, the purpose of the research was to study the contribution of lipid component membranes in the implementation of adaptive-compensatory reactions.

MATERIALS AND METHODS

The experimental studies were carried out using the semen material of Black-Motley bulls, Large White boars and Rhode Island breeds cocks, which were kept in conditions meeting modern veterinary requirements. Determination of the content of phospholipids, cholesterol and cerebrosides was carried out by the described methods (Кейтс, 1975). The isolation of plasma membranes was carried out according to the method that was improved by us (Иванов et al., 1983). Statistical processing of digital material was carried out using the Student's t-test.

RESULTS AND DISCUSSIONS

Fundamental researches in the field of cryobiology have quite definitely proved that lipids play an important and sometimes decisive role in a number of processes taking place in the cell in normal and in pathology conditions contributing to the stabilization of its functional homeostasis. In this regard were investigated cryogenic changes of phosphorlipids and cholesterol of the bull and boar gametes (Table 1).

Table 1. Phospholipids, cholesterol and their ratio in the process of cryopreservation of bull and boar semen

Investigated indicators in gametes			
Bull		Boar	
After dilution	After thawing	After dilution	After thawing
M±m	M±m	M±m	M±m
Phospholipids (Mole %)			
3.8 ± 0.06	2.3 ± 0.10*	3.6 ± 0.09	2.8 ± 0.04*
Cholesterol (Mole %)			
1.1 ± 0.04	0.9 ± 0.04*	1.3 ± 0.05	1.1 ± 0.03*
Molar ratio of phospholipids: cholesterol			
3.54	2.62	2.86	2.56

*Cryogenic changes are statistically authentic

From the table it follows that the amount of studied lipids in the diluted material is practically at the same level. The process of cryopreservation leads to a decrease in this indicator by a statistically significant amount, which may be due to: 1) the involvement of lipids in energy metabolism; 2) increased activity of phospholipases; 3) the enhancement of the free radical process of lipid peroxidation.

The decrease in the amount of cholesterol in gametes of bull and boar after their cryopreservation can apparently occur as a result of its "decompaction" in lipid bilayer of membranes or due to destruction of lipid micelles in the membrane structure (Белоус et al., 1982).

The phospholipide-cholesterol ratio undergoes similar changes. The obtained results convincingly show that the molar ratio of phospholipids: cholesterol in gametes changes in the direction of one after cooling and freezing of the semen, that is, in the direction of the ratio eliminating the phase transitions of lipids or, at least, moving it to the zone of lower temperatures. It should be noted that the rate of approach to one is the highest in the case of experimentation with the bull semen. This is further evidence of the prevalence of his cryoresistance compared to boar seed.

Considering the fact that the maximum activity of phospholipases occurs in the temperature range of the lipid phase transition (Katkov, 2002), as well as the fact that it is the initiating link in the basic biochemical rearrangements, it can be assumed that a decrease in the ratio of phospholipids: cholesterol is one of the mechanisms in the system of adaptation-compensatory reactions of gametes to the action of low temperatures. At the same time, it is necessary to assumed that along with the value of the ratio of phospholipids: cholesterol or the content of these components, their dynamics in the process of cryopreservation of the seed, as an important mechanism for adapting gametes to low temperatures, also becomes important. Nevertheless, the change in this ratio cannot be a positive phenomenon if it is associated only with the loss of phospholipids - the most important functional and structural components of biological membranes, since the loss of phospholipids leads to a significant deterioration in the physiological, morphological and biochemical state of gametes (Наук et al., 1991). Consequently, the positive effect of this mechanism can be manifested only in the presence of exogenous lipids, for example,

seminal plasma lipids, egg yolk, etc., and can also be caused by the processes of synthesis or resynthesis of endogenous substrates. Thus, the inclusion of a system of phospholipases, aimed at changing the ratio of phospholipids: cholesterol can be considered as an intrinsic protective function of the cell.

Phospholipids and cholesterol are able to realize lipid-lipid interactions.

Many researchers have attempted to explain the formation of the cholesterol-phospholipid complex by hydrogen bonds between the hydroxyl group of the sterol and the oxygen atoms of the phospholipid phosphate. However, this type of bond is unlikely, because, firstly, the ester groups of phospholipids should be hydrated; secondly, the inclusion of cholesterol in various phosphatidylcholine liposomes did not change the NMR spectrum (Богачет al., 1979).

The cyclopentaneperhydrophenanthrene structure of the sterol molecule is unique, since its cyclic part and side chain have great opportunities for the manifestation of intermolecular interactions, various reactions and transformations, which include the ability of hydrogen atoms to be replaced by different radicals, the presence of double bonds, several hydroxyl groups, carbonyl carboxyl groups in various combinations, spatial configurations (authors). Therefore, it is possible to form a very large number of individual compounds with different specific properties, including intermolecular interactions.

Due to the fact that the bulk of lipids of biomembranes are phospholipids, which can be fractionated, in the next series of experiments have been studied the cryogenic changes of individual phospholipids in plasma membranes of the cock gametes (Table 2).

As a result of the studies, the following phospholipid fractions were extracted from the rooster's gamete membranes: sphingomyelin, phosphatidylcholine, phosphatidylserine, phosphatidylethanolamine, cardiolipin and phosphatidic acid.

As a result of the studies, the following phospholipid fractions were extracted from the rooster's gamete membranes: sphingomyelin, phosphatidylcholine, phosphatidylserine, phosphatidylethanolamine, cardiolipin and phosphatidic acid.

Table 2. Cryogenic changes of phospholipids of plasma membranes of cock gametes

Phospholipids	Content of phospholipids (%) in	
	native membranes	thawed membranes
	M±m	M±m
Sphingomyelin	6.7 ± 0.15	7.3 ± 0.18
Phosphatidylcholine	13.4 ± 0.31	19.4 ± 0.05*
Phosphatidylserine	9.6 ± 1.30	7.7 ± 0.20
Phosphatidylethanolamine	10.2 ± 0.18	20.6 ± 0.15*
Cardiolipin	12.1 ± 0.32	0
Phosphatidic acid	48.0 ± 0.35	46.0 ± 0.29*

*Cryogenic changes are statistically authentic

In the largest amount are presented phosphatidic acid and phosphatidylcholine. These data indicate a massive saturation of the investigated lipids and a high biosynthetic potential in the process of spermatogenesis. The minor component of the membranes of gametes of the cock is sphingomyelin, which is based on glinozemnylinosport sphingosine (Крепс, 1981).

Cooling, freezing and thawing of semen significantly reduced the ratio of individual phospholipid fractions. Significant specific weight in lipid losses with decreasing temperature is occupied by phosphatidylserine, cardiolipin and phosphatidic acid. The analysis of the content of phosphatidylcholine and phosphatidylethanolamine indicates an increase in the percentage of these fractions, which is inconsistent with the literature data (Наук et al., 1991) and the results of the studies presented in Table 1.We believe that in this case we are not talking about increasing the number of these phospholipids, but a their less pronounced decrease in comparison with other lipids, about the changes in the chromatographic mobility of phospholipids and the complex structural and biochemical rearrangements in the process of cryopreservation of the cock semen.

The observed changes in the content of phospholipids of plasma membranes of rooster gametes during the cryopreservation testify to the development of complex adaptive-compensatory reactions, which are aimed at preserving the viability of gametes in the new storage conditions.

The adaptive role of lipids concerns not only those that make up the matrix of biological membranes, but also many lipids that are not included in membrane structures.

The gametes would not have coped with the problem of compensating the membrane processes necessary to maintain functional activity, if there was no universal biochemical mechanism based on the adaptive ability of lipids.

At organisms that are acclimatized to low temperatures notes a high ability to rebuild their lipid metabolism in such a way that the content of polyunsaturated fatty acids sharply increases in their cell membranes, which reduce the temperature of the phase-structural transitions of lipids (Огурцов,2012), and this creates favorable conditions for maintaining their liquid crystalline state, which is of important functional importance, since it ensures the functioning of lipid-dependent membrane-bound enzymes. The deepening of research in this direction necessitates the study of cryogenic changes and peculiarities of lipid components, such as fatty acids at preservation of the semen of various species of farm animals. In this aspect, studies have been conducted to study the fatty acid composition of plasma membranes of bull and boar gametes (Table 3).

Table 3. Fatty acid composition of plasma membranes in the process of cryopreservation of bull and boar gametes

Percentage of fatty acids in gamete membranes of:			
Bull		Boar	
After dilution	After thawing	After dilution	After thawing
M±m	M±m	M±m	M±m
Saturated fatty acids			
69.9 ± 1.98	67.5 ± 2.87	64.1 ± 0.93	65.1 ± 2.77
Unsaturated fatty acids			
A. Mono-unsaturated			
16.1 ± 0.91	23.0 ± 2.23*	23.3 ± 0.82**	21.8 ± 1.31
B. Di-unsaturated			
14.0 ± 1.34	9.5 ± 1.02	12.6 ± 0.45	12.5 ± 1.07
Total unsaturated fatty acids			
30.1 ± 1.61	32.5 ± 2.45	35.9 ± 0.93**	34.3 ± 1.93
Ratio of saturated: unsaturated fatty acids			
2.32	2.08	1.78	1.61

*Cryogenic changes are statistically authentic
** Breeds particularity are statistically authentic

The analysis of the data presented in the table shows the species specificity of the fatty acid composition of plasma membranes of freshly diluted gametes of the studied animal species. Thus, in the membranes of boar gametes, the total amount of unsaturated fatty acids is higher than that of the bull ($P<0.05$).The analysis of the data presented in the table shows the species specificity of the fatty acid composition of plasma membranes of freshly diluted gametes of the studied animal species. Thus, in the membranes of boar gametes, the total amount of unsaturated fatty acids is higher than that of the bull ($P<0.05$). Their increased content is due to a high number of mono-unsaturated ($P<0.01$) in particular oleic acid ($P<0.001$).This indicates a lower ordered laying of hydrocarbon chains, an increase of the liquid crystal phase in the membranes of boar gametes, which in turn should positively affect the physicochemical properties of lipids at low temperatures.

The smaller amount of unsaturated fatty acids in the plasma membranes of the bull gametes, with a greater cryoresistance compared to those in the boar, can be explained by the peculiarity of the phospholipid spectrum of the plasmatic membrane of gametes of the animal species under study. There are phospholipids in which the same molecule has in its structure not only

fatty acids capable of oxidation, but also reaction groups that can serve as traps for radicals and breaking off the chain of free radical lipid oxidation (Наук et al.,1991).

In the plasma membranes of the bull gametes, a tendency is observed for high content of saturated fatty acids with an even number of hydrocarbon atoms and a longer chain, which indicates their higher melting temperature of lipids than in the boar gametes.

The ratio of saturated: unsaturated fatty acids of plasma membrane is slightly higher in the gametes of the bull in comparison with those in gametes of the boar, which is not observed in a similar analysis of the results of experiments with gametes of freshwater and marine fish, with different possessing of cryostability (Drokin et al., 1996). The results of the conducted researches of fatty acid composition of plasma membranes testify to its change under the influence of cryogenic factors.

Similar data were obtained by Bulgarian researchers (Иванов et al., 1983) in the study of fatty acid composition of plasma membranes of ram gametes. The accumulated material allows us to note the manifestation of specific reactions characteristic of individual fatty acids during freezing and thawing of biological objects.

Changes in the composition of biological membranes appear to represent their adaptive response to changes in the environment. According to our data, the formation of such a response at the membrane level in bull gametes is mainly due to changes in the content of mono-unsaturated fatty acids. On adaptation, as a way to maintain the necessary degree of liquid and permeability of membranes with a decrease in ambient temperature, we meet in early reports (Selivonchick et al., 1977). Similar changes in the cryopreservation of semen of boar semen are not observed. Apparently this can be explained by its lower cryoresistance.

Thus, cold adaptation is accompanied by a greater unsaturation of fatty acids. This pattern is clearly manifested, despite the fact that the lipid composition of membranes is more specific, characteristic for gametes of various animal species. But always, depending on environmental conditions, saturated acids can be replaced by unsaturated (or vice versa), often resulting to an extension of the chain of fatty acids along with their desaturation. This leads to an increase in the concentration of long chain unsaturated acids in the case of adaptation to environmental conditions. Under other conditions, these changes may develop in the opposite direction.

CONCLUSIONS

The researches allow making the following conclusions:

1. As a result of the action of the temperature factor, take place adaptive-compensatory reactions in the plasma membranes of farm animals gametes.
2. At adaptation-compensatory reactions in the plasma membranes of the bull and boar gametes, in response to the action of the temperature factor, takes place the displacement of the phase transition of lipids to a lower temperature zone.
3. In plasma membranes, the amount of phospholipids predominates over the content of cholesterol.
4. At the stages of cryopreservation of the rooster semen, there is a decrease in the content of cerebrosides 1, 5, 6 and 8^{th} fraction, which is compensated by an increase in the number of cerebrosides of the third fraction.
5. Change of activity of phospholipase system is a protective reaction of gametes in response to the action of extreme factors of cryopreservation.
6. The change in the content of phospholipids and saturation of fatty acids of plasma membranes testifies to complex adaptive compensatory reactions aimed at maintaining the viability of gametes in the new conditions of their stay.

ACKNOWLEDGEMENTS

This research work was carried out with the support of Institute of Physiology and Sanocreatology and was financed from the Project 15.817.04.01A “Nutrition in accordance with constitution types. The impact of nutrition on the sanogenity of male gametes”.

REFERENCES

Drokin S.I., Dzyuba B.B., Kopeika E.F. et al., 1996.Comparative aspects of spermatozoa resistance of saltwater and freshwater fish species. Proceed. XIV work "Geneties resourse conservation", Pushchino, 94-95.

Katkov I.I., 2002. Electroporation of cells in applications to cryobiology. Problems of Cryobiology "Summary of 20–years experiences", 2, 3-8.

Selivonchick D.P., Johnson P.V., Roots B.I., 1977. Acyl and alkenyl group composition of brain subcellular fractions of Goldfish (*Carassius auratus* L) acclimated to different environmental temperatures. Neurotic micas Res., 4, 379-393.

Vie V., Van Man N., Gioconde M.C. et al., 2000. Near field microscopy approach to the heterogeneity of artificial and biological membranes. Protein, lipid and membrane traffic: pathways and targeting. J.A.F. Op den kamp (Ed.) IOSPress, 139-154.

Белоус А.М., Бондаренко В.А., 1982. Структурные изменения биологических мембран при охлаждении. Киев: Наукова думка, 256.

Богач П.Г., Финагин Л.К., Мирошниченко З.Н., 1979. Холестерин в плазматической мембране.Бихимия животных и человека. Киев: Наукова думка, 3, 39-47.

Иванов Н.Г., Профиров Я.И., Хинковска В.Ц. et.al., 1983. Биохимия на сперматозоидите мембране при ниски температури. Biol. Immunol. Reproduct, 7, 29-34.

Крепс Е. М., 1981. Липиды клеточных мембран. Л.: Наука, 339.

Кейтс М., 1975. Техника липидологии. Москва.: мир, 270.

Наук В.А., 1991.Структура и функция спермиев с-х животных при криоконсервации. Кишинев: Штиинца, 200.

Огурцов А.Н., 2012. Биологические мембраны, 186.

Scientific Papers. Series D. Animal Science. Vol. LXI, Number 1, 2018
ISSN 2285-5750; ISSN CD-ROM 2285-5769; ISSN Online 2393-2260; ISSN-L 2285-5750

RESEARCH REGARDING THE EFFECT OF DEUTERIUM DEPLETED WATER FROM DILUENT ON SOWS' FECUNDITY

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Abstract

In the effectuated research, we aimed to observe the effect of dilution of boar sperm with water with a 30 ppm deuterium content on sows' fecundity. The control batches (CB) were formed by 367 sows inseminated with doses based on bi-distillate water. Experimental batches (EB) were formed by 366 sows inseminated with doses based on deuterium-depleted water. The effect of deuterium depleted water was analysed function on utilised synthetic diluent (BTS and Merck), boars' age and season for sperm gathering. In relation with utilised diluent, fecundity was between 79.5% and 80.2% at CB and between 78.7% and 81.9% at EB. Fecundity of boars aged 1 year oscillated between 79.9% at CB and 79.8% at EB, and at boars with age of 3.5 years between 79.8% at CB and 80.8% at EB. In spring, fecundity was between 81.1% at CB and 82.2% at EB, and in summer between 78.6% at CB and 78.3% at EB. No matter of analysed factor differences between control and experimental batches were insignificant.

Key words*: sows, boar, fecundity, deuterium-depleted water.*

INTRODUCTION

Deuterium-depleted water (DDW) has a lower concentration of deuterium than occurs naturally (less than 145 ppm) (Rehakova et al., 2016). Deuterium concentration interest area for deuterium-depleted water is in the range 20-110 ppm. Deuterium-depleted water is obtained by isotopic distillation under vacuum (Mladin et al., 2013).

Physicochemical properties of the light water include the melting and boiling points, kinematic viscosity, density, the spin-spin proton relaxation time, self-diffusion coefficients, and the small-angle laser light scattering (Goncharuk et al., 2010).

Deuterium content of living animals body can be increased, with dramatical effect over the health state, through bioaccumulation process by continuously adding of heavy water in their nourishment. On the other hand, deuterium depletion has been mentioned in literature as a health stimulator and a new possible anticancer tool (Ştefanescu et al., 2010).

A series of experiments have shown that deuterium depleted water has beneficial effects for life. According to Boros (2016) studies, the naturally occurring isotope of hydrogen (^{1}H), deuterium (^{2}H), could have an important biological role. Deuterium-depleted water delays tumour progression in mice, dogs, cats and humans. In the former, the DDW is thought to diminish the deuteration of sugar-phosphates in the DNA backbone, helping to preserve stability of hydrogen bond networks (Boros et al., 2016**).** The biologically active role was also explained by prevention of acetaminophen induced hepatotoxicity in rat. This protection may involve the reduction of oxidative stresses (Rasooli et al., 2016).

Also, Dzhimak et al. (2015) argues that a physiological solution prepared on deuterium-depleted water during induced apoptosis activates the DNA repair system, significantly reducing the number of single-stranded DNA breaks, which, in general, indicates an increase in the efficiency of defensive systems of the cell.

Some studies show the effect of depleted water on the nervous system. Strekalova et al. (2015) conducted a correlation analysis between tap water deuterium content and rates of depression

in regions of the USA. Their data suggest that the deuterium content of water may influence the incidence of affective disorder-related pathophysiology and major depression, which might be mediated by the serotoninergic mechanisms. Mladin et al. are of the opinion that a deuterium desaturation treatment with deuterium depleted water might have a use in anxiety disorders. Another study on rats suggests that the administration of DDW may generate an improvement of the reference memory, as an index of long-term memory (Mladin et al., 2014).

Deuterium depleted water is proposed as an adjunct to cancer therapy (Boros, 2014; Krempels, 2008; Mirica, 2010).

Wang (2013) found that deuterium depleted water was an effective inhibitor of cancer cell proliferation. Deuterium depleted water is a new, nontoxic adjuvant therapeutic agent that suppresses the proliferation, migration and invasion of the cells of the nasopharyngeal carcinoma.

Some researchers have investigated the effect of depleted water on plant physiology. Thus Tanase et al. (2014) showed that deuterium-depleted water together with polyphenolic extracts from spruce bark can act in growth stimulation and also influence biosynthesis, the photosynthesizing pigments, and secondary metabolites in plants. Other researchers are of the opinion that plant physiological processes are optimal at concentrations of deuterium present in natural waters. Thus, Somlyai et al. (1993) are of the opinion that the deuterium concentration is either higher or lower than that of natural waters (150 ppm D), the growth of coleoptyl and root is hindered.

The role of water depleted in deuterium has also been demonstrated in fish reproduction. Pricope et al. (2003) showed that the reproduction of rainbow trout in a 1:1 solution of deuterium-depleted water and distilled water led to a significant increase in survival of roes during their embryonic development.

In mammals, the results of artificial insemination depend on the quality of the semen, the technique of inoculation and the good functioning of the female genital apparatus (Păcală, 2004). In pigs, the quality of the sperm depends on the age of the boars, the breed, the environmental conditions (Tăpăloagă et al., 2013) and the method of harvesting (Ciornei et al., 2012; Tăpăloagă et al., 2013).

The dilution medium for boar semen must provide the conditions for preservation (Buzan, 2015; Buzan, 2016) in order to achieve good results after insemination.

In this context, in which the biologically active role of depleted-deuterium water was identified, we aimed to investigate the effect of this water from the diluent on the sows' fecundity.

MATERIALS AND METHODS

From 4 boars of synthetic line LS 408 (2 boars -1 year old and 2 boars - 3.5 years old) was harvested the semen in April (16 ejaculates), May (24 ejaculates), July (16 ejaculates), and August (24 ejaculates). Harvesting was done through masturbation. After evaluation of the sperm quality, 4 types of doses were prepared with 4 billion mobile spermatozoa /dose. The sows were grouped in to 4 batches, depending on the type of diluent used to process the ejaculates, thus:

- CB 1 – control batches (the sows artificially inseminated with dose based on Merck III diluent and bi-distilled water);
- EB 1- experimentally batches (the sows artificially inseminated with dose based on Merck III diluent and deuterium-depleted water);
- CB 2 – control batches (the sows artificially inseminated with dose based on BTS diluent and bi-distilled water);
- EB 2- experimentally batches (the sows artificially inseminated with dose based on BTS diluents and deuterium-depleted water).

733 Camborough sows were inseminated between the first and fifth calves. For the first insemination, fresh dosages were used, and for the second, preserved for 12 hours at 17°C. Fecundity was calculated as a ratio between the number of pregnant sows at 63 days after insemination and the total number of sows inseminated. For the variance analysis, the Fisher test was used.

RESULTS AND DISCUSSIONS

Results obtained in function by the age of the boars.

The mean fecundity of artificially inseminated sows with semen harvested from young boars

was 79.9% for CB and 79.8% for EB, and sows' fecundity inseminated with doses obtained from 3.5 years old was 79.8% for CB 80.8% for EB (Table 1).

Table 1. The sows' fecundity in function by the age of boars

Batches	Boars of 1 year old			Boars of 3.5 years old		
	Artificial inseminated (no)	Pregnant (no)	Fecundity (%)	Artificial inseminated (no)	Pregnant(no)	Fecundity (%)
CB	164	131	79.9	203	162	79.8
EB	163	130	79.8	203	164	80.8
Insignificant differences $F(1;6) = 0.13 < F_{0.05}(1;6) = 5.99$				Insignificant differences $F(1;6) = 0.13 < F_{0.05}(1;6) = 5.99$		

The difference between CB and EB corresponding to boars aged 1 year was 0.1% in favor of CB (statistically insignificant) and between batches corresponding for 3.5 years old, 1% in favor of EB, also insignificant. These values demonstrate that the age of the boars does not influence the effect of deuterium-depleted water on the sows' fecundity.

Results obtained in function by the synthetic diluent used

The average fecundity of CB 1 sows was 80.2%, with 0.7% higher than that achieved at CB 2: 79.5% (Table 2). The very small difference between the results obtained using the two types of diluent did not have statistical significance ($F(1;6) = 0.23 < F_{0.05}(1;6) = 5.99$). This proves that the two diluents have a similar effect on sperm fecundity. The fecundity of EB 1 sows (82.1%) was slightly superior to that of sows in CB 1, the difference being insignificant ($F(1;6) = 0.04 < F_{0.05}(1;6) = 5.99$).

At EB 2, the level of fecundity was only 78.6%, even lower than the sows' fecundity in CB 2 (Table 2). An unsteady dynamics of the results obtained was observed by using deuterium-depleted water. Its use has insignificantly influenced the fecundity of the sows ($F(1;6) = 0.14 < F_{0.05}(1;6) = 5.99$). Under our investigations, the results of using deuterium depleted water were maximal in association with Merck III diluent. For experimental lots, the maximum level of fecundity was also achieved using Merck III diluent.

Table 2. The sows' fecundity in function by the synthetic diluent used

Batches	Boars of 1 year old			Boars of 3.5 years old			Total		
	A.I.* (no)	Pregnant (no)	Fecundity (%)	A.I.* (no)	Pregnant (no)	Fecundity (%)	A.I.* (no)	Pregnant (no)	Fecundity (%)
CB1	85	68	80.0	102	82	80.4	187	150	80.2
EB1	84	67	79.7	101	85	84.2	185	152	82.1
Insignificant differences $F(1;6) = 0,13 < F_{0,05}(1;6) = 5,99$									
CB2	79	63	79.7	101	80	79.2	180	143	79.5
EB 2	79	63	79.7	102	79	77.4	181	142	78.6
Insignificant differences $F(1;6) = 0,09 < F_{0,05}(1;6) = 5,99$									

A.I.* - artificial insemination

Results obtained in function by the season of the artificial insemination

In the spring season, mean fecundity of CB sows was 81.1% and in the summer, 78.6% (Table 3). Statistically, the difference was insignificant ($F(1;6) = 0.14 < F_{0.05}(1;6) = 5.99$). The level of fecundity of EB sows was 82.2% in the spring season and 78.3% in the summer. During the spring season was found an insignificant superiority of the fecundity of EB sows compared to that recorded in the CB, which was not preserved in the warm season, when the sows' fecundity in CB was superior to that recorded in EB (Table 3).

Although the lowest performances were found at sows in EB, in the months of the warm season, we believe that this is not due to the effect of deuterium-depleted water in the diluent on spermatozoon fecundity.

Table 3. The sows' fecundity in function by the season of the artificial insemination

Season	Batches	Artificial inseminated (no)	Pregnant (no)	Fecundity (%)
Spring	CB	180	146	81.1
	EB	180	148	82.2
Insignificant differences F (1;6) =0.13 < $F_{0.05}$(1;6) = 5.99).				
Summer	CB	187	147	78.6
	EB	186	146	78.3
Insignificant differences F(1;6) = 0.14 < $F_{0.05}$(1;6) = 5.99)				

CONCLUSIONS

The use of depleted water in semen diluents did not have a steady effect on the sows' fecundity. Inconstancy has been manifested both in relation to the age of the boars and in relation to the sowing season or the synthetic diluent used. The differences between control and experimental batches were insignificant.

REFERENCES

Boros L.G., D'Agostino D.P., Katz H.E., Roth J.P., Meuillet E.J., Somlyai G., Soleyman-Jahi S., Zendehdel K., Akbarzadeh K., Haddadi M., Amanpour S., Muhammadnejad S., 2014. *In vitro* assessment of antineoplastic effects of deuterium depleted water. Asian Pacific Journal of Cancer Prevention, 15(5), 2179-2183.

Buzan V., 2015. The protective effect of L-carnitine during the hypothermic storage of boar semen. Scientific Papers Series D, Animal Science, vol.LVIII, 201-204.

Buzan V., 2016. Phospholipids, phospholipid fractions and possibility of their stabilization during hypothermic storage of boar semen. Scientific Papers Series D, Animal Science, vol.LIX, 137-140.

Boros L.G., D'Agostino D.P., Katz H.E., Roth J.P., Meuillet E.J., Somlya G., 2016. Submolecular regulation of cell transformation by deuterium depleting water exchange reactions in the tricarboxylic acid substrate cycle. Medical Hypotheses, 87, 69-74.

Ciornei Șt., Roșca P., Grugociu D., 2012. Bacterial and fungal burden in boar semen. Research Journal of Biotechnology, 7(4), 23-27.

Dzhimak S.S., Basov A.A., Baryshev M.G., 2015. Content of deuterium in biological fluids and organs: influence of deuterium depleted water on D/H gradient and the process of adaptation. Biochemistry and Biophysics, 465(1), 370-373.

Goncharuk V.V., Lapshin V.B., Burdeinaya T.N., Pleteneva T.V., Chernopyatko A.S., Atamanenko I.D., Ul'yantsev A.S., Uspenskaya E.V., Samsoni-Todorov A.O., Taranov V.V., 2010. Physicochemical properties and biological activity of the water depleted of heavy isotopes. Journal of Water Chemistry and Technology, 33(1), 8-13

Krempels Krisztina, Somlyai Ildiko, Gabor A., 2008. Retrospective evaluation of the effects of deuterium depleted water consumption on 4 patients with brain metastases from lung cancer. Integrative Cancer Therapies, 7(3), 172-181.

Mirica Roxana Elena, 2010. Deuterium-depleted water in cancer therapy. Environmental Engineering and Management Journal, 9(11), 1543-1545.

Mladin C., Popescu A., Stefanescu I., Oubraham A., 2013. Deuterium depleted water-new studies about isotopic distillation obtaining process. Asian Journal of Chemistry, 25(14), 7976-7978.

Mladin C., Ciobica A., Lefter R., Popescu A., Bild W., 2014. Deuterium depletion induces anxiolytic-like effects in rats. Archives of Biological Sciences, 66(2), 947-953.

Mladin C., Ciobica A., Lefter R., Popescu A., Bild W., 2014. Deuterium-depleted water has stimulating effects on long-term memory in rats. Neuroscience Letters, 583, 154-158.

Păcală N., 2004. Biotehnologia însămânțării artificiale la vacă. Ed. Waldpress, Timișoara.

Pricope F., Stefanescu I., Titescu G., Caraus I., Ureche D., 2003. Effect of deuterium-depleted water on reproduction of rainbow trout. Environmental Chemistry Letters, 1(2), 149-151.

Rasooli A., Fatemi F., Akbarzadeh K., Dini S., Bahremand S., 2016. Synergistic protective activity of deuterium depleted water (DDW) and *Satureja rechingeri* essential oil on hepatic oxidative injuries induced by acetaminophen in rats. Journal of Essential Oil Bearing Plants, 19(5), 1086-1101.

Rehakova R., Klimentova J., Cebova M., Barta A., Matuskova Z., Labas P., Pechanova O., 2016. Effect of deuterium-depleted water on selected cardiometabolic parameters in fructose-treated rats. Physiological Research, 65, S401-S407, Suppl.3

Somlyai G., Jancso G., Jakli G., Vass K., Barna B., Lakics V., Gaal T., 1993. Naturally-occurring deuterium is essential for the normal growth-rate of cells. Febs Letters, 317(1-2), 1-4.

Tanase C., Boz Stingu Alina, Volf Irina, Popa V., 2014. Physiological and biochemical responses induced by Spruce bark aqueous extract and deuterium depleted water with synergistic action in sunflower (*Helianthus annuus* L.) plants. Industrial Crops and Products, 60, 160-167.

Tăpăloagă P.R., Șonea Al., Iancu Al., Mitrănescu Elena, 2016. Researches regarding age, breed and collecting season influence in quality and quantity boars semen, Scientific Papers Series D, Animal Science, vol.LVI, 161-165.

Stefanescu I., Manolescu N., Titescu Gh., Balint Emilia, Tamaian R., Feurdean V., 2010. Assessment of deuterium depletion in plymouth rock chickens'

bodies. Environmental Engineering and Management Journal, 9(11), 1477-1480.

Strekalova T., Evans M., Chernopiatko A., Couch Yvonne, Costa-Nunes J., Cespuglio R., Chesson L., Vignisse Julie, Steinbusch, H.W., Anthony D.C., 2015. Deuterium content of water increases depression susceptibility. The potential role of a serotonin-related mechanism. Behavioural Brain Research, 277, Special Issue, 237-244.

Strekalova T., Evans M., Chernopiatko A., Couch Yvonne, Costa-Nunes J., Cespuglio R., Chesson L., Vignisse Julie, Steinbusch, H.W., Anthony D.C., 2015. Deuterium content of water increases depression susceptibility. The potential role of a serotonin-related mechanism. Behavioural Brain Research, 277, Special Issue, 237-244.

Wang H.Q., Zhu B.H., He Z.W., Fu H., Dai Z., Huang G.L., Li B., Qin D.Y., Zhang X.Y., Tian L., Fang W., Yang H., 2013. Deuterium-depleted water (DDW) inhibits the proliferation and migration of nasopharyngeal carcinoma cells *in vitro*. Biomedicine & Pharmacotherapy, 67(6), 489-496.

Scientific Papers. Series D. Animal Science. Vol. LXI, Number 1, 2018
ISSN 2285-5750; ISSN CD-ROM 2285-5769; ISSN Online 2393-2260; ISSN-L 2285-5750

QUALITY AND QUANTITY PARAMETERS IN BUFFALO SEMEN PRODUCTION

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Abstract

Semen production depends upon different factors as age, breed, collecting and environmental conditions. The present research has in view to establish the influence of age upon the quantitative and qualitative parameters of semen from few of the Romanian buffalo reproducers. There were analyzed 12 native buffalo males, from different age category. At every semen collecting there were recorded the following morphologic sperm parameters: sperm volume, sperm concentration(x 10^9spz/ml), colour, mass activity, pH and motility. The influence of the age was made along two years, studying the dynamics of the sperm parameters. Statistical analysis was performed as per standard statistical methods. The results reveal the fact that the best performances were recorded in bulls up than three years old. The study clearly demonstrates that there is a variation in reproductive parameters in the buffalo bulls, which could be studied at the molecular level to unveil any genomic markers associated with low fertility and/or infertility. The males may be used to obtain semen by subjecting the young ones to training at an early age, thereby decreasing the initial age of semen donation.

Key words*: sperm collecting, qualitative and quantitative indices, buffalo.*

INTRODUCTION

Artificial insemination is practiced very little in Europe and East Asian countries like Iran and Egypt (Borghese, 2014). Also, buffalo livestock and strategies are reported for all the countries in Europe, where buffalo specie is reared and used for food production, as Italy, Romania, Bulgaria, Germany, Macedonia, United Kingdome, Greece, Serbia, Albania, Ukraine and Hungary. It was reported that the percent of buffaloes covered by AI programs is only 5% in Italy, 3.7% in Azerbaijan, 0.3% in Egypt and 0.1% in Romania. In Bulgaria, in the large cooperative state farms, it is used on 80% of the buffaloes. It was also stated that the diffusion of AI in buffaloes is difficult.

The buffalo rearing in Romania is still in an uncertain phase, which could be considered a developing phase (Tapaloaga, 2015). Despite of remarkable contribution of buffaloes breeders in the European context in the past, and a sinuously importance during the last 30 years that leads to no more than sixty thousand animals in the whole country, there is always shortage of scientific Information on this animal especially in the field of reproduction.

Although artificial insemination (AI) is being widely used in nearly all cattle / buffalo breeding countries, but the number of breeding bulls has greatly reduced and consequently the quality of the bulls has become a matter of vital importance for this species. The present study was planned with the following objective: to investigate the relationship between semen quality and quantity parameters in some Romanian buffalo bulls.

Artificial insemination facilitated the choice of using the best possible males of proven quality in improving the genetic base of the bovine population, thus conveying to the primary goal for breeding, the increasing of the productivity and the profitability of the commercial herds, by increasing the number of the offspring produced by selected genetically superior males. Due to the fact that buffalo is one of the main dairy animal in many countries of the world, but not in the European countries, excepting Italy or Bulgaria and taking into consideration that Romanian buffalo livestock decreased so much, meanwhile the consumers trend in buffalo milk products is increasing, it is of major importance for Romanian scientists

and animal breeders to focus on this species advantages (Koonjaenak, 2007; Al Dulaimi, 2015; Tapaloaga, 2015). The objective of this study was to investigate the influence of buffalo age on some quantity and quality traits and to relate them to the animal fertility, thus contributing to the success of buffalo artificial insemination. The buffalo bull age factor has been investigated concerning its effect on morphologic and morph metric sperm features by many authors (Biswajit, 2014). Moreover, sperm analyze has been also used in fertility evaluation of male and it is recommended as part of the domestic animal sperm files (Al Dulaimi, 2015; Al Dulaimi, 2014).

MATERIALS AND METHODS

The present study was conducted in the Department of Animal Reproduction, Faculty of Animal Science, University of Agronomic+ Sciences and Veterinary Medicine, Bucharest, Romania, on 12 Romanian buffalo bulls (*Bubalus bubalis*) divided into three age groups, with four bulls in each group, the first group consisted in less than 4 years aged bulls, the second one consisted in 5-6 years aged bulls and more than 7 years aged bulls. All bulls were kept under identical conditions of management, feeding and watering throughout the study period.

They were selected according to the normal sperm quality in routine tests. They were individually penned and fed, including the population households. All buffalo bulls were sexually active and under a weekly semen collection regime throughout the study period. The recorded data were analysed according to the statistical procedures. All data were nearly normal distributed. Hypothesis testing was performed by parametric tests which included analysis of variance (ANOVA). Semen from all the experimental bulls was collected every two days with the aid of an artificial vagina (AV). The semen collected was brought to the laboratory immediately and it was placed in a water bath at 37°C. Two collected ejaculates were pooled and evaluated for total volume, colour, mass activity, motility, pH and sperm concentration.

Semen volume was recorded directly in the collector glass. The colour of the semen was recorded as creamy, milky or watery, depending on the thickness of the semen and was assigned a numerical weight from zero to two for statistical analysis. A numerical weight of 2 was assigned to creamy, 1 to milky and 0 to watery samples. The pH of semen was recorded with a pH meter. The mass activity of spermatozoa was observed by placing an undiluted semen drop on glass slide under warm stage of Phase Contrast Microscope (10x) and the grading was recorded as: 0 = no mass motility; + = less than 20 percent of sperms showing progressive motion; ++ = 40 to 60 percent showing progressive motion with slow wave; +++ = 60 to 80 percent showing progressive movement with wave more intense and ++++ = 80 to 100 percent showing progressive movement with rapid wave waking eddies (+++ or more are recommended for A.I. purpose). Motility, as a percentage of individually motile spermatozoa, was estimated by examining a drop of diluted fresh semen (with 2.9% sodium citrate solution) under a microscope at 400×. Motility was scored on the basis of the percentage of spermatozoa with normal forward progressive movement, while those showing circling movements or those oscillating at one place were regarded as immotile. Sperm concentration was measured using a photo colorimeter. The means were compared, and correlation coefficients among different parameters were also worked out. The recorded data were analysed according to the statistical procedures. All data were nearly normal distributed. Hypothesis testing was performed by parametric tests which included analysis of variance (ANOVA).

RESULTS AND DISCUSSIONS

In the present study the colour of all semen samples was white, creamy with normal appearance. Normal colour of buffalo semen is white to creamy white (Johnson, 1997; Tapaloaga, 2003). The current study findings are also in agreement to Brohi (1993) in Kundhi buffalo bulls and Kumar (1993) in Indian buffalo bull semen and Tapaloaga et al. (2015) in Romanian buffaloes.

The mean (+SEM) of ejaculate volume of the semen was found to be 4.07 (±0.02) ml ranging from 3.85-4.33 ml (Table 1). Analysis of

variance showed no significant (P>0.05) difference between the bulls for ejaculate volume. The overall mean value of ejaculatory volume was 4.07 ml (SD = 0.16; CV = 3.05%). The intra-assay CV ranged between 2.89% and 3.21%. Ejaculatory volume varied between 3.72 ml and 4.33 ml among semen ejaculates and 3.42 ml and 4.69 ml among bulls. Mature bulls had higher mean values of ejaculatory volume (4.33±0.01) than those of the young (3.85±0.03) bulls (Table 1). No significant interactions were found between bulls, age group and ejaculate on ejaculatory volume.

The volume in the current study falls in the range reported by Sansone (2000) and Tapaloaga (2016). In current study all the bulls were of the age and young therefore variation in the ejaculate was not expected. The variation might be due to the difference in group age.

Table 1. Mean ejaculatory volume in Romanian buffalo bulls (ml)

Age group	Group 1 (n=4) N_1=4 N_2=32	Group 2 (n=4) N_1=4 N_2=36	Group 3 (n=4) N_1=4 N_2=32	Overall N_1=4 N_2=100
Mean	3.85	4.33	4.21	4.07
SD	0.18	0.14	0.18	0.16
SEM	0.03	0.01	0.02	0.02
CV (%)	2.89	3.21	3.08	3.05

The mean (+ SEM) pH value of fresh semen was 5.81(± 0.06) with the range of 5.72 - 6.01 (Table 2). A significant (P<0.05) difference was observed between the bulls for pH values. The mean pH value (5.72 ±0.06) found in the current study is slightly lower than the mean (6.16) reported by Brohi (1993) in Kundhi buffalo and Younis (1996) in the Nili Ravi buffalo (6.04-6.93). This might have been due to concentration of semen, season and hygienic conditions (Alvi-Shoushtari and Babazadeha-Hebashi, 2006).

However none of the pH level recorded in the present study falls in the lethal level for sperm cells (Mann and Mann, 1988). Time interval after collection and individual aliquot also influence the level of pH in fresh semen as was the case in present study.

Table 2. Mean sperm pH in Romanian buffalo bulls

Age group	Group 1 (n=4) N_1=4 N_2=32	Group 2 (n=4) N_1=4 N_2=36	Group 3 (n=4) N_1=4 N_2=32	Overall N_1=4 N_2=100
Mean	5.72	5.79	6.01	5.81
SD	0.46	0.64	0.49	0.55
SEM	0.03	0.03	0.02	0.06
CV (%)	4.07	3.86	4.87	4.51

For assessing the mass activity, swirling movement was observed. All the sample appeared to have score of +++ to ++++. For statistical interpretation these were given numerical values. The mean (+ SEM) numerical value was found to be 3.32 (± 0.05), which ranged from 3 to 4 (Table 3). Analysis of variance showed no significant (P>0.05) difference between the bulls for mass activity. Mass activity found (3.32 ±0.05) in the current study was higher than the reported values (2.65 ±1.14) in Nili Ravi bulls (Javed et al., 2000; Heuer et al., 1982) and in Indian bulls (2.54) (Vyawanare et al., 1989). The desirability can be attributed to the effect of climatic conditions, in which sperm might be more rigors due to preservation temperature.

Table 3. Mean sperm mass activity in Romanian buffalo bulls

Age group	Group 1 (n=4) N_1=4 N_2=32	Group 2 (n=4) N_1=4 N_2=36	Group 3 (n=4) N_1=4 N_2=32	Overall N_1=4 N_2=100
Mean	3.12	3.76	3.72	3.32
SD	0.46	0.64	0.33	0.55
SEM	0.03	0.03	0.02	0.05
CV (%)	4.87	3.23	4.32	4.36

The mean (+SEM) motility percentage of Romanian buffalo bull semen was 71.5 (±0.03), which ranged between 69.2-72.3% (Table 4). The motility percentage (60-75%) found in the current study in different bulls were higher beside the ones reported (63%) by Brohi et al. (1993) in Kundhi buffalo bulls and Jainuddin et al. (1982) in swamp buffalo. Higher values than the current figures have been reported in Murrah buffalo bull by Jainuddin et al. (1982). However assessment of mean percentage of motility using simple method is readily available and cost efficient and provides rapid means of semen evaluation in field conditions but objective type of semen assessments are still needed to be applied for precise analysis of semen sample (Molinia et al., 1994).

Table 4. Mean sperm motility in Romanian buffalo bulls

Age group	Group 1 (n=4) N_1=4 N_2=32	Group 2 (n=4) N_1=4 N_2=36	Group 3 (n=4) N_1=4 N_2=32	Overall N_1=4 N_2=100
Mean	69.2	72.3	71.7	71.5
SD	0.36	0.44	0.31	0.55
SEM	0.02	0.01	0.02	0.03
CV (%)	5.09	3,86	4.36	4.51

The mean (+SEM) sperm concentration of semen was found to be $1.65x10^9$ per ml, which ranged from 0.89-1.83 billion/ml (Table 5). Analysis of variance showed no significant (P>0.05) difference for semen concentration between the bulls.

The sperm concentration of the ejaculates found in the current study in Romanian buffalo falls in the range (1-4 x 10^9/ ml) reported in other breeds of buffalo (Arther, 2003; Rehman, 2012, Aguiar et al., 1994) excepting the younger bulls, in the first category, which recorded values slightly under 1 billion cells/ml. This indicates that the number of cells required for maintaining fertility level of the semen from Romanian bulls was acceptable to be used for AI programme, but taking into consideration further researches due to the fact that bulls before 3 years old have a lower sperm concentration.

Table 5. Mean sperm concentration in Romanian buffalo bulls ($x10^9$)

Age group	Group 1 (n=4) N_1=4 N_2=32	Group 2 (n=4) N_1=4 N_2=36	Group 3 (n=4) N_1=4 N_2=32	Overall N_1=4 N_2=100
Mean	0.89	1.83	1.74	1.65
SD	0.23	0.42	0.46	0.55
SEM	0.03	0.03	0.02	0.03
CV (%)	3.46	3.21	4.71	4.51

The overall mean value of sperm concentration was 1.65 x 10^9 (SD = 0.55; CV = 4.51%). The intra-assay CV ranged between 3.21% and 4.71%. Sperm concentration varied between 0.68 x 10^9 and 1.96 x 10^9 among semen ejaculates and 0.85 x 10^9 and 1.92 x 10^9 among

bulls. Older bulls had lower mean values of sperm concentration (1.74 x 10^9±0.03) than those of the young (0.89 x 10^9±0.03) bulls (Table 4). No significant interactions were found between bulls, age group and ejaculate on sperm concentration.

It is well known that spermatozoa differ in shape and dimensions among species and also between individuals (Thurston et al., 2001). Abnormal bull sperm morphology has always been correlated with reduced fertility. However, a number of studies have shown no correlations between sperm morphology and fertility (Linford, 1976) with clear associations between normal bull sperm morphology and fertility continuing to remain elusive (Johnson, 1997). Our results have brought a modest contribution to describing the morphologic traits in native buffaloes, with values close to the ones reported by other researchers from the Asian continent on crossbred and native Murrah buffalo bulls (Biswajit Roy).

CONCLUSIONS

On the basis of current study it was concluded that Romanian buffalo bulls' qualitative and quantitative semen parameters in correlation with other objective characters can be useful tools for developing a fertility index. The study clearly demonstrates that there is a variation in reproductive parameters in the bovine bulls, which could be studied at the molecular level to unveil any genomic markers associated with low fertility and/or infertility. The males may be utilized to obtain semen by subjecting the young ones to training at an early age, thereby decreasing the initial age of semen donation.

REFERENCES

Aghangari Y.T., 1992. Cryopreservation of ram semen for A.I. Ph.D. Thesis, Univ. Wales, Bangor.

Al Dulaimi M.K.H., Tapaloaga P.R., Tapaloaga D., 2015. Age related morphometric features of spermatozoa in ram, Journal of Biotechnology, 208, Supplement, p S37, ISSN 0168-1656.

Al Dulaimi M.K.H., Tapaloaga P.R., Tapaloaga D., 2014. Artificial insemination sperm fertility assessment by CASMA procedures in ram, Journal of Biotechnology, 185, Supplement, September, p S41, ISSN 0168-1656.

Al Dulaimi M.K.H., Tapaloaga P.R., Tapaloaga D., 2015. Results regarding some morphometric features of spermatozoa in ram, Agriculture and Agricultural Science Procedia, 6, 232-235, ISSN 2210-7843.

Alvi S.S.M., Babazadeha-Hebashi B., 2006. Seasonal variations in the characteristics of the Azerbaijani buffalo. Iran. J. of Vet. Res., 7, 1-14.

Aguiar P.H.P., Andrade V.J., Abreu J.J., Gomez N.B.N., 1994. Physical and morphological semen characteristics of buffalo aged from four to eight year old. Proc. 4th Int. Buffalo Congr.,Sao Paulo, Brazil., 3, 488-488.

Arther, 2003. Artificial Insemination. Arther's Veterinary Reproduction and Obstetrics 8th edition London, W.B. Sunder, Co., 751-758.

Biswajit Roy, 2014. A comparative study on sperm morfometry of crossbred and Murrah buffalo bulls, International Journal of Agricultural Sciences and Veterinary Medicine, 2(3), 149-155.

Borghese A, 2014. A Scientific Bulletin of Escorena, vol 10.

Brohi N.A., 1993. Sexual behaviour and semen characteristics of Kundhi buffalo bulls. M.Sc. Thesis. Department of Anim. Repro. Sindh Agriculture University Tandojam.

Heuer C.H., Bader M.A., Bajwa, 1982. Sperm morphology of the Nili- Ravi buffaloes. Pak.Vet. J., 2, 155-160.

Javed M.T., Khan A., Kausar R., 2000. Effect of age and season on some semen parameters of Nili-Ravi buffalo (*Bubalus bubalis*) bulls. Vet.Arhiv., 70, 83.

Jainuddin M.R., Bangso T.A., Dass S., 1982. Semen characteristics of the swamp buffalo (*Bubalus bubalis*). Anim. Repro. Sci., 4, 213-217.

Johnson W.H., 1997. The significance of bull fertility of morphologically abnormal sperm. Bull fertility, Veterinary Clinics of North America: Food Anim. Practice, 13, 255-270.

Linford E. et al., 1976. The relationship between semen evaluation methods and fertility in bull. Journal of reproduction and fertility, 47, 283-291.

Mann T., Mann L.W., 1988. Male reproductive function and semen. Themes and trends in physiology. Biochemistry and investigative Andrology. Spring-Velog, Berlin, New York.

Molinia F.C., Evans G., Casares P.I., Maxwell W.M.C., 1994. Effect of monosaccharides and disaccharides in Tris-based diluents on motility, acrosome integrity and fertility of pellet frozen ram spermatozoa. Anim Reprod Sci., 36, 113– 122.

Rehman Z.U., Samo M.U., Qureshi T.A., Khan S., Qureshi M.S., Khan F.A., Bahadadr S., 2012. Studies on the freezability of Kundhi Buffalo bull semen. The Journal of Animal and Plant Sciences, 22(2), 18-23, ISSN 1018-7081.

Thurston I. et al., 2001. Morphologically distinct sperm subpopulations defined by Fourier Shape Descriptors in fresh ejaculates correlated with variation in boar semen quality following cryopreservation. J. Androl., 22, 382-394.

Sansone G., Nastri M.J.F., Fabbricini A., 2000. Storage of buffalo (*Bubalus bubalis*) semen. Anim. Repro. Sci., 55-76.

Tapaloagă P, 2003. Handbook of animal reproduction, Allprint Publishing House, Bucharest.

Tapaloaga D, Tapaloaga P.R., 2015. Study Regarding Age-Related Morphometric Features of Buffalo

Sperm, Agriculture and Agricultural Science Procedia, 6, 272-276.
Vyawanare R., Chauhan R.A.S., Nema S.P., Poswal M.L., 1989. Studies on seminal attributes, enzyme leakage and preservability of buffalo semen, Ind. Vet. J., 66, 1128-1132.
Younis M., 1996. Studies on semen quality, freezability and fertility of buffalo bulls during low and peak breeding seasons. Ph.D. Dissertatiot of Anim.Repro. University of Agriculture, Faisalabad, Pakistan.
Seri Koonjaenak, Vichai Chanatinart, Suneerat Aiumlamai, Tanu Pinyopumimintr, Heriberto Rodriguez-Martinez, 2007. Seasonal variation in semen quality of swamp buffalo bulls (*Bubalus bubalis*) in Thailand. Asian J Androl., 9(1), 92–101.

TECHNOLOGIES OF ANIMAL HUSBANDRY

Scientific Papers. Series D. Animal Science. Vol. LXI, Number 1, 2018
ISSN 2285-5750; ISSN CD-ROM 2285-5769; ISSN Online 2393-2260; ISSN-L 2285-5750

SLAUGHTER AND QUANTITATIVE CHARACTERISTICS OF THE MEAT FROM LAYING HENS

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Abstract

The aim of the research was to obtain comparative indicators of the slaughtering and quantitative characteristics of the laying hens from different hybrid lines. For the examination were used hybrid lines from ISA Brown, for gaining eggs with brown shell, and DeKalb, for gaining eggs with white shell. The total number of examined individuals is 40, 20 of each hybrid line, with an average age of 84 weeks. After the slaughter, the measurements of each carcass were made and the participation of the basic parts in the mass of the clean carcass was determined. The dressing percentage of clean carcasses without internal organs and skin of hens from the hybrid line ISA Brown is 49.80%, while the dressing percentage of chicken from the hybrid line DeKalb is 46.80%. The average mass of chests (white meat with bone) is 234.65 g in ISA Brown, or 26.95% in the mass of the carcasses, while in the DeKalb hybrid line the average mass of the chest is 197.20 g with 26.86% participation in the mass of carcasses.

Key words*: hybrid lines, slaughter, carcass, quality.*

INTRODUCTION

The poultry industry, which is mainly focused on egg production, shows constant development in the last twenty years, and the private individual farms which increase their activity in this sector are the greatest contributors.

The egg and broilers production in small family agricultural economies has long tradition and representation in the Republic of Macedonia and it is also a part of the rural heritage. In egg production part, the main goal is keeping the current situation of total self-sufficiency on the domestic market with domestic production through keeping and advancing of the domestic consumption.

According to data from (Official Gazette of R. Macedonia, 2015), the total number of laying hens is 1,352,564 and the average egg production per laying hen is 150.

In total production, 109,977 are from individual producers and 93,406 is a part of egg production in the business sector.

Total production of eggs in Pelagonija region is 37,291 - 8,767 eggs are produced by individual producers, and 28,524 eggs are produced by business entities.

Numerous hybrid lines are used for production of eggs for consummation. They are divided in two groups: heavy and light hybrid lines which differ by live weight. Heavy hybrid lines have bigger weight - above 1550 g, and the light hybrid lines weigh 1350 g (Todorovski, 2016).

Despite the fact that the laying hens are, first of all, intended for egg production, still, they are being slaughtered for meat production after their exploitation. Meat from laying hens is used in meat industry for production of various types and kinds of sausages.

The bones from the parts of the carcass which are characterized with bigger percentage of meat (chicken breast bone, thigh and drumstick) are being removed, and that meat is used for production of sausages with bigger quality, while other parts of the carcass are used for production of mechanically deboned meat (MDM), which is used for production of sausages with lower quality (Vasilev and Kitanovski, 2005).

MATERIALS AND METHODS

Laying hens from the hybrid lines ISA brown and DeKalb which are excluded from

production due to end of the production cycle, 84 weeks old, were taken as material for examination.

Both of the hybrid lines are procured as day old chicks and they are bred in special object for floor system breeding. The chicks are bred on a mat from sawdust, with controlled feeding, according to recommendations from hybrid lines' producers.

During the breeding, the chicks get all the vaccines recommended from the Veterinary Institute.

The resettlement of pullets in the objects with cages is performed when they are 16 weeks old, in order to avoid stress.

Hens in production objects are fed with same food, which is being changed, depending on the age, water available all the time and in same ambient conditions. Because of high egg laying percentage and favourable market conditions, the exploitation period is extended and they are kept until 84 weeks old.

The examination of the slaughtering and the quantitative characteristics of laying hens were conducted in two parts. Examination of the slaughtering characteristics of hens was conducted in the first part, and examinations of the chemical composition of meat were conducted in the second part. Before slaughtering, the hens were subject to starving in period of 8 hours.

Thereby, the following measurements and activities were performed:

Weighing live weight of the hens, Slaughtering, Weighing bloody hen with head, calculating the amount of blood, Weighing the head, Weighing head, feathers, skin and legs up to the knee ankle, Calculating dressing percentage, Weighing internal organs, Weighing wings, Weighing drumstick and hip with bone, Weighing the meat from drumstick and hip without bone, Weighing white meat with bone, Weighing the back with the neck and all the tissues which belong to it.

The cutting of slaughtered hen's carcass on main pieces is made according to the principles and criteria for classification and categorization of meat, which are applied for cutting the hen carcasses.

The main parts of the carcass according to the general criteria for classification and categorization of meat is made according to generally accepted criteria for cutting chicken carcasses. The chicken meat is categorized in three quality categories, whereas chest and drumstick with hip belong to first category. The wings belong to second category, and the third category is comprised of back with pelvis.

The comparison of the data between two groups was held by using t- test ($p<0.05$).

RESULTS AND DISCUSSIONS

After removal of inner organs, a pure carcass is obtained. The average weight of chicken carcasses from ISA Brown hybrid line is 870.40 g, and the average weight of chicken carcasses from DeKalb hybrid line it is 734.55 g. The difference in average weight of pure carcasses is 135.85 g in favour of ISA Brown, and it is statistically significant ($p>0.01$) (Tables 1 and 2).

The obtained weight of pure carcass is slaughter yield, which in our case is nearest to yield of processing of poultry carcasses intended for barbecue (grill processing). In our case, the yield is without skin, since it is removed along with subcutaneous fat tissue.

Yield of pure carcasses without inner organs and skin of chicken from ISA Brown hybrid line is 49.80%, while the yield of chicken from DeKalb hybrid line is 46.80%. The results for the slaughter yield that are obtained in our examinations, show significant differences in comparison to other authors. In the researches of Nikolova and Bogosavljević-Bošković (2011) for determination of quality of the broiler carcass from two hybrid lines slaughtered at different age, the yield was presented through three kinds of carcass processing: classic processing, „ready to roast" and „ready to grill". The authors determined that the age factor has biggest impact on the yield in classic processing ($p<0.05$), as they were processed in our researches.

Similar results for slaughter yield were conducted by Zivkovic (1994) of broilers intended for barbecue from the hybrid line Hibro which is 63.3%.

Table 1. Slaughter yield of hens from ISA Brown hybrid line

	Carcasses without blood		Carcasses without head		Carcasses without skin		Clean carcasses	
	g	%	g	%	g	%	g	%
x	1695.35	97.00	1629.00	93.25	1276.55	73.04	870.40	49.89
min	1472.00		1416.00		1111.00		746.00	
max	1907.00		1830.00		1437.00		978.00	
Sd	123.65		120.58		106.87		57.72	
Cv	7.29		7.39		8.37		6.63	

Table 2. Slaughter yield of hens from DeKalb hybrid line

	Carcasses without blood		Carcasses without head		Carcasses without skin		Clean carcasses	
	g	%	g	%	g	%	g	%
x	1528.90	96.07	1457.45	91.15	1168.35	73.41	734.55	46.17
min	1225.00		1170.00		865.00		588.00	
max	1849.00		1764.00		1416.00		912.00	
Sd	175.17		166.93		157.06		85.79	
Cv	11.45		11.45		13.44		11.67	

Table 3. Share of basic parts in the weight of pure carcass of chicken from ISA Brown hybrid line

	Pure carcass	Wings		Thigh and drumstick		Chest		Back and neck	
		g	%	g	%	g	%	g	%
x	870.40	85.70	9.84	293.90	33.76	234.65	26.15	256.15	30.46
min	746.00	65.00		250.00		179.00		213.00	
max	978.00	99.00		327.00		268.00		305.00	
Sd	57.72	8.66		23.00		21.55		25.10	
Cv	6.63	10.11		7.82		9.18		9.80	

Table 4. Share of basic parts in the weight of pure carcass of chicken from DeKalb hybrid line

	Pure carcass	Wings		Thigh and drumstick		Chest		Back and neck	
		g	%	g	%	g	%	g	%
x	734.55	66.85	9.10	232.3	32.62	197.20	26.86	238.20	32.42
min	588.00	53.00		186.00		145.00		174.00	
max	912.00	80.00		287.00		254.00		309.00	
Sd	85.79	8.12		29.00		28.86		33.12	
Cv	11.67	12.15		12.48		14.63		13.90	

Once the weight of pure carcass is obtained, it is cut in basic parts, and the weight of the parts is measured. The results obtained from our examinations are given in Table 3 and Table 4.

From Tables 3 and 4, it could be ascertained that the share of wings in the weight of pure carcass from ISA Brown hybrid line is 9.84%, i.e. 9.10% from DeKalb hybrid line. The difference in average weight of wings between the hybrid lines of laying hens is 18.85 g in favour of ISA Brown. Our obtained results for the average weight of wings of chicken from both hybrid lines differ from those obtained by other researchers. Thus, according to examinations conducted by Ljubojević et al. (2011), it is determined that the share of wings in the total weight of the carcass from hybrid line Ross is 7.65% in male and 8.12% in female individuals.

The share of chicken thigh and drumstick in pure carcass is 33.76%, i.e. 31.62%. The differences in the average weight of thighs and drumsticks of the examined chicken are statistically significant ($p<0.05$).

In the carcasses of young chicken Rašeta and Dakić (1994) have determined that the share of the thigh and drumstick in the total weight of the carcass is from 29.8 % to 32.3 %, and they are in correlation with the results from our examinations. In their researches, Ljubojević et al. (2011), have stated that the share of the drumstick of male broilers from Ross hybrid line is 9.35 %, and the share of the thigh is 9.06%, while the share of the drumstick of female broilers is 9.10%, and the share of the thigh is 8.51%.

In the conducted examination about the impact of selenium and vitamin E in the nutrition of broilers from hybrid line Cobb 500, Marković et al. (2009) have ascertained that the share of thigh and drumstick in the total weight of carcass of slaughtered broilers is 28.74, which is similar to the one in our researches.

The stated difference in the average weight of the chest (white meat with bones) of chicken from ISA Brown and DeKalb hybrid lines is 37.45 in favour of ISA Brown at the level of ($p<0.01$).

The share of the back and neck in the weight of pure carcass of hens from ISA brown hybrid line is 29.42 %, i.e. 32.42% of laying hens from DeKalb hybrid line. The difference of average weight of the back and neck is in favour of ISA Brown, in both absolute and relative indicators, and it is statistically significant at the level of ($p<0.05$)

Similar results are obtained in the researches of Pavlovski et al. (2009), Hopić et al. (2002), Blagojević et al. (2009).

CONCLUSIONS

On the basis of conducted researches about slaughter and quantitative characteristics of meat from laying hens from ISA Brown and DeKalb hybrid lines, it could be ascertained that the hybrid line and method of breeding, in direction of improvement of genetic characteristics of the hybrids, have impact on the slaughter yield, the quality, the nutritive and the biological value of poultry.

REFERENCES

Blagojević N., Pavlovski Z., Škrbić Z., Likić M., Milošević N., Perić L., 2009. The effect of genotype of broiler chickens quality in extensive rearing system, Acta Veterinaria, 59(1), 91-97.

Hopić S., Pavlovski Z., Škrbić Z., Likić M., 2002. Metode određivanja konformacija trupa pilića, Biotehnologija u stočastvu, 3-4, 45-49 .

Marković R., Blatić M., Peturujkic B., Sefer D., Todorovic E., 2009. Uticaj selena I vitamin E na kvalitet I prinos trupova brojlera. Tehnologija mesa, 3-4, 194-199.

Nikolova N., Bogosavljević-Bošković S., 2011. Carcass quality of broiler chicken of two hybrid line slaughtered in different age. Macedonian Journal of Animal science, 1(1), 95-100.

Official Gazette of R. Macedonia, 2015.

Rašeta J., Dakić M., 1994. Higijena mesa (Higijena zivine i jaja). Univerzitet u Beogradu,Veterinarski Fakultet, Beograd.

Pavlovski Z., Škrbić Z., Likić M., Vitorović D., Petričević V., Milošević N., 2009. Nacked neck chicken of Serbian and foreign origin: carcass characteristics. Biotechnology in animal husbandry, 5-6 pp. 1023-1032.

Todorovski S., 2015. Carcass and Slaughter characteristics of the meat from laying hens. Master's Thesis, Faculty of Biotechnical Sciences, Bitola, 9 p.

Vasilev K., Kitanovski D., 2005. Technology on poultry meat and eggs. Faculty of Biotechnical Sciences, Bitola.

Živković J., 1994. Higijena i tehnologija mesa - kakvoča i prerada. Veterinarski fakultet, Zagreb, Croatia.

Ljubojević D., Božić A., Bjedov S., Milošević N., Stanačev E., 2011. Efekat ekstrudiranog kukuruza u ishrani na konformaciju trupova brojlera. Tehnologija mesa, 52(2), 205-211.

Scientific Papers. Series D. Animal Science. Vol. LXI, Number 1, 2018
ISSN 2285-5750; ISSN CD-ROM 2285-5769; ISSN Online 2393-2260; ISSN-L 2285-5750

INFLUENCE OF DIFFERENT WATER SOURCES ON SOMATIC CELL COUNT AND COMPOSITION OF BOVINE RAW MILK

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Abstract

The objective of this study was to investigate the effects of water sources on somatic cell count (SCC) and composition of bovine raw milk. The examinations were carried out in a private Holstein farm located in Konya province of Turkey. Before and after changing water supply of the farm (BCW and ACW), all analysis results were recorded. At the BCW period, parameters were noted to be; antimony (Sb): 15.4 µg/L, arsenic (As): 40.98 µg/L, blurriness: 3.3 NTU, mercury (Hg): 2.385 µg/L and iron (Fe): 293.6 µg/L. The values of the same compounds at seven days later ATC time were measured to be <5µg/L, <10 µg/L, 0.74 NTU, 1.457 µg/L and 320.3 µg/L, respectively. According to tank milk test results during seven days before and after the process, the means for dry matter, fat, protein, lactose, density, freezing point, mineral and logarithmic SCC (logSCC) were calculated to be 11.89±0.099 and 12.02±0.111%; 3.01±0.020 and 3.47±0.034% (P<0.001); 3.30±0.044 and 3.17±0.036% (P<0,05); 4.83±0.090 and 4.64±0.063%; 1.0315±0.00099 and 1.0299±0.00071 g/ml; -0.461±0.0042 and -0.466±0.0093°C; 0.673±0.0129 and 0.686±0.0205%; 5.349±0.0453 and 5.228±0.0246, respectively. Changes in water supply caused an increase in fat percentage but, a decline in the protein percentage of tank milk. Individual milk samples of 18 cows at BCW and those collected from 16 cows at ACW shown that mineral increased (P<0.05) but logSCC decreased (P<0.01). Obtained findings here might be attributed to adverse effect of change in Sb, As and Hg on the metabolism.

Key words*: water supply, dairy cow, milk, somatic cell count, milk compound.*

INTRODUCTION

According to enhanced industry, people and different animal species may be influenced by waste materials and contaminated feed/water sources those including toxic matter and thus, animal health may adversely be affected and society may be exposed to severe health problems (Phillips et al., 2003; Crout et al., 2004; Licata et al., 2004; Ozmen and Mor, 2004; Patra et al., 2008; Sanchez de la Campa et al., 2008).

This case also causes an important environmental problem for wild animals and habitat. Such that, excessive amount of heavy metals those known as beneficial elements for human body may create drastic poisoning and toxicity (Rana, 2010; Sigrist et al., 2010).

These elements pass to the surroundings by mining, industrial activities or exhaust gases and blend to groundwater and land (Sanchez de la Campa et al., 2008).

In spite of these kind of contaminations are mostly caused by heavy metals such as arsenic (As), cadmium (Cd) and lead (Pb), no clinical symptom via drinking water or feeds has been declared in dairy cattle (Bhattacharya et al., 2009; Datta et al., 2010). Besides, it has been informed that as in taken by water or feeds substantially discarded from the cow's body during the milking process (Fangstrom et al., 2008; Datta et al., 2010) Gharibi et al. (2012) indicated that Cd, Pb, chrome (Cr) and mercury (Hg) have been accumulated in different organs and products of cattle and moreover, these elements transfer to human's body by consuming animal products.

Bilandzic et al. (2011), who investigated heavy metal residues in milk produced in Croatia, pointed out the high Pb level and suggested to dense control with sampling milk and pasture.

Really, producing raw milk in healthy conditions plays an important role for human health.

In this sense, tracking harmful effects of industrial wastes for drinking water and feeds may be seen a major issue.

In this study, changes of milk composition and revealing possible toxic effects in cows consumed water sources including different heavy metal ingredients were investigated.

MATERIALS AND METHODS

Raw milk samples collected from Holstein cows raised in a private farm in Konya region of Turkey and water samples taken from two different sources were used to be the study materials.

The farm had a semi-open barn system and lactating cows were milked twice a day in a separate unit.

The animals were fed with wheat stalk, dried alfalfa hay, corn silage and dairy cattle feeds. Water requirements of examined cows were satisfied by automatic drinking bowls.

Due to milk fat level of tank milk had dropped, water source was commented the possible reason of this case and thus, water samples were analyzed with EPA 200.7 standard using inductively coupled plasma optical emission spectrometry (ICP-OES) technique in an accredited laboratory.

Raw milk samples were collected from both tank milk and each cows individually during morning milkings for seven days.

Sample tubes with threaded were coded by the origin of the sample and the date and then, all samples were kept at -18°C in a deep-freezer until the test time.

Similarly, new drinking water samples were analyzed after changing the source. Thus, new milk samples were collected from the tank and cows and storaged in the freezer for seven days post-two days of changing the water source. The milk samples were protected using ice-boxes and immediately transferred to the laboratory for analyses. To tests, bovine raw milk samples were incubated at 35^{o}C in a water bath. While dry matter, fat, non-fat dry matter, protein, lactose, density, freezing point and minerals were analyzed by Funke Gerber Lactostar device, somatic cell counts (SCC) were determined by Somatic Cell Counter DCC (DeLaval Group, Sweden).

Milk analysis results were evaluated in two groups: before and after changing water source. Due to abnormal variance had been observed among the SCC values, all SCC data were converted to logarithm 10 base (log10) for statistical evaluations. Independent sample-*t test* was used to compare the results using SPSS 17.0 for Windows packet program.

RESULTS AND DISCUSSIONS

Water analysis results before (BCW) and after (ACW) the changing of water source are presented in Table 1. As seen, Sb, As, Hg and Fe levels had reached to higher than TS 266 standard that declared by Turkish Standards Institution. Such that, As, Sb and Hg were found to be four, three and two and a half times higher than those accepted as the highest threshold levels, respectively.

Also, Fe level was measured as 50% higher than its standard. During the BCW, Sb, As and Hg levels were noted to be 15.4 µg/l, 40.8 µg/l and 2.385 µg/l, respectively.

Really, the exceed levels of each mineral might be an important risk factor for of animal and human health. Many researchers (Phillips et al., 2003; Crout et al., 2004; Licata et al., 2004; Ozmen and Mor, 2004; Patra et al., 2008; Sanchez de la Campa et al., 2008; Javed et al., 2009) pointed out to this case and mentioned the problems related to human health.

For instance, Pérez-Carrera et al. (2016) determined As concentration in some regions of Argentina as >10 µg/l that informed over the admissible level.

In this study, similar analyses were repeated after changing the water source and it was observed that Sb, As and Hg levels largely dropped but Fe increased (Table 1).

Datta et al. (2010), who determined the As levels in contaminated (0.047 mg/l) and control (0.015 mg/l) water sources, emphasized the risk of consumption of raw milk produced in their investigation area.

In this study, while Sb and As levels shown to be lower than threshold according to TS 266 by ranging between <5 and <10 µg/l, Hg decreased about 1 mg/l and diminished up to 1.457 µg/l when compared to initial source.

Nevertheless, eliminating any problems via investigating contamination reasons might be suggested.

Comparatively, a rise in an increment in Fe level might not be assumed to be an adverse effect on both the animal health and milk composition when compared to other elements.

Fe levels were measured as 293.6 µg/l and 320.3 µg/l in the BCW and ACW periods, respectively. Of the findings, especially As, Hg and Sb might be regarded as the components

those had potential to cause toxic effect and to damage animal's health. This case could be seen an intimated process between udder tissues of the animal and human health due to raw milk including these elements.

However, both final results might be presumed as acceptable for TS 266 standard and thus, no adverse effect for animal health or milk might be expected. But, blurriness values of BCW and ACW were obtained to be 3.3 NTU and 0.74 NTU, respectively.

In spite of these levels were observed within the suitable ranks, the severe dropping was found as attractive.

For two periods, tank milk SCC and composition levels are given in Tables 2 and 3. As seen, fat percentage was changed from 3.01% to 3.47% and this level was statistically significant ($P<0.001$). In contrast, protein percentages significantly ($P<0.05$) reduced.

These changes might be explained by high Sb, As and Hg those determined in the drinking water. Besides, relatively low fat ratio was evaluated within the acceptable thresholds for Holstein cattle due to dropping heavy metals to the normal limits.

In this study, mean of logSCC was calculated to be 5.288±0.0299, no significant effect of CWS on SCC was observed.

Change of milk fat and protein percentages are presented in Figure 1. Similarly, changing the water supply had no significant effect on the other components.

As seen in Table 3, fat and protein levels of individual milk samples had similar trends. However, mineral level significantly increased ($P<0.05$) in the ACW period. Also, SCC of individual samples significantly decreased after this process ($P<0.01$). This finding might be commented by the negative effects of consuming water with heavy metals and also, causing a physiologic stress on body resistance because of predisposition of these sources to more microbial load. Such that, elevated SCC could be assumed as a response against microbial contamination.

Distribution of mineral and logSCC levels are given in Figure 1.

Table 1. Analyse results before and after changing water supply

		Before			After		
Parameters	Unit	Result	TS 266	Method	Result	TS 266	Method
Al	µg/L	<45	200	EPA 200.7	<45	200	EPA 200.7
NH_4	mg/L	0.064	0.5	SM 4500-NH3 B.F	0.016	0.5	SM 4500-NH3 B.F
Sb	µg/L	**15.4**	5	EPA 200.7	**<5**	5	EPA 200.7
As	µg/L	**40.8**	10	EPA 200.7	**<10**	10	EPA 200.7
Cu	µg/L	<5.4	2000	EPA 200.7	<5.4	2000	EPA 200.7
Blurriness	NTU	**3.3**	5	SM 2130 B	**0.74**	5	SM 2130 B
Hg	µg/L	**2.385**	1	EPA 200.7	**1.457**	1	EPA 200.7
Fe	µg/L	**293.6**	200	EPA 200.7	**320.3**	200	EPA 200.7
EC (cond.)	µs/cm	627	2500	SM 2510 B	605	2500	SM 2510 B
Cd	µg/L	<3.4	5	EPA 200.7	<3.4	5	EPA 200.7
Ag	mg/L	<7		EPA 200.7	0.007		EPA 200.7
Cr	µg/L	<6.1	50	EPA 200.7	<6.1	50	EPA 200.7
Pb	µg/L	<10	10	EPA 200.7	<10	10	EPA 200.7
Mn	µg/L	<1.4	50	EPA 200.7	<1.4	50	EPA 200.7
Ni	µg/L	<15	20	EPA 200.7	<15	20	EPA 200.7
ph		7.65	6.5-9.5	SM 4500 B	7.69	6.5-9.5	SM 4500 B
Flavour		Accept			Accept		
Smell		Accept		SM 2150 B	Accept		SM 2150 B
Mg	meq/L	1.17			0.52		

Table 2. Components of tank milk

Supply	N	TDM (%)	Fat (%)***	NFDM (%)	Protein (%)*	Lactose (%)	Density (g/ml)	FP (^oC)	Mineral (%)	LogSCC
Before	7	11.899	3.01	8.88	3.30	4.83	1.0315	-0.461	0.673	5.349
After	7	12.02	3.47	8.55	3.17	4.64	1.0299	-0.466	0.686	5.228
Mean	14	11.95	3.24	8.72	3.23	4.74	1.0307	-0.463	0.679	5.288

*P<0.05; ***P<0.001

Table 3. Components of individual milk samples

Supply	N	TDM (%)	Fat (%)	NFDM (%)	Protein (%)	Lactose (%)	Density (g/ml)	FP (°C)	Mineral (%)*	LogSCC**
Before	18	12.11	3.11	9.00	3.35	4.954	1.0327	-0.468	0.687	5.356
After	18	12.11	3.24	8.88	3.29	4.872	1.0321	-0.475	0.731	5.045
Mean	36	12.11	3.17	8.94	3.32	4.912	1.0324	-0.471	0.709	5.201

*P<0.05; **P<0.01

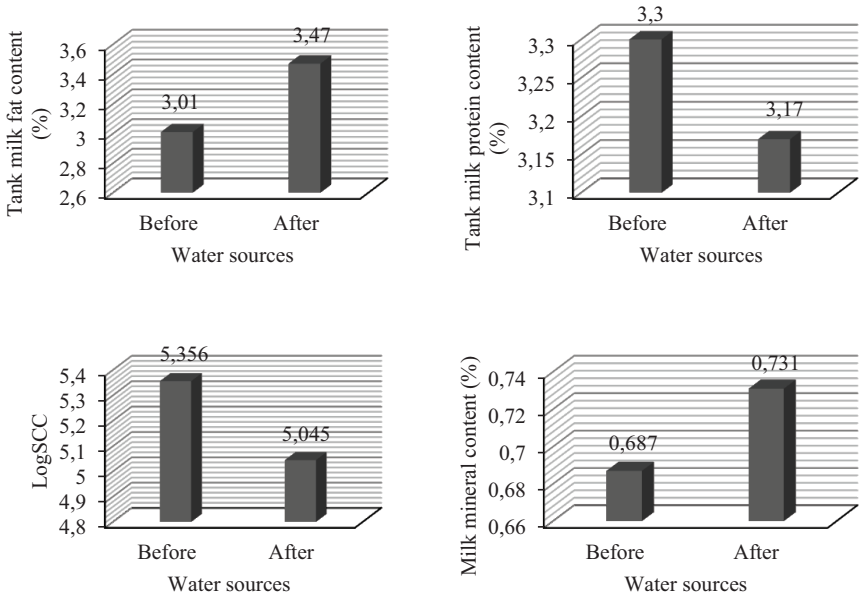

Figure 1. Distribution of investigated components before and after changing water supply

CONCLUSIONS

Finally, it was revealed that Sb, As and Hg levels of drinking water source had higher than those declared by the standards and this case was regarded as the potential reason of decreased fat percentage of tank milk.

In this sense, changing the drinking water source removed the adverse impacts and fat ratio turned to above for Holstein cattle.

While mineral levels of individual milk samples increased after this process, SCC also positively affected.

Actually, these findings indicate to involving the possibility of industrial wastes and heavy metals to the underground waters in the region where this investigation had been conducted.

However, further field studies in that location are advised to confirm revealed findings here.

REFERENCES

Bilandzic N., Dokic M., Sedak M., Solomun B., Varenina I., Zorka Knezevic Z., Benic M., 2011. Trace element levels in raw milk from northern and southern regions of Croatia. Food Chem., 127, 63-66.

Bhattacharya P., Samal A.C., Majumdar J., Santra S.C., 2009. Transfer of arsenic from ground water and paddy soil to rice plant a micro level study in West Bengal. World J AgriSci., 5, 425-431.

Crout N.M.J., Beresford N.A., Dawson J.M., Soar J., Mayes R.W., 2004. The transfer of 73As, 109Cd and 203Hg to the milk and tissues of dairy cattle. J.Agric.Sci., 142(2), 203-212.

Datta B.K., Mishra A., Singh A., Sar T.K., Sarkar S., Bhatacharya A., Chakraborty A.K., Mandal T.K., 2010. Chronic arsenicosis in cattle with special reference to its metabolism in arsenic endemic village of Nadia district West Bengal India. Science of the Total Environment, 409, 284-288

Fangstrom B., Moore S., Nermell B., Kuenstl L., Goessler W., Grander M., Kabir I., Palm B., Ariffeen S.E., Vahter M., 2008. Breast feeding against arsenic exposure in Bangladshi infants. Environ Health Perspect, 116, 963-969.

Gharibi H., Sowlat M.H., Mahvi A.H., Mahmoudzadeh H., Arabalibeik H., Keshavarz M., Karimzadeh N., Hassani G., 2012. Development of a dairy cattle drinking water quality index (DCWQI) based on fuzzy inference systems. Ecological Indicators, 20, 228-237.

Javed I., Jan I., Muhammad F., Rahman Z., Khan M.Z., Aslam B., Sultan J.I., 2009. Bull Environ ContamToxicol., 82, 616-620.

Licata P., Trombetta D., Cristani M., Giofre F., Martino D., Calo M., 2004. Levels of „toxic" and „essential" metals in samples of bovine milk from various dairy farms in Calabria, Italy. Envir. Int., 30, 1-6.

Ozmen O., Mor F., 2004. Acute lead intoxication in cattle housed in an old battery factory. Vet. Hum. Toxicol., 46(5), 255-256.

Patra R.C., Swarup D., Kumar P., Nandi D., Naresh R., Ali S.L., 2008. Milk trace elements in lactating cows environmentally exposed to higher level of lead and cadmium around different industrial units. Science of the Total Environment, 404, 36-43

Pérez-Carrera A., Alvarez-Gonçalvez C.V., Fernández-Cirelli A., 2016. Transference factors as a tool for the estimation of arsenic milk concentration. Environ. Sci. Pollut. Res., 23, 16329-16335.

Phillips C., Gyori Z., Kovacs V., 2003. The effect of adding cadmium and lead alone or in combination to the diet of pigs on their growth, carcass composition and reproduction. J. Sci. Food Agric., 83, 1357-1365.

Rana T., Das A.T., Bhattacharya S., Bandyopadhyay D., Pan S., Das S.K., 2010. Effect of chronic intake of arsenic-contaminated water on blood oxidative stress indices in cattle in an arsenic-affected zone. Ecotoxicology and Environmental Safety, 73, 1327-1332.

Sánchez de la Campa A. M., de la Rosa J. D., Sánchez - Rodas D., Oliveira V., Alastuey A., Querol X., Gómez Ariza J.L.,2008. Arsenic speciation study of PM2.5 in an urban area near a copper smelter. Atmospheric Environment, 42, 6487-6495.

Sigrist M., Beldoménico H., Repetti M.R., 2010. Evaluation of the influence of arsenical livestock drinking waters on total arsenic levels in cow's raw milk from Argentinean dairy farms. Food Chemistry, 121, 487-491.

Scientific Papers. Series D. Animal Science. Vol. LXI, Number 1, 2018
ISSN 2285-5750; ISSN CD-ROM 2285-5769; ISSN Online 2393-2260; ISSN-L 2285-5750

COMPARATIVE STUDY ON EVOLUTION OF REPRODUCTION INDICES IN HOLSTEIN COWS FROM ROMANIA

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Abstract

The objective of this study was to evaluate the evolution of the main reproduction indices registered by the Holstein cows from Romania, in regard with the correlation between their values and the economic efficiency of the exploitation of this category. The data processed for this paper were collected between 2012 and 2017, and the comparison of the results was made with the results obtained in Romania 20 years ago by another author. The indicators evaluated were: the calving interval, defined as the number of days passed between two consecutive calves, the dry period, represented by the period in which the cows aren't milked and the age of the first parturition, expressed in days. The comparison of the data was done based on the following statistics, determined using the Excel software: mean, mean error, standard deviation, and coefficient of variability. The data presented in this paper were collected from 59 farms located throughout Romania and correspond to 40,770 complete lactations. The overall trend of the values recorded by these indices was improving over time.

Key words*: age of the first parturition, calving interval, dry period, Holstein livestock, reproduction indices.*

INTRODUCTION

The success of any genetic improvement program of the Holstein breed population of dairy cows exploited in Romania is directly dependent on knowing the breeding parameters. Thus, in order to establish the objecttives of genetic improvement, we must fully understand the state of the Romanian Holstein livestock.

Another valence of knowing these indicators has very important economic implications. Any farmer wants his livestock to show productive and reproductive precocity manifested by reducing the age at first calving, maintaining an optimal value for the calving interval (the ideal value is equal to or less than 365 days) and achieving a correct correlation between the productivity of animals, the system of exploitation and the duration of the dry period.

In this context, the present paper, along with other such works, may be one of the premises of a future genetic improvement program that takes into account the complicated relationship between the production conditions in Romania, the possibilities of the Romanian Holstein population and the characteristics of the milk market in the country characterized by the price volatility offered to the producer and the crises with various causes that periodically affect this market.

MATERIALS AND METHODS

For this comparative study, we used the data presented in a paper published in the past by Murat in 1997 that treated the same subject, and the comparison was made with the results obtained from data generated between 2012 and 2017 with the purpose of the Official Control of Production by the HolsteinRo Cow Breeders Association.

To estimate the values of each reproductive indices, we used the mean, average error, standard deviation and coefficient of variability.

The statistical accuracy of the results was provided by a database that involved more than 40,000 records represented by the lactations of a total of over 25,000 cows from the Holstein breed exploited in about 60 farms across Romania. Statistical data processing was done with the Excel program.

RESULTS AND DISCUSSIONS

Age at first calving (A.F.C.) is an important indicator of the precocity of milk production. For the period considered by Jeana Murat and I. Murat in the 1997 paper, it was recorded an average of 965 days for the active population. The trend of this indicator was upward, rising from 833 days in 1971 to 912 days in 1994, which led to a proportional increase in the unproductive period of livestock with a negative impact on economic performance.

For the period under review, the results obtained from the statistical processing are shown in Table 1.

Table 1. Evolution of the A.F.C.

Year	n	$X \pm s_x$	S	V%
2011	680	817.34 ± 6.30	164.28	20.10
2012	1464	817.40 ± 4.96	189.86	23.23
2013	2435	823.57 ± 4.14	204.33	24.81
2014	4677	811.58 ± 2.37	161.83	19.94
2015	6531	796.94 ± 1.88	151.59	19.02
2016	7487	781.81 ± 1.39	120.25	15.38
2017	33	821.91 ± 15.49	88.96	10.82
Mean	**23307**	**799.71 ± 1.01**	**154.88**	**19.37**

The trend recorded by this indicator for the period between 2011 and 2017 is shown in Figure 1. There is a general downward trend.

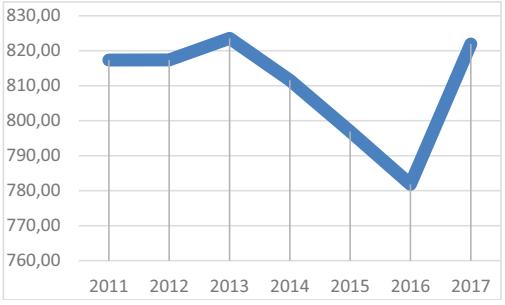

Figure 1. Trend of A.F.C.

The average for A.F.C. for the current period is 799.91 ± 1.01 days with a standard deviation of 154.88 days and a coefficient of variability of 19.37%, indicating a relative homogeneity of the values. The maximum value was recorded in 2013 and was 823.57 ± 4.14 days. Also this year was the highest coefficient of variability (24.81%). In 2016, the minimum value for A.F.C. was reached. This was 781.81 ± 1.39 days with a standard deviation of 120.25 days and a coefficient of variation of 15.38%.

Calving interval (C.I.) is the indicator that provides information on the rhythm of reproduction in dairy cows and results by summing up the gestation duration and service period (Vidu, 2002).

Jeana Murat and I. Murat determined an average value, for the period between 1971 and 1994, of 438 days, which varied between 393 days in 1971 and 455 days in 1984. They also noted the sinuous character of the curve describing this indicator in the succession of lactations, especially between lactation 6th and 12th (Figure 2).

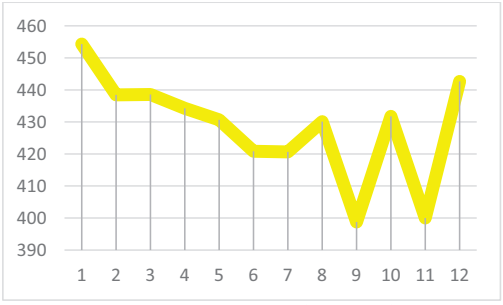

Figure 2. Sinuous character of the curve describing C.I. in the succession of lactations in period from 1971 to 1994

For the period between 2011 and 2017, C.I. had an average of 427.85 ± 1.07 days with a standard deviation of 111.56 days and a coefficient of variation of 26.07% (Table 2).

Table 2. Evolution of the C.I.

Between lactations	n	$X \pm s_x$	S	V%
1 and 2	5039	422.86 ± 1.39	98.40	23.27
2 and 3	3046	431.56 ± 2.15	118.63	27.49
3 and 4	1694	432.63 ± 3.00	123.35	28.51
4 and 5	777	428.50 ± 4.45	124.02	28.94
5 and 6	265	440.38 ± 8.14	132.51	30.09
6 and 7	94	437.26 ± 13.20	127.25	29.10
7 and 8	31	443.23 ± 18.73	104.30	23.53
8 and 9	12	469.50 ± 36.90	127.83	27.23
Total Mean	**10958**	**427.85 ± 1.07**	**111.56**	**26.07**

The variability of this indicator is relatively large, both overall and for each lactation in

part, which can be an advantage in the activity of genetic improvement of the Holstein cow population in Romania.

The lowest value of C.I. was recorded between 1st and 2nd lactation and was 422.86 ± 1.39 days with a standard deviation of 98.40 days and a coefficient of variation of 23.27%. The longest duration of C.I. was performed between lactations 8 and 9 and was 469.50 ± 36.90 days with a standard deviation of 127.83 days and a coefficient of variability of 27.23%.

Compared to the results published in 1997, there is a reduction in the graphic curve sinuosity and a more pronounced tendency to increase the value of C.I. (Figure 3).

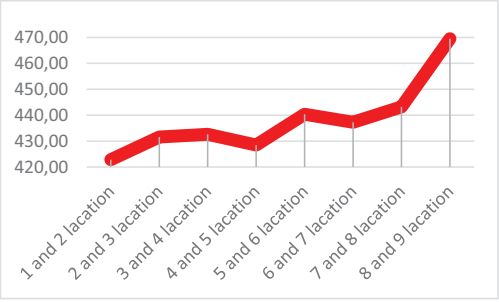

Figure 3. Sinuous character of the curve describing C.I. in the succession of lactations in period from 2011 to 2017

In neither case, the C.I. did not reach the optimum between 365 and 400 days. This situation is due to the duration of service period, which appears to be arrhythmic, character imprinted to the C.I. to. Starting from the premise that gestation duration is about 270 to 280 days for Holstein population in Romania, we can estimate an average service period duration of 147.85 - 151.85 days.

Dry period (D.P.) is the third reproduction indicator described in this paper and can be defined as the period, expressed in days, which flows from the end of a lactation to the next calving (Velea and Mărginean, 2012).

In 1997, Jeana Murat and I. Murat found for D.P. an average value of 95.91 ± 0.27 days with a standard deviation of 31.58 days and a 32.91% variability coefficient. The value of the variability coefficient suggests a reduced homogeneity of this indicator, which, as in the case of C.I., may be beneficial in the work of genetic improvement of the Holstein population of Romania.

For the current period, the statistic of D.P. is shown in Table 3.

Table 3. Evolution of the D.P.

Between lactations	n	$X \pm s_x$	S	V%
1 and 2	5666	62.30 ± 0.51	38.41	61.66
2 and 3	3574	80.80 ± 1.29	76.93	95.21
3 and 4	3114	81.96 ± 1.40	77.90	95.05
4 and 5	361	81.97 ± 3.91	74.26	90.59
5 and 6	133	90.66 ± 6.97	80.39	88.67
6 and 7	54	83.70 ± 7.98	58.64	70.06
7 and 8	20	89.35 ± 12.17	54.44	60.93
Total Mean	**12922**	**73.13 ± 0.56**	**63.86**	**87.33**

The mean value of D.P. is 73.13 ± 0.56 days, with a standard deviation of 63.86 days and a very high variability coefficient of 87.33%, which suggests an increased heterogeneity of the data string that determined these values. D.P. lasts the most between the 5th and 6th lactation when it reaches 90.66 ± 6.97 days, with a standard deviation of 80.39 days and a coefficient of variation of 88.67%. The minimum duration is between lactations 1 and 2 and is 62.30 ± 0.51 days, with a standard deviation of 38.41 days and a coefficient of variation of 61.66%.

Figure 4 shows the curve appearance describing the duration variation of D.P.

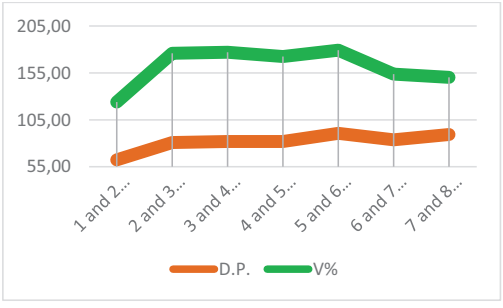

Figure 4. Sinuous character of the curve describing D.P. and variability of V% in the succession of lactations in period from 2011 to 2017

The interval in which the value of the coefficient of variability, in the lactation

sequence, varies is between 60.93% (between 7th and 8th lactation) and 95.21% (between 2nd and 3rd lactations).
Figure 4 suggests a positive correlation between the evolution of D.P. and the value of the coefficient of variability.

CONCLUSIONS

Age at first calving decreased from 912 days, as it was in 1994 to 799.71 ± 1.01 days, as it is today. This indicator decreased by 112.29 days during the analyzed period.
The duration between two calves was reduced by 14.79 days from 442.64 ± 1.03, the value published in 1997, to 427.85 ± 1.07, as is currently the case.
The dry period decreased from 95.91 ± 0.26 days, as it was in 1997, to 73.13 ± 0.56 days, as it is now.
In the case of the A.F.C. there was a small value of the coefficient of variability, of only 19.37%, a value explained by the influence of the biological limitations.
For the other two indicators studied (C.I. and D.P.) there was a high and very high variability revealed by coefficients of variability of 26.07% and 87.33%, respectively. It is possible that these values can be explained by the variable and deficient management existing on Romanian farms. This situation creates a favorable ground for the genetic improvement of reproduction of the Holstein population in Romania.
In synthesis, the three indicators studied for this paper have improved over the last 20 years as follows: A.F.C. improved by about 12.31%, C.I. improved by about 3.34%, and D.P. with 23.75%.

ACKNOWLEDGEMENTS

This study was conducted using the data collected by the HolsteinRo Cow Breeders Association with the occasion of the Official Control of Milk Production of the Holstein cows from Romania.

REFERENCES

Murat Jeana, Murat I., 1997. The Monography of the Romanian Black Spoted Breed. Agricultural Technology Publishing House, Bucharest.
Velea C., Mărginean Gh., 2012. Bovine Breeding Treaty. Risoprint Publishing House, Cluj-Napoca.
Vidu Livia, 2002. Research on the exploitation of dairy cows in standard farms for the private sector, U.S.A.M.V., phD Thesis, Bucharest.

Scientific Papers. Series D. Animal Science. Vol. LXI, Number 1, 2018
ISSN 2285-5750; ISSN CD-ROM 2285-5769; ISSN Online 2393-2260; ISSN-L 2285-5750

RESEARCH ON EVOLUTION OF MILK PRODUCTION AT NATIONAL AND EUROPEAN LEVEL

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Abstract

The paper aims to present the evolution of Milk Production during the period 2007 -2016 in Romania and also in the European Union. It is based on the statistical data provided by the National Institute of Statistics, the Food and Agriculture Organization of the United Nations, Eurostat and other open sources. During the analyzed period, livestock has continuously decreased in Romania, compared to the year of Romania's accession in the European Union (2007), in 2016 the livestock (dairy cow) was 418,109 less, representing a smaller percentage of 25.82% of livestock in 2016 as compared to 2007. In comparison to the production of cow milk achieved in the first year of Romania's accession to the European Union, production of 2016 is lower by 184,420 tons, respectively by 16.22%. Regarding the productivity of the milk quantity, in relation to the number of existing dairy cows, it is noticed that the productivity increased in 2016, ie if in 2007 there was a production of 1,136,372 tons of milk with a number of 1,619,241 dairy cows, in 2016 there was a production of 951,952 tons of milk with a total of 1,201,132, respectively milk production per dairy cow increased from 0.70 tons in 2007 to 0.79 tons of milk per cow in 2016, the productivity increase being about 13% expressed as a percentage.

Key words*: dairy cows, evolution, milk production, Romania.*

INTRODUCTION

In Romania, agriculture traditionally occupies an important position in the national industry, representing the field that generates food and raw materials for the agro-food industry. The massive fragmentation of property, the existence of a large number of cows holdings with a reduced number of animals, reduced productivity and high self-consumption of own products in households generate important structural problems in Romanian agriculture. Due to the low level of labor productivity and given that the Romanian food industry fails to provide enough products to cover the high demand for food products, the Romanian agricultural industry cannot compete with some EU Member States that have a developed industry,with high productivity.

Of agricultural products, the dairy sector is the most difficult and delicate problem of the national economy; it is difficult because in the period following 1989, little progress has been made in terms of the effectiveness of this sector, incomparably less than in the rest of the European countries and delicate, because decisive, immediate restructuring and modernization measures have to be taken of the sector.

MATERIALS AND METHODS

In order to characterize the evolution of milk production, the following indicators were used: number of cattle stock, of which dairy cows and heifers, milk yield and total milk production.

The period analysed in this study was 2007-2016.

The data, collected from the Romanian and international institutions already mentioned have been processed in data mining form, resulting in metadata.

RESULTS AND DISCUSSIONS

Milk is produced on a large scale in the European Union, totaling 165 million tonnes in 28 EU Member States in 2014 (Eurostat 2015 source), with the European Union contributing around 24% to total world production (FAO statistics 2015). Under these circumstances,

theEU is a major player on the world milk market, as can be seen in Figures 1 and 2, which are realised from informations available on the Eurostat website.

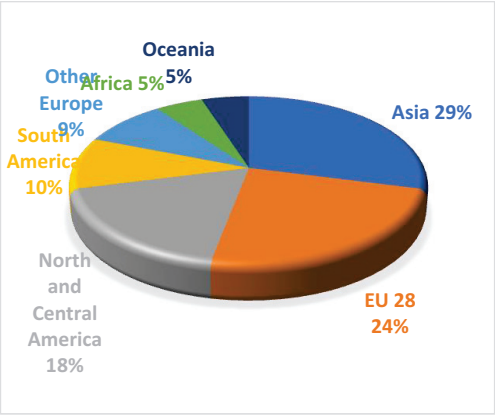

Figure 1. Milk production in 2015 by region

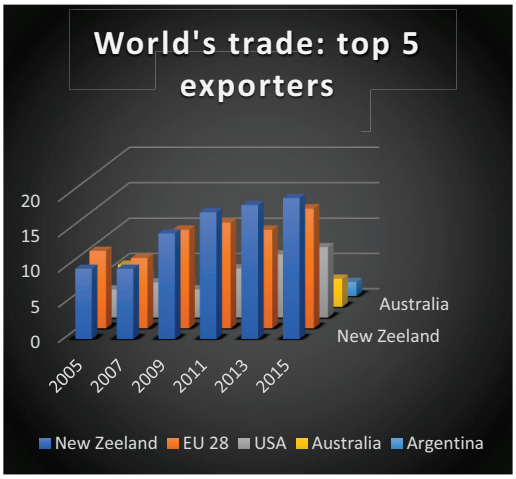

Figure 2. First 5 exporters of milk by region/country in 2015

Germany and France are the main producers within the European Union, producing almost 40% of total EU production. Other major Community producers are the United Kingdom (10%), the Netherlands (8%), Poland and Italy (both with 7%).

Next, before presenting and analyzing the evolution of milk production in our country, we will present some statistical data regarding the evolution of dairy cattle heads in Romania, given that the number of dairy cows has an impact on milk production in a way directly, together with the productivity of each animal, after the date of accession of Romania to the European Union until 2016 (the last year with statistical data), using as the annual reference date 1 June, with informations available on the website of the National Institute of Statistics and the Ministry of Agriculture and Rural Development (Table 1).

Table 1. The number of dairy cows in Romania at the reference date of June 1 of each marked year

Year	Number of cows	Diferences +/- from the last year
2007	1,619,241	+ 3.105
2008	1,578,909	- 40.332
2009	1,512,329	- 66.580
2010	1,431,406	- 80.923
2011	1,181,140	- 250.266
2012	1,187,578	+ 6.438
2013	1,193,771	+ 6.193
2014	1,192,024	- 1.747
2015	1,200,915	+ 8.891
2016	1,201,132	+ 217

From the analysis of the data presented above, the following conclusions can be drawn:

- Compared to the year of Romania's accession to the European Union (2007), in 2016 the national dairy herd numbered less by 418,109 heads, representing a smaller percentage of 25.82% of animals in 2016 as compared to 2007;
- Since 2007, the dairy herd has experienced a sharp decline, reaching a critical level of 250,266 animals in minus in 2011 compared to 2010;
- From 2012, the stock of livestock has grown, reduced in number but constant, except in 2014.

From the analysis of the data presented above, obtained from informations available on the website of the National Institute of Statistics and the Ministry of Agriculture and Rural Development we can present the folowing observations:

- The cow's milk production in our country declined annually, starting with the first year after Romania's accession to the European Union, with the exception of 2014, when it grew by 13%, and of 2016 when it increased by 3.6% compared to the previous year;

Table 2. The production of cow's milk produced in Romania between 2007 and 2016

Year	Production (tones)	Diferences from last year (+/-) in tons
2007	1,136,372	+3,250
2008	1,051,481	-84,891
2009	991,588	-59,893
2010	903,750	-87,838
2011	897,348	-6,402
2012	887,854	-9,494
2013	882,381	-5,473
2014	996,653	+114,272
2015	915,874	- 80,779
2016	951,952	+32,655

- As compared to the production of cow's milk in the first year of Romania's accession to the European Union, the production of 2016 is lower by 184,420 tons, ie by 16.22%;
- From the point of view of the productivity of the quantity of milk, in relation to the number of existing dairy cows, it is noticed that the productivity increased in 2016, ie if in 2007 there was a production of 1,136,372 tons of milk with a flock of 1,619,241 dairy cows, in 2016 there was a production of 951,952 tons of milk with a total of 1,201,132, respectively milk production per dairy cow increased from 0.70 tons in 2007 to 0.79 tonnes of milk per head of cow in 2016, the productivity increase being about 13% expressed as a percentage (Tables 1 and 2).

European level

As far as dairy cows are concerned at European level, the number of dairy cows in the EU (with 28 Member States) in 2016 was 23.5 million head, a decrease of 0.2% (46,890) compared to the year 2015.

Germany held the largest number of dairy cows in all EU countries in 2016 and 4.2 million heads respectively, representing 17.9% of the total EU-28 dairy cow population. Malta continues to be the smallest milk producer with only 6,500 dairy cows in 2016, up 2.0% over 2015. More than half of the EU-28 countries have reduced their number of cows in 2016, with Germany registering the largest absolute decrease - 66,940 cows less than in 2015.

The Netherlands recorded the largest absolute increase in the number of dairy cows in the EU-28 in 2016, up 77,000 (4.5%) compared to 2015.

Britain accounted for 8.2% of all dairy cows in the EU-28 in 2016, reaching 1.9 million heads, with an increase of 2,000 cows (0.1%) over the previous year.

Milk production within the European Union

Over a period of 30 years, the dairy sector in the European Union has been operating under the milk quota scheme, which was introduced in 1984 to manage the issue of over-milk production.

After five years of preparation, in order to ensure a smooth transition, milk quotas disappeared on 1 April 2015.

In the European Union of 2015, the production of raw cow's milk slightly increased (+0.8%), the price of farm milk decreased, and the number of dairy cows remained stable (+0.2%). However, at national level, the dairy herd grew strongly in several countries (+9.9% in Ireland and +6.6% in the Netherlands), while it decreased in 18 Member States. Belgium, Denmark and the United Kingdom recorded similar but less significant increases. Cow's milk production followed national trends in cows' milk (+14% in Ireland and +5.3% in the Netherlands). In many EU countries, the decrease in the number of dairy cows was offset by a rise in productivity. Crude milk production declined in only seven Member States. The increase in cow's milk production was the most spectacular in Greece, where the 18% decrease in the dairy herd had no impact on milk production, although it related to a smaller flock.

Dairy cow milk production increased by 1.5% in the EU between 2014 and 2015, reaching almost 6,900 kg per dairy cow. In the Czech Republic, Spain, Hungary and Poland, milk production increased by 320 - 420 kg per capita, also reflecting the development of the most productive farms and the cessation of milking in the least productive.

In 2015, 168.2 million tonnes of milk were produced in the European Union, of which 96.8% of cow's milk.

More than one fifth (21.0%) of the total cow's milk collected by dairy factories in the EU-28 in 2015 was collected in Germany, while slightly more than one-sixth of the total (16.7%) was collected by dairy factories in France, as can be seen in the following graph, which shows the distribution of cow's milk collected from Member States (Figure 3).

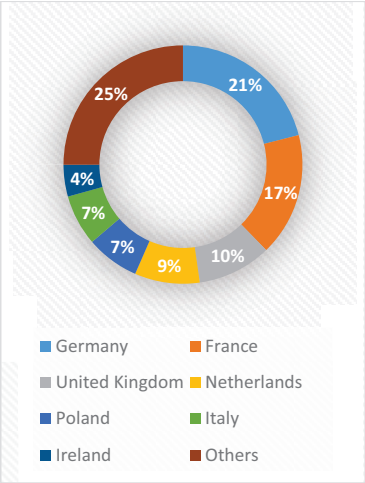

Figure 3. The distribution of collected cow's milk per Member State (Eurostat website)

Table 3. The production of cow's milk on farms at national and regional level in the European Union in 2014 - 2016

Country/ Collection year	**2014**	**2015**	**2016**
France	26,341.58	26,461.05	25,618.50
Netherlands	13,093.70	14,024.00	15,090.00
United Kingdom	14,887.80	15,272.60	14,732.54
Italy	12,492.51	12,499.27	13,321.70
Poland	:	10,876.26	11,142.63
Turkey	8,725.10	9,033.60	9,304.50
Denmark	5,122.40	5,278.90	5,385.00
Switzerland	3,544.24	3,489.13	3,436.35
Austria	3,151.19	3,215.11	3,271.64
Sweden	2,962.38	2,956.48	2,883.99
Finland	2,367.03	2,398.13	2,389.71
Portugal	1,947.16	2,008.99	1,935.43
Norway	:	1,590.00	1,594.00
Greece	1,394.60	1,350.73	1,421.00
Romania	**:**	**1,070.42**	**1,153.83**
Serbia	833.28	864.57	847.36
Estonia	735.91	729.70	719.75
Bulgaria	572.65	566.31	609.63
Slovenia	552.48	572.30	583.66
Cyprus	209.69	220.62	251.28
Albania	113.90	125.20	125.00
Malta	42.77	41.57	43.13
Montenegro	26.10	24.10	24.50

According to the chart below, generated by Eurostat, Romania occupies the thirteenth position regarding the collection of cow's milk among the Member States of the European Union (Table 3).

The phenomen of the increase in total and at the same time of the milk production, of course in different proportions, does not manifest in the continents that comprise the vast majority of the industrialized countries of the world, but here there are decreases in cows, but with strong growth of average milk production per cow.

ACKNOWLEDGEMENTS

This research work was carried out with the data and information's available from the National Statistics Institute, Food and Agriculture Organization of the United Nations, and other open sources.

CONCLUSIONS

In 2016 the milk production in Romania was lower by 184,420 tons, respectively by 16.22%, compared to the production of cow milk achieved in the first year of Romania's accession to the European Union.

The productivity of the milk quantity, in relation to the number of existing dairy cows increased about 13% in 2016, from 0.70 tons in 2007 to 0.79 tons of milk per cow in 2016.

REFERENCES

http://www.fao.org
http://www.meat-milk.ro
http://www.madr.ro
http://www.cota-lapte.ro
http://ec.europa.eu/eurostat
http://www.insse.ro
http://www.roaliment.ro

Scientific Papers. Series D. Animal Science. Vol. LXI, Number 1, 2018
ISSN 2285-5750; ISSN CD-ROM 2285-5769; ISSN Online 2393-2260; ISSN-L 2285-5750

DECREASING OF AMMONIA GAS LEVEL IN BROILER BREEDING WITH PHOSPHORIC ACID METHOD

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Abstract

The aim of this study is to test the new, non-marketable system for reducing the most common ammonia gas from the harmful gases in the broiler house and gain them to the farmers. This new system is designed to decrease the ammonia level in the poultry house environment without ventilation. The study was carried out in a broiler breeding house with a capacity of 3000, length of 21 m, a wall height of 3.3 m and a width of 10 m. The phosphoric acid system consists of 2 barrels with a volume of 70 liters placed on top of each other, 5 M phosphoric acid solution and polyethylene balls with three different surface areas. A 190 m^3 h^{-1} capacity fan is installed on the top of the system. It is aimed to reduce the ammonia level by passing the poultry air through this planned system. The phosphoric acid placed in the system is wetting the polyethylene balls and the ammonia in the poultry air passing through the surface of the polyethylene balls is trying to be trapped by the acid in these surfaces. This system, which has never been tried before, is thought to be successful in reducing ammonia levels in broiler chickens.

Key words*: ammonia, broiler, phosphoric acid, polyethylene ball.*

INTRODUCTION

Ammonia (NH_3) is an important pollutant gas that is emitted from poultry farms as a result of the microbial spoilage of the uric acid in poultry fertilizer. It prevents economic poultry husbandry with adverse impacts on animal and human health during the breeding period in coops resulting from an increase in the gas and dust density especially in periods when ventilation is difficult (Okuroğlu, 1987; İpek et al., 2002). The problem of eliminating contaminated air and odors inside the poultry housings has not been solved yet and it is still widely discussed (Nowakowicz-Dębek et al., 2016). Air change of about 0.5-6.0 m^3 h^{-1} kg^{-1} is required for attaining the optimum environmental conditions in poultry housings (Wlazlo et al., 2016). This results in a significant amount of pollutant gas emission to the atmosphere. Ammonium ions (NH_4^+) that form in litter may develop as melted ammonia on the surface, ammonium ions (NH_4^+) and free ammonia (NH_3). The ammonia gas coming from the litter surface spreads during a convection process. While it can generally remain for relatively shorter periods of time in the atmosphere ranging from a few hours to a few days, ammonium ions in aerosol form may remain in the atmosphere for up to 15 days (Wlazlo et al., 2016). Ammonia emissions have increased dramatically in the 20th century, doubling or tripling at certain locations in the world. Studies that aim to limit the amount of nitrogen compounds released from poultry fertilizer are being carried out in many research centers in Europe and America (Jones et al., 2013; Zhang et al., 2010).

Various chemical applications such as zeolites, superphosphate, phosphoric acid, iron sulfate, acetic acid etc. are used for reducing ammonia emissions from animal manure. These chemical applications reduce ammonia emissions but may also result in different forms of pollution. For example, phosphoric acid addition may increase the phosphor content of litter (Singh et al., 2009).

The objective of the study was to try out a new system that is not in the market for reducing the ammonia gas which is the most widespread deleterious gas generated in the broiler housing and to make it available for the producers. In

this regard, the applied Phosphoric Acid Setup has been developed within the scope of this study. The objective for developing this system was to reduce the level of ammonia without affecting the ambient temperature.

MATERIALS AND METHODS

The study was carried out at the deep litter broiler housing with a length of 21 m, height of 3.3 m and width of 10 m with a capacity of 3000 located at the Süleyman Demirel University, Faculty of Agriculture Research and Application Center. Rough sawdust was used as litter material during the husbandry period. Ventilation hatches with dimensions of 1.45 x 5.6 m were placed on the south and north walls. In addition, another ventilation hatch with a length of 0.5 x 21 m was placed on the roof. Ross hybrid chicks used frequently in broiler husbandry were used as live material in the study and the chicks were taken into the poultry housing when they were one day old. Careful attention was given to the requirements of broiler chicken.

Ammonia remover system with phosphoric acid solution was tested in the study. A phosphoric level of 5 M phosphoric acid solution was added. Three different applications were tested for this purpose with an objective of decreasing ammonia level. The prepared phosphoric acid setup along with ammonia, temperature and moisture sensors placed at intervals of 1.5 m were placed inside the poultry housing as shown in Figure 1 after which measurements were carried out at the chick and human level. The first sensor was placed right next to the system (0 m) and the other sensors were placed at intervals of 1.5 meters along the long axis of the poultry housing. Sensor height from the ground was arranged depending on animal development. Two sensors were placed at a height of 1.7 m for determining the ammonia levels at the human level one of which was right next to the phosphoric acid setup with the other at a distance of 3 m (Top 0 m and Top 3 m).

Application 1. A plastic barrel with a volume of 70 l was used in the system. Eighteen holes were drilled on the barrel with diameters of 3.3 cm starting from 14 cm above the bottom of the barrel. Plastic pipes with lengths of 8 cm were placed in these holes for preventing acid seepage. A single hole with a diameter of 11.5 cm was drilled on the top of the system and a fan with a capacity of 190 m^3 h^{-1} was placed here. A ½ polypropylene water pipe was made into a circle and placed right below the upper lid with 60 holes of 2 mm diameter drilled on it.

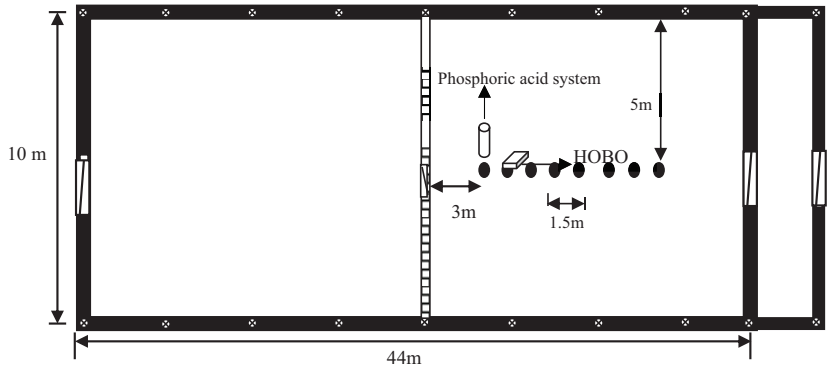

Figure 1. View of the phosphoric acid system and ammonia sensors

A total number of 80 plastic balls with diameters of 8.5 cm were placed inside the barrel for ensuring that the air passing through the barrel gets into contact with a larger surface area, thereby reaching a total interior surface area of 1.82 m^2. A single hole was drilled under the barrel which was connected to a pipe that was in turn connected to a pump. The outlet of the pump was connected to the circular pipe placed on top of the system. In short, the system wets the balls inside with phosphoric acid and the ammonia inside the air that passes in contact with the surface of the balls is tried to be absorbed by way of the acid on these surfaces.

Application 2. Whereas in the second application two plastic barrels were placed on top of each other and the system was operated with an increased surface area. Thus, the surface area was increased from 1.82 m^2 to 2.27 m^2 and measurements were obtained with doubled interior volume (Figure 2).

Application 3: A total number of 300 plastic balls with smaller diameters (4 cm) were also included in the system for increasing the total surface area. The surface area that the air flowing inside gets in contact with was increased from 2.27 m^2 to 3.78 m^2 following the final system change after which measurements were taken. Patent study was carried out for this developed system and the details of the system will be presented in another article.

Figure 2. Designed phosphoric acid system

RESULTS AND DISCUSSIONS

Application 1. The applications for the phosphoric acid method used in the study were tried to be depicted graphically. As can be seen in Figure 3, the system was operated when the ammonia level measured by the 0 m sensor right next to the setup reached 25 ppm. The researchers specify the ammonia level limit value for the poultry housing as 25 ppm (Choiniere and Munroe, 1997). Values decreased in the 0 m and upper 0 m sensors right after the system was operated, whereas the values acquired from the 1.5 m sensor increased. This is most likely due to an ammonia gas concentration difference inside the poultry housing and the onset of gas diffusion from ammonia rich regions towards the system. As a result, decreases and increases occurred in the other sensors which were not at sufficient levels for explaining the effectiveness of the system. However, it can be observed as a result of examining Figure 4 which depicts the average values that the first sensor yields lower values in comparison with the sensor right next to it thus indicating that the system has decreased the level of ammonia. Low ammonia levels detected around the locations of 9 m and 10.5 m sensors can be explained by the entry of fresh air into the system when the gate near these sensors is opened and closed while controlling the system.

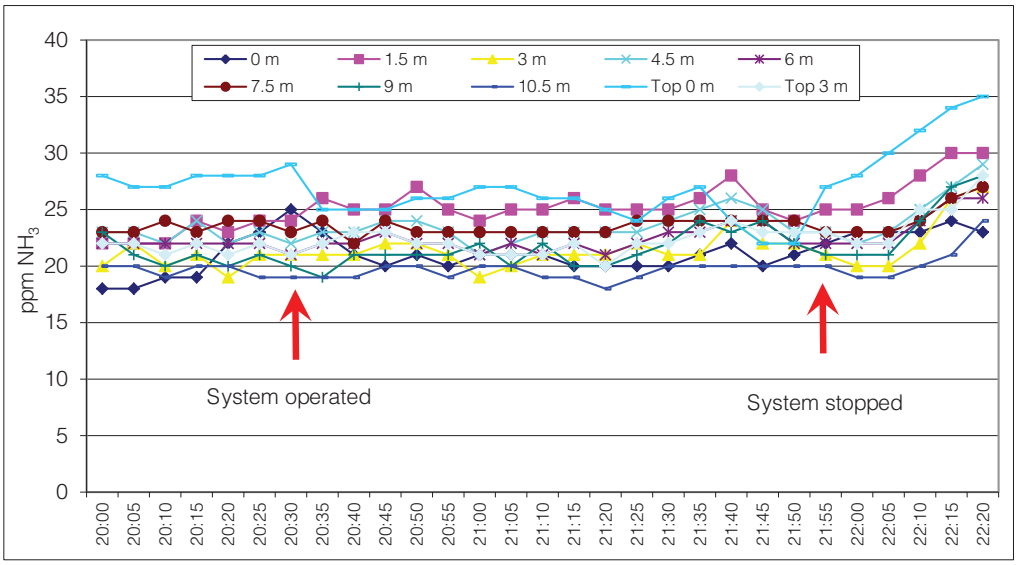

Figure 3. Ammonia values measured by application 1

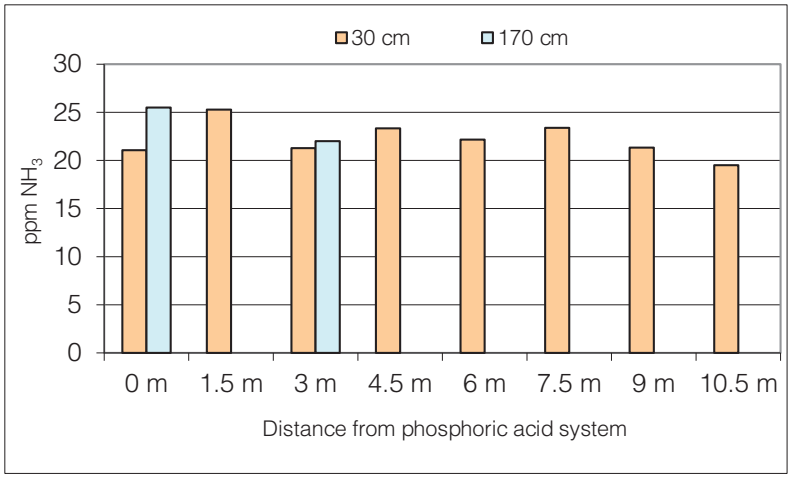

Figure 4. Average ammonia values obtained by application 1

It can be indicated upon examining the values acquired from 0 m and upper 0 m sensors which yielded the highest decrease during the operating time of the phosphoric acid system that a decrease of about 5 ppm took place which would not be too significant for decreesing the level of ammonia inside the poultry housing. However, this result may also be due to the failure to prevent the gas flow and diffusion inside the poultry housing. It is also apparent that there is a need to place a greater number of systems for determining the impact of the system on the whole area. It can be suggested to use temperature values of around 24-26°C for about 3 week old broiler chicks upon examining the temperature values for the poultry housing (Büyüktaş et.al., 2016). It was determined that these values have been met upon an examination of the measured temperature values. In addition, it is also suggested that the proportional moisture value for the interior of the poultry housing should not be lower than 40 % and higher than 70% (Çelik, 2011). This value should be between 60% and 70% for a good development and feathering (Büyüktaş et al., 2016). An examination of the proportional

moisture values put forth that the suggested values have been met for the animals during the measurement period.

Application 2. Surface area was increased from 1.82 m^2 to 2.27 m^2 thus trying to determine the level of ammonia in order to ensure that the system operates more effectively. The ammonia values and average values determined for the system have been given in Figures 5 and 6.

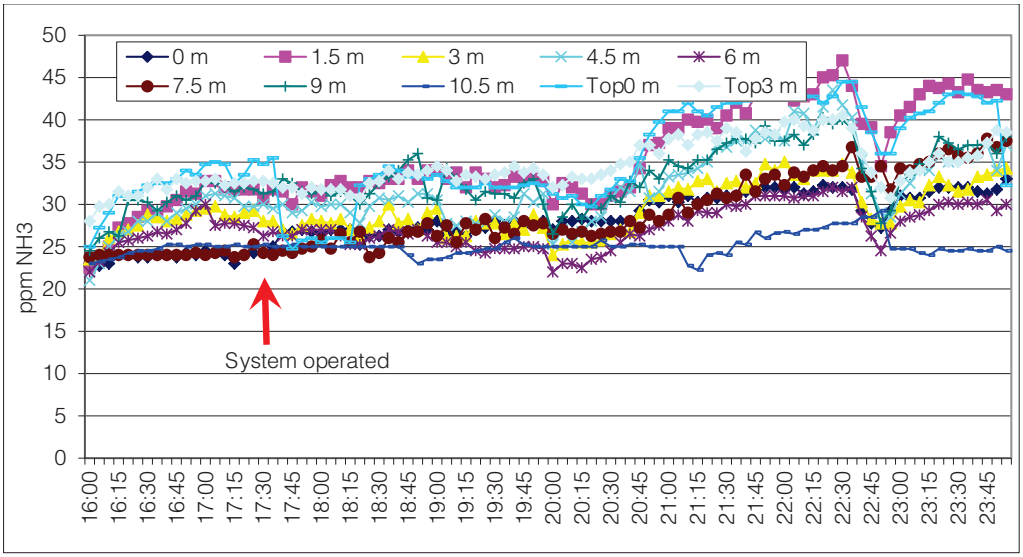

Figure 5. Ammonia values measured by application 2

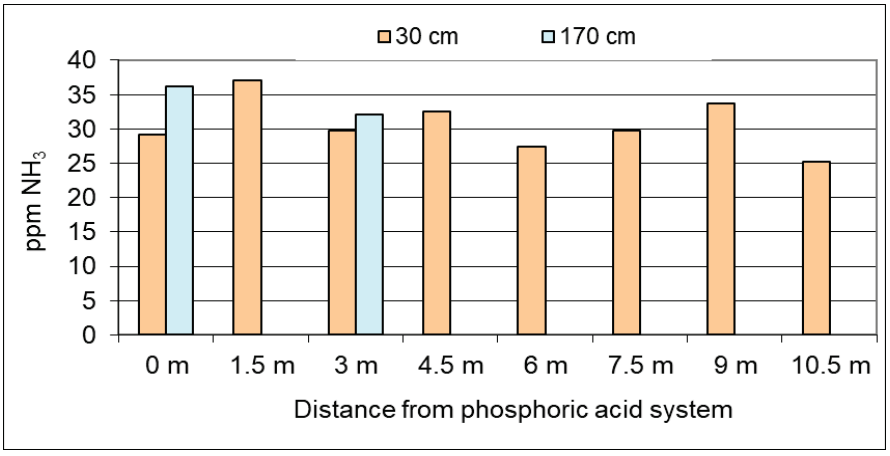

Figure 6. Average ammonia values obtained by application 2

It was observed that the ammonia values inside the poultry housing increased in direct proportion with the manure amount from the animals depending on their development. Because, Ritz et al. (2006) indicate the source of ammonia as excrements of animals left in the environment. Figure 6 indicates that system effectiveness increased with increased surface area and in particular, the sensor at the top 0 m (170 cm) has been found to have reduced ammonia levels at around 10 ppm. However, this decrease was not observed in the 0 m sensor. The decrease in the upper 0 m placed at around human height level took about 1 hour and it was observed that the ammonia values started to increase in the following period due to a rapid release of ammonia resulting from heated litter after the heaters were turned on. It

was observed upon an examination of the average values given in Figure 6 that the decrease in the ammonia values near the system was more distinct in comparison with application 1. This finding is an indication that the development of the system shall be beneficial. Temperature and moisture values for the measurement period were tried to be kept at around the values suggested for the animals in this measurement application.

Application 3. It was observed as a result of an examination of the data acquired during application 2 that the effectiveness of the system inside the poultry housing decreased the ammonia levels but that the decrease was not at the expected level which indicates the necessity of remodifying the system in order to obtain more distinctive results. 300 balls with smaller diameters (4 cm) were added to the system for increasing the total surface area in the system and measurements were continued. The surface area that the air passing through the system gets in contact with was increased from 2.27 m^2 to 3.78 m^2 with the final change in the system. Data acquired from measurements on the final revised version of the system have been given in Figure 7. Averages for the related values have been given in Figure 8.

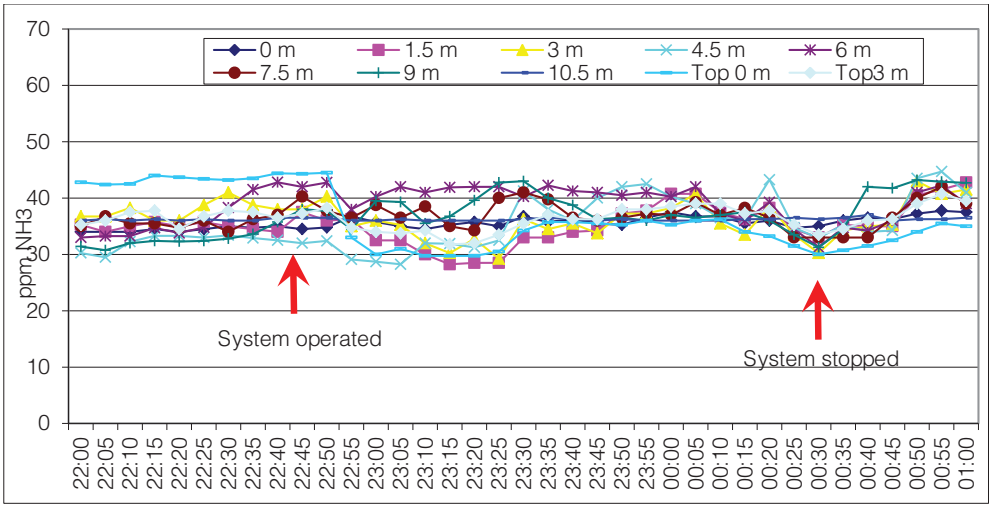

Figure 7. Ammonia values measured by application 3

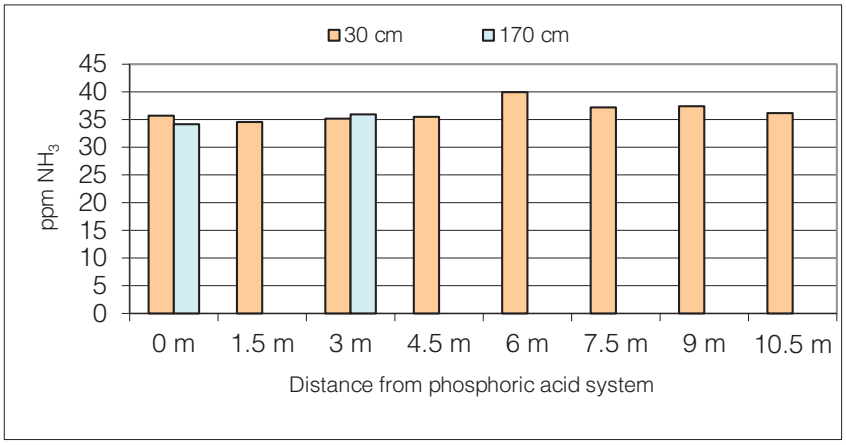

Figure 8. Average ammonia values obtained by application 3

As can be seen from Figure 7, values obtained from the Upper 0 m sensor placed at human height level started decreasing suddenly after the phosphoric acid setup was turned on and the system yielded a decrease from a peak measurement value of 45 ppm to around 30 ppm.

This distinctive decrease could not be attained in other sensors.

The Upper 0 m sensor is located nearest to the outlet of the phosphoric acid system.

Therefore, the ammonia level in this region starts decreasing suddenly once the system is started.

Whereas the other sensors are affected later from the decrease and all sensors indicate a decrease in the ammonia level inside the poultry housing as a function of time after the system is started.

The system was shut down for demonstrating its efficiency since the ammonia level inside the poultry housing did not decrease rapidly after the system was started.

In this scope, the system was shut down at 00:30 after which a tendency to increase was observed in the values.

Thus, it was concluded that the increase of ammonia level inside the poultry housing was prevented during the time that the system was running.

The poultry housing was not ventilated to allow for ammonia levels of 25 ppm during this measurement period.

Therefore, the measurement was started when the average ammonia level reached around 35 ppm.

Ammonia values increase significantly in any closed animal barn if the ambient air is not changed or sufficient ventilation is not provided. Levels of ammonia and other deleterious gases are especially high during winter time due to limited ventilation conditions. Values of around 60 ppm were measured in the poultry housing where the study was carried out.

Development of animals is affected adversely when the ammonia level exceeds the suggested values and live animal gains (Malone, 1985; Atasoy, 2000).

Hence, precautions should be taken for improving the ambient air in poultry housings and to eliminate its adverse effects.

It is important to show that the system is effective as a result of operating in high concentrations of ammonia in the poultry house. The ammonia level in the poultry housing never decreased below 25 ppm during the time period when the third application took place.

The system was operated during this measurement when the ammonia levels were quite high and the change as a function of time was tried to be determined (Figure 9).

Poultry housing ammonia levels decreased significantly right after the system was operated. Decrease was greater and more distinct at the Upper 0 m from among the measurement points. This is due to the fact that the measurement point is close to both the system as well as the system outlet. Similarly, distinctive decreases were recorded by the 0 m sensor which is the closest sensor to the system at the animal level.

The observed decrease stopped after some time and the poultry housing ammonia level was determined to be below 50 ppm.

When the values obtained from the sensors at the human level were examined in Figure 10 depicting the average values, it was observed that the ammonia level was lower at the point closer to the system.

Average ammonia levels inside the poultry housing remained at levels much below the peak value of 60 ppm.

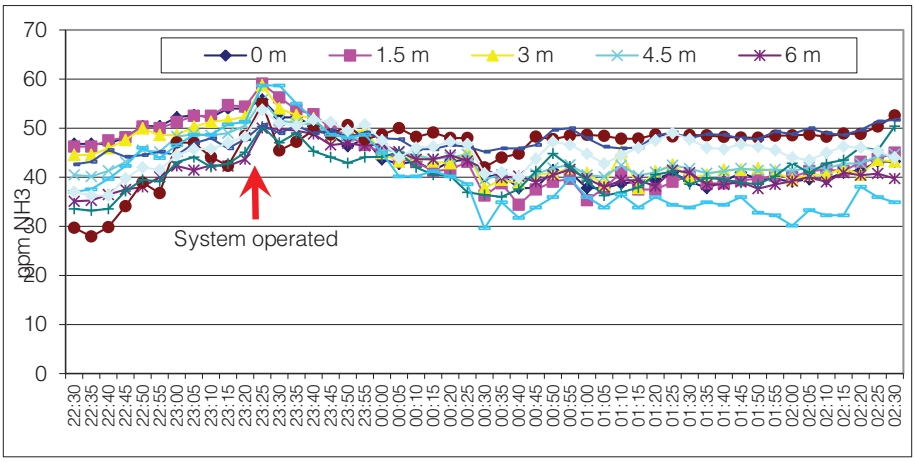

Figure 9. Ammonia values measured by application 3

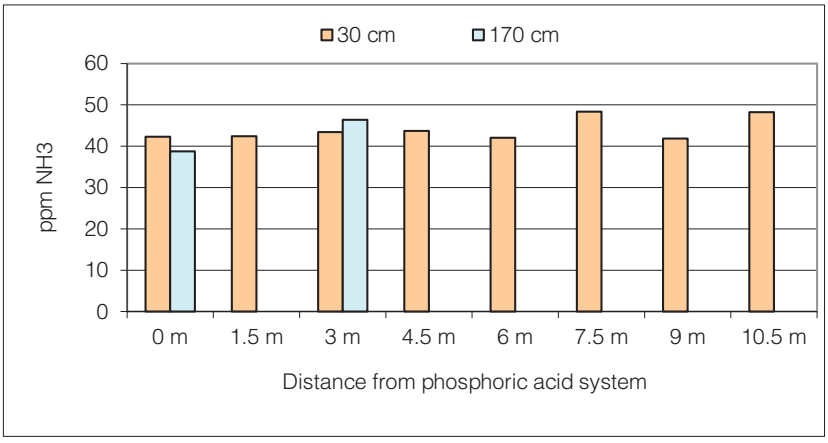

Figure 10. Average ammonia values obtained by application 3

CONCLUSIONS

The phosphoric acid system is designed on the basis of holding the ammonia inside the poultry house by trying different surface area applications. It was concluded based on the values obtained as a result of the study that the phosphoric acid setup used is successful in reducing the ammonia level inside the poultry housing and that a different system setup may be beneficial for ensuring that the system operates more effectively. It was especially concluded that the ammonia level can be reduced to values below the critical value of 25 ppm by placing more than one system inside the poultry housing. It was not possible to place more than one phosphoric acid system in the poultry housing in the study since it was planned to place only one phosphoric acid system. Therefore, system effectiveness was different at each point of the poultry housing. It was observed that the ammonia levels measured by sensors close to the system decreased rapidly. It was thus concluded that the system which is not out in the market yet and which has been tried for the first time is successful and that there is a need to further improve the system in order to increase its effectiveness.

ACKNOWLEDGEMENTS

This research was supported by The Scientific and Technological Research Council of Turkey (TUBITAK) with the Project number 107 O 114.

REFERENCES

Atasoy F., 2000. Tavuk Yetiştiriciliğinde Altlığın Kullanılması ve Önemi, Lalahan Hayvancılık Araştırma Enstitüsü Dergisi, 40(1), 90-97. (In Turkish).

Büyüktaş K., Atılgan A., Tezcan A., 2016. Tarımsal Üretim Yapıları, Süleyman Demirel Üniversitesi Yayınları, Yayın, No.101, ISBN:978-9944-452-98-4, Isparta. (In Turkish).

Choiniere Y., Munroe A.J., 1997. Air Quality inside Livestock Barns, Ministry of Agriculture and Food, AGDEX 400/717, Factsheet, Ontorio İpek A., Karabulut A., Canbolat, Ö., Kalkan, H., 2002. Değişik Altlık Materyalinin Etlik Piliçlerin Verim Özellikleri ve Altlık Nemi Üzerine Etkileri, Uludağ Üniversitesi Ziraat Fakültesi Dergisi, 16(2), 137-147.

Çelik M., 2011. Farklı İklim Koşullarında Enerji Tutumlu Bir Kümesin Yapımı İçin Uygun Yapı Elemanlarının Bilgisayar Yardımıyla Belirlenme Olanakları Üzerinde Bir Çalışma, Yüksek Lisans Tezi, Çukurova Üniversitesi, Tarım Makinaları Anabilim Dalı, 144s, Adana.

İpek A., Karabulut A., Canbolat Ö., Kalkan H., 2002. Değişik Altlık Materyalinin Etlik Piliçlerin Verim Özellikleri ve Altlık Nemi Üzerine Etkileri, Uludağ Üniversitesi Ziraat Fakültesi Dergisi, 16(2), 137-147.

Jones L., Nizam M.S., Reynolds B., Bareham S., Oxley E.R.B., 2013. Upwind impacts of ammonia from an intensive poultry unit. Environ. Pollut., 180, 221-228.

Malone G.W., 1985. A Profile of Ammonia and Select Litter Parameters in Delmarys Broiler Houses, Delmarys Broiler Housing and Flock Supervisers Conference, U.S.A., 16-21.

Nowakowicz-Dębek B., Pawlak H., Maksym P., Wlazło Ł., Bis-Wencel H., Sasáková N., Stasińska B., 2016. Study on the chemical composition of the air in fur animal farms. Przem. Chem., 95(4), 770-772.

Okuroğlu M., 1987. Hayvan Barınaklarında Zararlı Gazlar, Toz ve Etkileri, Et ve Balık Endüstrisi Dergisi, 8(49), 19-24. (In Turkish).

Ritz C.W, Mitchell B.W., Fairchild B.D., Czarick M., Worley J.W., 2006. Improving In-House Air Quality In Broiler Production Facilities Using An Electrostatic Space Charge System, The Journal Of Applied Poultry Research, 15(2), 333–340**.**

Singh A., Casey K.D., King W.D., Pescatore A.J., Gates R.S., Ford M.J., 2009. Efficacy Of Urease Inhibitor To Reduce Ammonia Emission From Poultry Houses. J. Appl. Poult. Res., 18, 34-42.

Wlazlo L., Nowakowicz-Debek B., Kapica J., Kwiecien M., Pawlak H., 2016. Removal Of Ammonia From Poultry Manure By Aluminosilicates. Journal of Environmental Management, 183, 722-725.

Zhang Y., Dore A. J., Ma L., Liu X.J., Ma W.Q., Cape J.N., Zhang F.S., 2010. Agricultural ammonia emissions inventory and spatial distribution in the north China plain Environ. Pollut., 158, 490-501.

Scientific Papers. Series D. Animal Science. Vol. LXI, Number 1, 2018
ISSN 2285-5750; ISSN CD-ROM 2285-5769; ISSN Online 2393-2260; ISSN-L 2285-5750

RESEARCH ON MORPHO-PRODUCTIVE APTITUDES OF A GOAT'S POPULATION OF CARPATHIAN BREED FROM ARGEŞ AREA

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Abstract

The purpose of this paper is to investigate the evolution and situation of Carpathian goat breed population from Argeş County. Carpathian breed is the oldest, most widespread, primitive, rustic, resistant and very heterogeneous breed in terms of morphological characteristics. The biologic material is represented by 25 females and 6 males belonging to Carpathian breed. The livestock presents the following morphological characteristics: live weight (39.03±0.52 kg females and 51.42±0.91 kg males), whiter height (61.11±0.76 cm females and 68.23±0.45 cm males), trunk length (65.57±0.66 cm females and 77.94±0.65 cm males), thoracic width (15.05±0.35 cm females and 18.64±0.4 cm males), thoracic perimeter (66.30±0.57 cm females and 76.82±0.42 cm males), shin-bone perimeter (6.83±0.09 cm females and 8.08±0.15 cm males), resulting a dolicomorphe body type. The average production of milked milk per month is 31.68 kg during May-October and the chemical composition of the goat milk reveals a dry matter content of 13.58%, (fat 4.41%, protein 3.57%), which represents a qualitative goat milk, especially when is taken into account that is a local and rustic breed.

Key words*: Carpathian breed, body measurements, body weight, goat milk.*

INTRODUCTION

Goat breeding is in our country a traditional activity taken into account the large area of natural grassland, spread in all landforms that cannot be operated more efficiently than through sheep and goats breeding.

Goats are animals that are using very well the cheap feed such as grass, roughage and woody plants, are implying relatively low costs compared with other species, and are providing valuable foods for human consumption.

In Romania, goats were considered „the poor's cow", being also a survival niche of the poor families.

Carpathian goat breed is a natural breed that has her origins in the Carpathians Mountains as a descendent of Capra Prisca, being a very robust breed and is spread in the South or Eastern Europe (Mason, 1996).

This breed represents 90% of the entire goat livestock from Romania, being encountered in all the rural areas of the country. From a morphological and productive point of view, the breed shows a great variability, mainly due to the different rearing conditions and the low level of genetically improvement of the populations (Vlad et al., 2009).

The breed is adapted to the local climate, management system and to our type of vegetation with a quite facile and profitable rearing. Carpathian breed can be exploited both for the milk and meat production.

The milk production is small towards medium compared to other breeds, 200-250 litres obtained over a lactation period of 196 days; being a fat milk, with a concentration of 4.5-6.5% fat and in some situations reaching 10%.

The meat has an important nutritional value, being similar to the sheep meat. Goat skin is used in the manufacture of footwear, clothing and leather goods. Through the wide range of products supplied (milk, meat, hides, hair production etc.) goats bring their contribution in covering the needs of the population in terms of high quality animal products and with an affordable price (Taftă, 2010).

MATERIALS AND METHODS

The purpose of this paper was to investigate the main characteristics of the Carpathian breed in

a population represented by 25 females and 6 males, belonging to a private farm from Argeș County.

The main measurements were aimed to highlight the morphological characteristics and productive parameters of this breed. Observations were focused on conformation aspects and primary milk production, especially in terms of quantity and quality.

As regards the body measurements, the focus was on the back height, the oblique body length, thoracic width, thoracic perimeter and shin bone perimeter. The females and males belonging to this population were also weighted.

The following measurements were carried out on our animals:

- The individual weighting of the males and females was carried out with a mechanical weighing machine (precision of ± 1 kg);
- Measurements of length and height were made with the zoometry machine;
- The measurements of width were made with the compass;
- The measurements of the perimeters were made with the tailor's ribbon;
- The thoracic perimeter was measured with the ribbon immediately on the back of the shoulders;
- The perimeter of shinbone was measured with the ribbon at the right anterior leg in the middle of the shinbone;

The following body indexes were used (Furtunescu, 1958):

Index of lateral body format (I_L):

$$I_L = \frac{\text{Oblique trunk length}}{\text{Whiter height}} \times 100$$

Index of transversal body format (I_{BF}):

$$I_{BF} = \frac{\text{Thoracic width}}{\text{Whiter height}} \times 100$$

Index of bone system (I_{BS}):

$$I_{BS} = \frac{\text{Shinbone perimeter}}{\text{Thoracic perimeter}} \times 100$$

Indicator of massiveness (I_M):

$$I_M = \frac{\text{Thoracic perimeter}}{\text{Whiter length}} \times 100$$

For milk production, the research was based on the information's obtained from the official control of production (OCP). The fat and protein content were analyzed, using the LACTOSCOPE equipment with infrared technology.

RESULTS AND DISCUSSIONS

The biologic material is represented by 25 females and 6 males belonging to Carpathian goat breed. It was analysed throughout productive performances in the actual condition of exploitation.

Body weight. The research results are as follows: the average females weight 39.03 ± 0.52 kg and the average males weight 51.42 ± 0.91 kg are values that are common for this breed and within the range of values reported by other authors for this breed (Taftă, 2002) (Table 1).

Table 1. Body weight of Carpathian goat breed population

Gender Category	n	$X \pm s_x$	S	V%
Females	25	39.03 ± 0.52	2.62	6.71
Males	6	51.42 ± 0.91	2.22	4.32

Somatometric measurements. The aim of the body size measurements, was to highlight the corporal conformation both for females and males: whiter height 61.11 ± 0.76 cm for females and 68.23 ± 0.45 cm for males; oblique trunk length 65.58 ± 0.69 cm for females and 77.93 ± 0.65 cm for males; thoracic width 15.76 ± 0.35 cm for females and 18.64 ± 0.43 cm for males; thoracic perimeter 66.30 ± 0,57 cm for females and 76.82 ± 0.42 cm for males; shinbone perimeter 7.03 ± 0,04 cm for females and 8.08 ± 0.15 cm for males.

All this measurements are the most important characters, with a large grade of variability, representing the morphological body type and offers us the opportunity to calculate some important indexes.

The body measurement results show that the analysed animals belong to a low-medium size category of this breed, more suited for the milk production.

The values are almost similar with those from other authors Priseceanu H. et al., 2015.

In order to assess the proportionality and the development harmony of the different body regions or parts, as well as of the productive capabilities assessment, based on the body measurements performed, some body indexes have been calculated, which represent the ratio between two dimensions morpho-physiologicaly correlated (Table 2).

The lateral body format index values, expressed by the trunk length value reported to the wither height and the transversal body format index, obtained by reporting the thoracic width to the whiter width, show that the animals fall into the dolichomorphe body type, which is body suited for milk production (Călin, 2004).

Also, the bone system index, with a value greater than 10%, indicates a population with well-developed bones, belonging to the morpho-productive type for milk.

The massiveness index shows that these animals have a body development characteristic to the milk production type, with a relatively low massiveness (Table 2).

Table 2. Body indexes

Crt. no.	Specification	Value (%)
Females		
1.	Index of lateral body format	107.30
2.	Index of transversal body format	25.78
3.	Index of bone system	10,06
4.	Index of massiveness	124.83
Males		
1.	Index of lateral body format	114.23
2.	Index of transversal body format	27.31
3.	Index of bone system	10.52
4.	Index of massiveness	112.59

Milk production. According to the classical principles the milking is executed using normal milking technique, taking into account more or less the hygienic principles.

The average milk production was 31.68 kg per month. During May-October period, the average milk production was amounting to 190.12 kg, as it is shown in Table 3.

Table 3. The milk production

Crt. no.	Lactation month	Argeş Farm	
		kg	% by total
1.	May	38.94	20.48
2.	June	41.63	21.89
3.	July	38.07	20.02
4.	August	30.65	16.12
5.	September	25.39	13.35
6.	October	15.44	8.14
7.	Total milked production	190.12	
8	Total milk/lactation/ simple parturition (kg)	220.87	
9.	Total milk/lactation/twin parturition (kg)	238.28	
10.	Average milked milk production /month (kg)	31.68	
11.	Lactation period (days)	196.27	

It has to be taken into consideration that the milk consumed by kids, obtained both from single or twin parturition, was not included and it is shown separately (Table 4).

Table 4. The amount of milk in suckling period depending on the type of birth

Crt no.	Specification	MU	Parturition type	Argeş Farm
1.	Milk consumed by kids in the 0-28 days period	kg	Simple	14.23
			Twin	21.37
2.	Milk consumed by kids in the 28 days - 2 months period	kg	Simple	16.52
			Twin	26.79
3.	Total milk consumed by kids from simple parturition 0-2 months	kg	-	30.75
4.	Total milk consumed by kids from twin parturition 0-2 months	kg	-	48.16

The lactation curve, in the case of this breed, must decrease slowly, after the sixth or seventh month of lactation (Figure 1).

As it can be observed in Figure 1, the lactation reveals the medium potential of this population as regards the milk production, which can be caused by a poor management or a lack of knowledge in dairy goat exploitation and a poor nutrition.

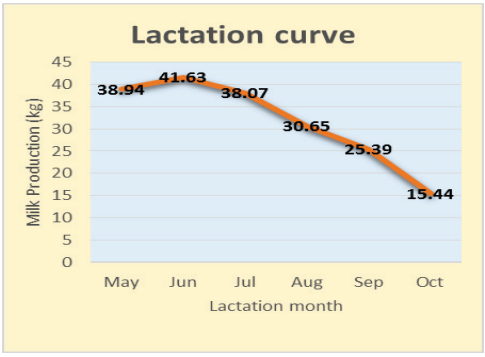

Figure 1. Lactation curve

The chemical composition during the lactation period is represented as follows: dry matter 13.58%, out of which: fat 4.41% and protein 3.57%, representing a qualitative production (Table 5).

Table 5. Chemical composition of milk

Specification	Argeş Farm
Water (%)	86.42
Dry matter (%)	13.58
Fat (%)	4.41
Protein (%)	3.57
Lactose (%)	4.69
Minerals (%)	0.91

From the analyses which has been carried out it is evident that the composition of milk fat in the cow milk and the one from goat milk are not significantly different, with the exception of the last month of lactation when the fat content has increased considerably (Table 6). According to our data, it can be observed that compared to the milk production, which is decreasing, the fat and protein content are increasing until the last month of lactation.

Table 6. Evolution of milk fat content

Crt. no.	Lactation month	Argeş Farm	
		%	kg fat/month
1.	May	3.47	1.35
2.	June	3.69	1.53
3.	July	3.84	1.46
4.	August	4.56	1.40
5.	September	4.83	1.23
6.	October	6.08	0.94
7.	**Average**	**4.41**	**7.91**

Our research reveals a normal curve for the fat content during the 6 months of milking that is increasing in each month of the lactation (Figure 2).

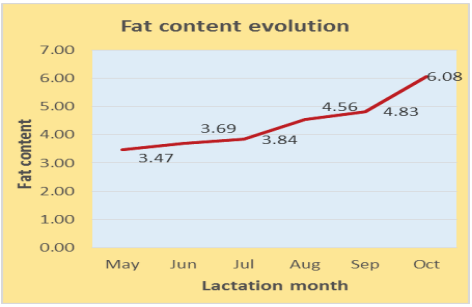

Figure 2. Fat content evolution

The same principle can be applied also for the protein content regarding the increasing trend and with values that are similar to the ones reported by other authors for this breed (Vlad I. et al., 2009) (Table 7).

Table 7. Evolution of protein content

Crt. no.	Lactation month	Argeş Farm	
		%	kg protein/month
1.	May	2.96	1.15
2.	June	3.26	1.36
3.	July	3.36	1.28
4.	August	3.58	1.10
5.	September	3.96	1.01
6.	October	4.29	0.66
7.	**Average**	**3.57**	**6.56**

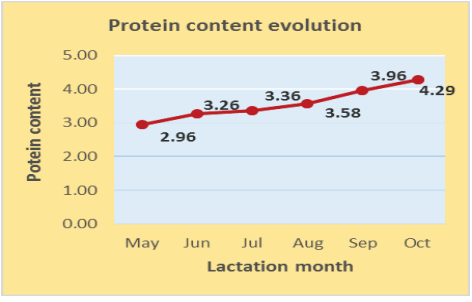

Figure 3. Protein content evolution

Pădeanu (2002), suggested that compared to sheep milk which has a higher dry matter content, goat milk has less dry mater and a lower protein and fat percentage.

CONCLUSIONS

The lateral body format index values, expressed by the trunk length value reported to the wither height and the transversal body format index, obtained by reporting the thoracic width to the whiter width, show that the animals fall into the dolichomorphe body type, which is a body suited for milk production.

The bone system index, with a value greater than 10%, indicates a population with well-developed bones, belonging to the morpho-productive type for milk. The massiveness index shows that these animals have a body development characteristic to the milk production type, with a relatively low massiveness.

The morphological values are common for this breed and within the range of values reported by other authors for these breeds.

The average production of milked milk, without the milk used for kids, is 190.12 kg, which reveals the medium potential of this population as regards milk production. As regards the fat and protein content, the research shows a normal curve during the 6 months of milking that are increasing in each month, until the end of lactation.

As a final conclusion it can be concluded that the values from this research are almost similar to those reported by other authors for the Carpathian breed.

REFERENCES

Călin I., 2004. Technology of rising sheep and goats. University ”Lucian Blaga” Publishing House, Sibiu, 72-81.

Furtunescu Al., 1958. General Animal Breeding. Ministry of Agriculture and Forestry State Agro-Forestry Publishing House. Bucharest, Vol. I, 146.

Mason I.L., 1996. A World Dictionary of Livestock Breeds, Types and Varieties. Fourth Edition. C.A.B International, 273.

Pădeanu I., 2002. Sheep and goat production. Mirton Publishing House, Timisoara, 400-409.

Priseceanu H., Călin I., Tăpăloagă D., Răducuță I., Tăpăloagă P., 2015. Researches regarding morphologic features in some goat populations from the south of Romania. The 35th International Scientific Session of livestock, Bucharest, 405-409.

Vlad I., Maftei M., Marmandiu A., Stanciu M., 2009. Research of Carpathian goat breed productive performances in the south-eastern part of the country. Scientific Symposium Environmental Protection and Food Safety, Priorities and Perspective, ISSN 2067-1989, Targoviste, 417-421.

Taftă V., 2002. Production and reproduction of goats, Ceres Publishing, Bucharest, pp. 73-98.

Taftă V., 2010. Sheep and goats breeding. Ceres Publishing House, 20-24.

Scientific Papers. Series D. Animal Science. Vol. LXI, Number 1, 2018
ISSN 2285-5750; ISSN CD-ROM 2285-5769; ISSN Online 2393-2260; ISSN-L 2285-5750

RESEARCH ON THE INFLUENCE OF SOIL AMENDMENTS AND LIVESTOCK MANAGEMENT ON SURFACE WATER QUALITY FROM TELEORMAN COUNTY

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Abstract

These studies are the point to the proposal of solutions to minimize the risk of methemoglobinemia in infants or of other diseases or chronic fluid in adults, which may be caused by the presence of these nutrients in the waters described. The research has been done in 3villages (Măldăieni, Roşiori de Vede, Peretu). For each research site, based on soil, surface/wells and manure samples were determined: the type and soil texture and supply status with major nutrients, soil reaction, fertilizers and pesticides, surface/wells quality, quantities and quality of manure from fresh and fermented samples. The samples were taken of surface water and from the well simultaneously. Surface water quality varies from class II quality close to class III. Water fountain concentrations of nitrogen forms, in all samples analyzed, were very close or exceed of maximum admissible values. Testing the significance of differences observed between the average values of the samples from the sites of research was done with the Student test for each analyzed compartment.

Key words*: amendments, soil structure, surface and wells water.*

INTRODUCTION

Methaemoglobinaemia is diagnosed when the percentage of methemoglobin exceeds 1% of the normal hemoglobin in which the ferric ion was ionized in the heme group to the ferric ion. (Tudor, 2009). Methaemoglobinaemia is characterized by the inability to bind oxygen to hemoglobin with functional anemia and lack of oxygen release to tissues. The classic presentation of methemoglobin is cyanosis in the presence of normal pulmonary oxygen pressure, with chocolate cholesterol that does not become pink by exposure to oxygen (Costache, 2016). Symptoms are proportional to the methemoglobin level and include skin color changes and blood color changes at levels up to 15% (Denshaw-Burke, 2017). As levels rise above 15%, neurologic and cardiac symptoms arise as a consequence of hypoxia. Levels higher than 70% are usually fatal. The application of excess chemical fertilizers in this area combined with poor manure management and lack of sewerage leads to a load of nitrates in surface and fountain water with negative effects on the health of the consuming population (Chirilă, 2012).Infants are most affected by drinking nitrate rich water. Although the maximum admissible limit is 50 mg/l, pediatricians recommend a maximum concentration of 25 mg/l or a safe source of water. Considering the complexity of the issues that the topic involves, in the present study the concentrations of different forms of nitrogen in the surface water and the fountain water (if there are nutrients) were determined in order to assess their vulnerability to pollution with nitrates or nitrites. These studies have been corroborated with soil analyzes, with quantitative and qualitative assessments of manure used as natural fertilizer or those derived from free-range cattle on herds, in order to minimize the risk of methaemoglobinaemia in young infants or other waterborne or chronic diseases at adults, which could be caused by the presence of these nutrients in the abovementioned waters.

MATERIALS AND METHODS

For the present study, a designated area was chosen from Teleorman County, comprising 3 localities that are part of the Vedea catchment area. These are: Măldăieni, Roşiori de Vede and Peretu. In all localities, sewerage and drinking water supply are poor, but for all, communal dwellings are used for grazing cattle.

The research has been done in the whole locality, and took soil samples to determine the type and soil texture and soil supply status with major nutrients (N, P, K). Based on these results and knowing the types of main crops and livestock structure of economic, at villages level, recommendations were made about avoiding the risks of pollution of surface water by nitrates from agricultural and livestock activities. The data on the distribution of the current sources of pollution of the surface waters with manure from the agro-zootechnical sector and especially from cattle were obtained on the basis of direct observations and data from the County Office for Pedology and Agrochemistry.

The structure of the exploited animals, by age and species category, but especially of the bovine species, is presented synthetically, for all three localities, as well as the recommended amendments (the relief, structure, texture and soil reaction are similar).

Working methodology for surface and well water samples: sampling was done using quantitative methods. The conservation and processing of water was performed using STAS methods. For each operational site samples of surface water were taken from 3 regions of the commune, respectively from the upstream side - the part located at the entrance of the communal water, upstream, at the exit of the river from the commune (downstream) and from the central part of the commune, on the water line. Fountain water was sampled from wells located in the city, near the surface water harvesting points.

Analysis of NH_4^+ ion. The "indophenol blue" reaction was used to determine the ammonium ion. The principle of the method is that the phenol reacts with ammonia in the presence of an oxidizing agent (such as sodium hypochlorite, sodium isocyanurate) and forms, under alkaline conditions, a colored compound absorbing the radiation at l = 660 nm.

Analysis of the NO_3^- ion. For the determination of the nitrate ion, the spectrophotometric method using the salicylic acid chromogenic agent was used. Although this method is time-consuming and subjected to interference from organic matter and nitrite ion, for samples of water analyzed, it has yielded good results due to its low content in interfering compounds.

Analysis of NO_2^- ion. Principle of the method: nitrite reacts with sulphanilic acid in the acidic environment resulting diazonium salt coupled with 2 naphthylamine to form a red colored azo compound.

Harvesting of manure samples was: for the chemical analysis of solid slurry, 10 individual samples were taken from each site surveyed. In the case of these solid fertilizers, the individual samples mixed to obtain an average sample, the values obtained from the mean sample analysis characterizing the chemical composition of the entire batch of fertilizer from which it was sampled (Budoi, 2000). For the chemical determinations of the chemical composition of manure from dairy farms as well as those from individual households, the content of ammoniacal nitrogen, nitric nitrogen and total nitrogen was determined. Total nitrogen determinations were made using the Kjeldahl method, for fertilizers known to have a low nitrate content and the Cope modified Kjeldahl method for nitrate fertilizers.

Description of operational sites

From the point of view of the surface waters, on the Măldăieni cadastral territory there are no flowing waters with a permanent flow. The depth of the ground water varies depending on the relief, thus, on the plain and slopes over 10 m, in valleys deeper at 1.5-6.0 m.

Of the total surface (7284.53 ha), 7089.31 ha are occupied by agricultural land, 68.17 ha pastures, 114.16 hectares of vineyards and 12.89 ha of unproductive land. The area cultivated with cereal grains is 3872 ha. As far as the application of fertilizers is concerned, 890 t have been applied at the level of Măldăieni commune. Of the 890 t, 608 t were nitrogenous fertilizers, 281 t of phosphate and 2 t of potash. No natural fertilizers were applied. Of the pesticides, 5 kg of insecticides, 64 kg of fungicides and 182 kg of herbicides were applied, of which 222 kg for maize.

In Măldăieni there are 395 cattle, of which 369 cows and heifers and 26 calves 0-6 months, which are raised in the households, but they also use the communal settlement for early spring free stabbing until late autumn.

The territory of Roşiori de Vede belongs to the water catchment area of the Vedea River with variable flow (very low in summer and higher spring when snow melts), good water for irrigation and drinking for animals. The groundwater level ranges from one relief to the other, being lower in the plain (> 10 m) at shallow depth in the meadow (3-5 m on flat forms and 1.5-3 m on depression forms). Of the total surface area (5902 ha), 5407.51 ha are occupied by agricultural land, 416.18 ha, pastures, 26.33 ha of vines, 10.12 ha of orchards and 41.86 ha of unproductive land. At the level of Roşiori de Vede, the area cultivated with cereal grains is 3564 ha. As far as the application of fertilizers is concerned, 798 t have been applied at the level of Roşiori de Vede on an area of 7513 ha. Of the 798 t, 596 t were nitrogenous fertilizers and 201 t phosphate. No natural fertilizers were applied. Also, 1266 kg insecticides, 2659 kg fungicides and 2557 kg herbicides were applied, of which 241 kg for maize. There are 630 cattle in this locality, of which 458 cows and heifers and 145 calves 0-6 months, which are raised in the households of the population, but they also use the communal harbor for early free spring stabbing and until late autumn. Within the cartographic territory of Peretu, the hydrographic network has the shape of a tree, by the affluence of the tributary network, being characteristic of a region with a flat structure.

For the whole meadow, the water of these rivers plays the role of regulator of the ground water table, which varies from 1.5 to 4 m. Of the total surface area (6207 ha), 6114.37 ha are occupied by agricultural land, 44.48 ha, pastures, 48.08 hectares of vineyards and 0.07 ha of meadows. At the level of Peretu commune, the area cultivated with cereal grains is 3784 ha cultivated. In this locality there are 211 cattle, out of which 180 cows and heifers and 31 calves 0-6 months, which are grown in the households of the population, but they also use the communal settlement for early free spring stabbing until late autumn. Among the wheat grain species in all localities, corn, wheat, durum wheat, barley, barley and spring and autumn oats are grown, and as oily plants, sunflower and rape. Vegetables, as well as fodder, are mostly grown in their own grazing, on relatively small surfaces, and those for the seed are grown only by commercial companies.

RESULTS AND DISCUSSIONS

Results on soil reaction and nutrient status

In Măldăieni locality, the soil reactivity - is strongly and moderately acidic - on 51% of the area (3726 ha), surface to be adjusted with calcium carbonate ($CaCO_3$) - 4 t/ha, to correct the reaction; weakly acidic and neutral on 47% of the area (3426 ha) - favorable for the growth and development of plants; slightly alkaline - on 2% of the surface (150 ha), surface to be treated with phosphogypsum - 6 t/ha, to correct the reaction. The state of supply of assimilable phosphorous soils (P mobile) - is considered to be poorly supplied on 10% of the area (728 ha), on average 25% of the area (1821 ha) and well and very well supplied on 65% of the area (4734 ha).The state of supply of the soils with assimilable potassium (K mobile) - considered medium on 10% of the surface (728 ha) and good and very good on 90% of the area (6556 ha).The state of natural nitrogen fertility - is poorly supplied on 22% of the area (1602 ha), on average 78% of the area (5682ha).

In the Roşiori de Vede locality, the soil reaction - strongly and moderately acidic - on 45% of the surface (2656 ha), surface to be fined with calcium carbonate ($CaCO_3$) - 4 t/ha, to correct the reaction; weakly acidic and neutral on 51% of the area (3010 ha) - favorable to plant growth and development; slightly alkaline - on 4% of the surface (236 ha), surface to be treated with phosphogypsum - 6 t/ha, to correct the reaction. The state of supply of soils with assimilable phosphorus (P mobile) - is considered to be poorly supplied on 33% of the area (1948 ha), on 40% of the surface (2361 ha) and well and very well supplied on 27% of the area (1593 ha). The state of supply of soils with assimilable potassium (K mobile) - considered medium on 12% of the area (708 ha) and good and very good on 88% of the area (5194 ha). The state of natural nitrogen fertility - is poorly supplied on 58% of the area (3423 ha), on average 42% of the area (2479 ha). The state of supply of soils with assimilable phosphorus (P mobile) - is considered to be poorly supplied on 33% of the area (1948 ha), on 40% of the surface (2361 ha) and well and very well supplied on 27% of the area (1593 ha).

In Peretu, the soil reaction - is moderately acidic - on 3% of the surface (187 ha), surface

to be fined with calcium carbonate ($CaCO_3$) - 4 t/ha, to correct the reaction; slightly acidic and neutral on 97% of the area (6020 ha) - a favorable reaction to the growth and development of plants.

The state of supply of soils with assimilable phosphorus (P mobile) - is considered poorly supplied on 27% of the surface (1676 ha), 47% of the area (2917 ha) and well supplied with 26% of the area (1614ha).The state of supply of soils with assimilable potassium (K mobile) - considered medium on 36% of the area (2234 ha) and good and very good on 64% of the area (3973 ha). The state of natural nitrogen fertility - is poorly supplied on 40% of the area (2482 ha), on average 58% of the area (3600 ha) and very good on 2% (125 ha).

Results of surface and well water samples.

In the determinations made in the study, the concentrations of the biogenic elements: ammonium, nitrates, nitrites, phosphorus, were reported at the maximum admissible concentrations accepted by the legislation in force (Law 311/28.06.2004 and Law 458/2002) fountain and the specifications in Order 161/16.02.2006 regarding the quality of surface water. These concentrations are summarized in Table 1.

Table 1. Maximum admissible concentrations

Indicator	M.U.	Well water	Surface water class quality I	II	III	IV
$N\text{-}NH_4$	mg N/l	0.5	0.4	0.8	1.2	3.2
$N\text{-}NO_2$	mg N/l	0.15	0.01	0.03	0.06	0.3
$N\text{-}NO_3$	mg N/l	11.2	1	3	5.6	11.2

The results of the chemical analyzes for surface and well water from operational site 1 are shown in Table 2.

Table 2. Calculated statistic valuables for operational site 1

	Surface water			Well's water		
$N\text{-}NH_4$ (mg N/l) n=3	**Upstream**	**Middle**	**Downstream**	**Upstream**	**Middle**	**Downstream**
X	0.84	0.846667	0.853333	0.416667	0.406667	0.423333
Sx	0.005774	0.003333	0.006667	0.006667	0.012019	0.003333
s	0.01	0.005774	0.011547	0.011547	0.020817	0.005774
c.v.%	1.190476	0.68191	1.353165	2.771281	5.118851	1.36382
$N\text{-}NO_2$ (mg N/l) n=3	**Upstream**	**Middle**	**Downstream**	**Upstream**	**Middle**	**Downstream**
X	0.029667	0.029667	0.030667	0.153667	0.15	0.152333
Sx	0.000667	0.001202	0.001202	0.000333	0.000577	0.000667
s	0.001155	0.002082	0.002082	0.000577	0.001	0.001155
c.v.%	3.892249	7.016852	6.788041	0.375716	0.666667	0.758009
$N\text{-}NO_3$ (mg N/l) n=3	**Upstream**	**Middle**	**Downstream**	**Upstream**	**Middle**	**Downstream**
X	3.44	3.353333	3.45	9.996667	9.89	9.98
Sx	0.028868	0.052387	0.026458	0.074461	0.055076	0.095394
s	0.05	0.090738	0.045826	0.12897	0.095394	0.165227
c.v.%	1.453488	2.705896	1.328283	1.290133	0.964549	1.655582

There is a slight increase in ammoniacal nitrogen concentration in surface waters, but these concentrations do not change the class of river segment quality (2^{nd} class). For fountain water, the concentration of this parameter is within normal limits, but it is notice that the downstream concentrations are higher than upstream determination values, most probably due to diffuse source of pollution ($N\text{-}NH_4$). In case of $N\text{-}NO_3$ and $N\text{-}NO_2$ ions can be observed a decrease of concentration from upstream to downstream. The results of surface and well water chemical analyzes from operational site 2 are shown in Table 3.

For surface water, the recorded values of the parameters analyzed fall within the CMA for Class II. The results of the chemical analyzes for surface water and wells from operational site 3 are shown in Table 4.

For the first operational site, surface water falls in the second category of quality and well water parameters are within the maximum limits. If operational sites 2 and 3 class surface water remains unchanged, although a slight increase of the concentration parameters from one site to another.

Table 3. Calculated statistic valuables for operational site 2

	Surface water			Well's water		
N-NH_4 (mg N/l) n=3	**Upstream**	**Middle**	**Downstream**	**Upstream**	**Middle**	**Downstream**
X	0.8698	0.853333	0.8623	0.423333	0.433333	0.436667
Sx	0.005774	0.003333	0.005774	0.003333	0.003333	0.003333
s	0.013	0.005774	0.011	0.005774	0.005774	0.005774
c.v.%	1.162791	0.676582	1.162791	1.36382	1.332347	1.322176
N-NO_2 (mg N/l) n=3	**Upstream**	**Middle**	**Downstream**	**Upstream**	**Middle**	**Downstream**
X	0.030667	0.030667	0.031333	0.154	0.153	0.154
Sx	0.001202	0.001667	0.000882	0.000577	0.000577	0.000577
s	0.002082	0.002887	0.001528	0.001	0.001	0.001
c.v.%	6.788041	9.41332	4.875081	0.649351	0.653595	0.649351
N-NO_3 (mg N/l) n=3	**Upstream**	**Middle**	**Downstream**	**Upstream**	**Middle**	**Downstream**
X	3.46	3.436667	3.463333	9.98	9.973333	10.14667
Sx	0.020817	0.003333	0.020276	0.095394	0.103976	0.079652
s	0.036056	0.005774	0.035119	0.165227	0.180093	0.137961
c.v.%	1.042067	0.167997	1.014019	1.655582	1.805741	1.359672

Table 4. Calculated statistic valuables for operational site 3

	Surface water			Well's water		
N-NH_4 (mg N/l) n=3	**Upstream**	**Middle**	**Downstream**	**Upstream**	**Middle**	**Downstream**
X	0.86	0.86	0.866667	0.44	0.446667	0.45
Sx	0.005774	0.005774	0.003333	0.005774	0.003333	0.003333
s	0.01	0.01	0.001774	0.01	0.005774	0.005774
c.v.%	1.162791	1.162791	0.666173	2.272727	1.292575	1.322176
N-NO_2 (mg N/l) n=3	**Upstream**	**Middle**	**Downstream**	**Upstream**	**Middle**	**Downstream**
X	0.031333	0.031	0.033	0.154	0.153667	0.154667
Sx	0.000882	0.001528	0.000577	0.000577	0.000333	0.000333
s	0.001528	0.002646	0.001	0.001	0.000577	0.000577
c.v.%	4.875081	8.534682	3.030303	0.649351	0.375716	0.373287
N-NO_3 (mg N/l) n=3	**Upstream**	**Middle**	**Downstream**	**Upstream**	**Middle**	**Downstream**
X	3.463333	3.466667	3.476667	10.14667	10.09667	10.16
Sx	0.020276	0.008819	0.023333	0.078811	0.054874	0.086217
s	0.035119	0.015275	0.040415	0.136504	0.095044	0.149332
c.v.%	1.014019	0.440632	1.16245	1.345308	0.941339	1.469802

Table 5. Testing the significance of observed differences between the mean values of nitrogen in surface water and fountain water ($t_{4;0.05}$=2.776; $t_{4;0.01}$=4.604; $t_{4;0.001}$=8.610; $t_{4;0.2}$=1.533)

N-NH_4	Surface water									Well water								
	Upstream			Middle			Downstream			Upstream			Middle			Downstream		
Spec.	S1	S2	S3	S1	S2	S3	S1	S2	S3	S1	S2	S3	S1	S2	S3	S1	S2	S3
S1	-	2.45	2.45	-	1.41	1.41	-	0.76	0.76	-	0.89	2.68	-	2.14	2.14	-	2.83*	2.83*
S2		-	0		-	0		-	0		-	2.83*		-	0		-	0
S3			-			-			-			-			-			-
N-NO_2	Surface water									Well water								
	Upstream			Middle			Downstream			Upstream			Middle			Downstream		
Spec.	S1	S2	S3	S1	S2	S3	S1	S2	S3	S1	S2	S3	S1	S2	S3	S1	S2	S3
S1	-	0.73	1.51	-	0.49	0.49	-	0.45	0.45	-	0.5	0.5	-	3.67*	3.67*	-	1.89	1.89
S2		-	0.45		-	0		-	0		-	0		-	0		-	0
S3			-			-			-			-			-			-
N-NO_3	Surface water									Well water								
	Upstream			Middle			Downstream			Upstream			Middle			Downstream		
Spec.	S1	S2	S3	S1	S2	S3	S1	S2	S3	S1	S2	S3	S1	S2	S3	S1	S2	S3
S1	-	0.56	0.66	-	1.59	1.59	-	0.4	0.4	-	0.14	1.38	-	0.71	0.71	-	1.34	1.34
S2		-	0.11		-	0		-	0		-	1.34		-	0		-	0
S3			-			-			-			-			-			-

Regarding the analysis of well water, for the first operational site, the values are close to the maximum allowable, but at sites 2 and 3, increases in these concentrations upstream and downstream concentrations exceeding the maximum admissible even in the case of nitrites. These observations are supported and conducted statistical tests (Student). Note the significant differences in the concentrations of ammonia nitrogen in water fountain between all operational sites, both on the upstream and downstream sector. In case of nitrites values Student test detected significant differences between S1 site and sites 2 and 3 in the middle.
In ecology, practice higher values of α (20%) to minimize the probability of degradation observed when it exists. Such an event affects not just a few individuals, but an entire community or the entire life of a community, so this strategy is the right solution (Dragomirescu, 1998). Using this level of significance, slight differences between the concentrations of ammonia nitrogen and nitrates, between first site and the other two sites, in the case of the surface water and between the concentration of ammonia nitrogen and nitrite, in the case of well water.

Results of slurry analysis.
Cattle manure is the cheapest and most complex fertilizer for soil fertilization. Bovine manure improves soil structure, promotes propagation of useful microorganisms and activity, increases the water retention capacity of the soil, causing large increases the yield per hectare (Georgescu, 1983).
For the quantitative assessment of manure, the following parameters were considered as the basis of calculation (Georgescu, 1983):
average weight of a dairy cow - 550 kg;
- feces per day and head - 25 kg;
- kg of urine per day and per head - 13 kg;
- feces kg + urine per day and per head - 38 kg;
- complex fecal density + urine - 0.8 kg/l;
- volume of feces + urine per day and head - 0.0475 m^3 (Tables 6, 7).
In fresh manure, the concentrations of nitrogen-containing forms ($N\text{-}NH_4$, $N\text{-}NO_3$) are lower than in manure, and in nitrogenous debris nitrogen losses due to oxidation-reduction processes occur in the mass manure during the fermentation process.

Table 6. The amount of solid and liquid manure obtained for all operational sites from cows and heifers

Site	Dairy cows	Solid manure (kg)	Liquid manure (l)	Solid manure (kg)	Liquid manure (l)
		24 hours		1 year (average 180 days in shelter)	
1	369	9225	4797	1660500	863460
2	458	11450	5954	2061000	1071720
3	180	4500	2340	810000	421200

Table 7. The amount of solid and liquid manure obtained for all operational sites from calves

Site	Calves 0-6 months	Solid manure (kg)	Liquid manure (l)	Solid manure (kg)	Liquid manure (l)
		24 hours		1 year (average 90 days in shelter)	
1	26	312	130	28080	11700
2	145	1740	725	156600	65250
3	31	372	155	33480	13950

In the fresh manure, concentrations of the relevant types of nitrogen ($N\text{-}NH_4$, $N\text{-}NO_3$) is smaller than in the case of manure, and the normal semifermented records the loss of nitrogen due to of oxidation-reduction to occur in the manure mass during fermentation.
This explains the phenomena occurring biodegradable waste mass in the fermentation process when microorganisms work is very intense, so the rate of nitrification phenomena - denitrification (Man, 1989).
For each site, the results were collated and solutions and recommendations, aimed at compliance with: storage accordance manure on concrete platforms with surfaces and storage capacity sufficient to maximum surface fertilization with manure within the limit of 170 kg nitrogen/hectare, periodic evaluation of the status of soil fertility, avoid use well water in infants and vulnerable (in sites which surpassed the maximum allowable concentrations), recommendations for use of public water supply and connection to sewer .

CONCLUSIONS

From the circumstances described in the sites in which the activity of the locals (growth of animals does not comply with the EU regulations, the use of pasture, the improper use

manure, vegetable intensive and empirical in terms of technology, etc.) explain these concentrations and the presence of nitrite in water fountain.

Along with the lack of technology in households and a better information regarding the hazard of nitrates in the water fountain, the social component also has a high share of pollution of surface water because sewage ineffective or lack thereof, and because refusal of the population to be connected to the public water supply and sanitation.

ACKNOWLEDGEMENTS

This research work was carried out with the support of Ministry of Agriculture and Rural Development, from Project ADER 8.1.1.

REFERENCES

Budoi Gh., 2000. Agrochemistry, vol I, II, Didactic and Pedagogical Publishing House, Bucharest, 57-63.

Chirila P., 2012. Don't feed the cancer. Christiana Publishing House, Bucharest, 14-15.

Cristea R., 2009. All about nitrates, nitrites and amonia in children feeding (www. Qbebe.ro).

Costache I., 2016. Nitrite poisoning in infants (pediatricblog.info/2016).

Denshaw-Burke M., 2017. Methemoglobinemia. Emedicine.medscape.com.

Dragomirescu L., 1998. Biostatistics for beginners Constellations Publishing House, Bucharest, 149-150.

Georgescu Gh., 1983. Cattle exploitation Book, vol. I, Ceres Publishing House, Bucharest, 214-220.

Man C., 1989. Water, health and animal production, Ceres Publishing House, Bucharest, 79-85.

Tudor Anca, 2009. Childhood acute methemoglobinemia generated by the water fountain, National Report, www.insp.gov.ro.

Scientific Papers. Series D. Animal Science. Vol. LXI, Number 1, 2018
ISSN 2285-5750; ISSN CD-ROM 2285-5769; ISSN Online 2393-2260; ISSN-L 2285-5750

THE ROLE OF COMMERCIALLY PRODUCED BUMBLEBEES IN GOOD AGRICULTURAL PRACTICES

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Abstract

Insufficient pollination due to low temperatures, high humidity, low light intensity and isolated atmosphere is one of the major problems in greenhouse vegetable production. These unsuitable conditions cause to insufficient production of fertile pollen, low pollen dynamism and finally serious pollination problems in greenhouses. Before 1990s, plant growth regulators which are also called as hormone spray were frequently used for greenhouse crop pollination. However, there is a worldwide interest to use the bumblebees as a pollinator of many crops in recent years. Due to their excellent pollinator behavior, bumblebees are indispensable element for especially greenhouse tomato production. Using of bumblebee also contribute to necessity of Good Agricultural Practices such as environmental sustainability, economic viability, social acceptability and food safety and quality. In this experiment, we aimed to evaluate the importance of commercially produced bumblebees in terms of environment friendly agriculture.

Key words*: bumblebee, crop pollination, food safety, good agricultural practices.*

INTRODUCTION

The industrialization of agriculture and development of synthetic chemicals have allowed growers to increase yields. Traditionally crop protection in greenhouse horticulture has also been based on the use of pesticides. In the early 1980s, growing concern for the human health and environment has led to try to find some alternatives to chemicals (Yılmaz et al., 2002; van der Velden et al., 2012). The concept of Good Agricultural Practices (GAP) has evolved in the context of a rapidly changing and globalizing food economy and as a result of the concerns and commitments of a wide range of stakeholders about food production and security, food safety and quality, and the environmental sustainability of agriculture. Good Agricultural Practices (GAP) are practices that address environmental, economic and social sustainability for on-farm processes and result in safe and quality food and non-food agricultural products (Ersoy et al., 2017; FAO, 2003). The use of bumblebee colonies to pollinate greenhouse crops is more effective than mechanical vibration or plant growth regulators (Dasgan et al., 2004). They have also led to improved integrated pest management (IPM) practices, resulting in a large reduction in the use of pesticides and other spray chemicals. Therefore the use of bumblebees for greenhouse pollination helps improve the safety and quality of greenhouse products and seems to be one of the Good Agricultural Practices. Evaluation of importance of commercially produced bumblebees in terms of Good Agricultural Practices was aimed in this review.

MATERIALS AND METHODS

Papers that examined the importance, year round rearing processes, pollination effectiveness and invasive potential of bumblebees were reviewed. The history of commercial rearing, use of these bees in greenhouse, their effects on fruit quality and quantity, and possible effects of bumblebee commercialization on ecology were explained.

RESULTS AND DISCUSSIONS

The history of commercial bumblebee rearing

The history of bumblebee research related to domestication and the importance of bumblebees as pollinating insects in agriculture have a long history that is over a hundred years

old (Sladen, 1912). But, after understanding the importance of bumblebees in greenhouse production, the year round rearing of bumblebees was achieved in the Netherlands and Belgium due to the efforts of commercial companies approximately 30 years ago. Since then, commercially reared bumblebee colonies have been used on a large scale for greenhouse pollination and demand for the bees by growers has been very high. Therefore, the number of colonies used is being increased rapidly year by year. In 2004, the total number of colonies of all species and on all continents sold was estimated to be around one million (Velthuis and van Doorn, 2006). Commercially reared colonies have been used in many countries, including some outside of its native range (Kraus et al., 2011). Although, commercial companies have not shared any production data we estimated that current worldwide sales of commercial bumblebee colonies has reached some three million colonies. For example, in Turkey, the number of colonies used as pollinators of greenhouse crops have increased rapidly year by year and reached to about 250,000 colonies yearly in 2017.

This high demand worldwide also caused to increase of interest for commercial rearing of bumblebees. However, lack of knowledge and inexperience about mass and commercial rearing of bumblebee can be important risks for new investors. The most important step is to have the technical knowledge and experience for commercial rearing. Additionally, the producers should have convenient rearing laboratory, required materials and equipments, hibernated queens for starting the rearing activity, official authorization for production and marketing, production planning according to sales forecast, healthy and quality queen rearing for sustainable production and experienced marketing staff for success in commercial rearing (Gosterit and Gurel, 2014).

Currently, about 250 species of true bumblebees have been identified (Williams, 1998). *Bombus terrestris* is the most commonly commercially reared species. This species is also one of the most abundant and widespread bumblebees throughout continental Europe and many Mediterranean and Atlantic islands (Chittka et al., 2004). *B. terrestris* includes nine subspecies (Rasmont et al., 2008). Although many of them were used in the early years of commercial rearing, *B. t. dalmatinus* proved to have superior characteristics in terms of mass rearing (Velthuis and van Doorn, 2006).

Colony foundation from queens, obtaining of young queens and males from colonies, mating of queens and males, and diapause control are main stages in commercially rearing of bumblebees. Colony supplier or breeders which have their own rearing process follow the natural life cycle of bumblebees and realize these stage in controlled conditions. Success of these stages directly affects the sustainability of mass rearing.

Bumblebee pollinatıon in greenhouses

The greenhouse industry is a very important segment of agriculture. One of the major problems in greenhouse vegetable production during winter is insufficient pollination due to low temperatures, low light intensity and isolated atmosphere. Insufficient production of fertile pollen and low pollen dynamism cause serious pollination problems in greenhouses. Studies have shown that a lack of pollination can significantly reduce fruit yield in greenhouse tomatoes and sweet peppers, especially early in the growing cycle (Abak et al., 1997). In recent years, a worldwide trend has been to use the bumblebees as a pollinator of many crops, including tomatoes, due to yield increase and enhancement of fruit quality. Bumblebees which work very long hours, forage from dawn to dusk even on cold, rainy or foggy days and are therefore very efficient pollinators. Bumblebee pollination has a positive effect on the yield, fruit weight, fruit volume and the number of seeds in fruits (Banda and Paxton, 1991).

Effects of bumblebee pollination in greenhouses were examined by different researchers for different crops. According to some previous reports, bumblebee pollination decreases the need for manual pollination, increases yield and quality of the greenhouse tomato crops (Ahmad et al., 2017), helps to produce more well-shaped fruit and the total marketable fruit production on greenhouse strawberry (Dimou et al., 2008), increases the fruit yield, fruit quality, and seed set of the pepper (Ercan and Onus, 2003).

Currently, bumblebees which are indispensable pollinator for especially greenhouse tomato completely replaced by mechanical vibration and plant growth regulators. They have some advantages as pollination agency: they pollinate the flowers through a method called "buzz pollination", a rapid vibrating motion which releases large amounts of pollen onto the bee; they have not sophisticated communication system of honeybees, therefore, in greenhouses: they are less likely to leave your crop for more attractive flowers; unlike honeybees, bumblebees are attracted to flowers with narrow corolla tubes, such as blueberries and cranberries; they mainly forage for pollen rather than nectar, and transfer more pollen to the pistils with each visit; they visit many more blooms per minute than honeybees; and they are much less aggressive than honeybees (Morandin et al., 2001; Dasgan et al., 2004; Velthuis and van Doorn, 2006; Ahmad et al., 2015). The use of bumblebee for pollination agency also indirectly contribute to decreasing of pesticide use.

Commercial bumblebee colonies can be easily purchased by all growers throughout the year. A commercial bumblebee colony which in small cardboard box should include a healthy queen, 60 to 70 worker bees and large brood area. Males and young queens have not begun to produce. The price of the colonies depends on the country or commercial firm. The number of colonies required per surface unit depends on the crop, variety, season, plant density, and type of glasshouse or tunnel. One colony can pollinate 1500-2000 m^2 of tomatoes for about 40 days, effectively. At the end of this period, colony life ends and colony is replaced with a new one.

Impact of commercially produced bumblebee colonies on ecosystem

Bumblebees are an important pollinator of wild flora as well as agricultural crops and are increasingly used as an effective commercial pollinator in greenhouses crops mainly in tomatoes all over the world. Although five species of bumblebees are reared commercially on a large scale, the Eurasian *B.terrestris* L. is the most reared subspecies for commercial pollination and has been used outside its natural distribution area. Very early after commercial introduction, it was recognized that this species is invasive and may disturb local ecosystems (Goulson, 2003). There are many invasive characteristics of *B. terrestris* such as high migration ability, early seasonal emergence, high adaptability under adverse climatic conditions in various habitat, polylectic foraging strategies and regulation of life cycle in a year in newly colonized area (Dafni et al., 2010). It is known that, commercial *B. terrestris* colonies produce more queens than local populations. A single *B. terrestris* colony may produce more than a hundred of new queens which may escape from greenhouses and found nest in native flora (Gosterit and Baskar, 2016). The invasion and the increase in population of introduced *B. terrestris* in the new areas have caused some problems, such as competition with native pollinators for floral resources and nest sites, the introduction of parasites and pathogens, and hybridization with native species. Therefore their potentially effects on the environment are also being observed carefully (Goka et al., 2001). It should not be forgotten that *B. terrestris* has colonized the native ecosystems of some countries where it does not occur naturally, including Japan, New Zealand, Tasmania, Chile and Israel as a result of commercial introductions (Macfarlane and Gurr., 1995; Ruz and Herrera, 2001; Hingston et al., 2002; Inoue et al., 2008; Dafni et al., 2010).

CONCLUSIONS

Bumblebees are excellent pollinators of tomatoes, peppers, eggplants and other crops grown in greenhouses. Bumblebee pollination increases the yield and the quality of the fruits and reduces the need for insecticide application. Therefore, we recommend the using of commercial bumblebee colonies in greenhouses as one of the Good Agricultural Practices. On the other hand, its spreading speed, adaptation to different conditions, hybridization with native species, competition with native pollinators for resources and potential for pathogen spillover need to be taken into consideration. While the use of bumblebees is recommended, some regulations also must be made such as using covering nets, killing colonies and then burning the hive after

use, using queen excluder and using colonies with health certificate to prevent escaping of *B. terrestris* from greenhouses and decrease their negative ecological impacts.

REFERENCES

Ahmad M., Bodlah I., Mehmood K., Sheikh U.A.A., Aziz M.A., 2015. Pollination and foraging potential of european bumblebee, *Bombus terrestris* (Hymenoptera: Apidae) on tomato crop under greenhouse system. Pakistan Journal of Zoology, 47, 1279-1285.

Abak K., Dasgan H.Y., Ikiz O., Uygun N., Kaftanoğlu O., Yeninar H., 1997. Pollen production and quality in pepper grown in unheated greenhouses during winter and the effects of bumblebee pollination on fruit yield and quality. Acta Horticulturae, 437, 303-307.

Banda H.J., Paxton R.J., 1991. Pollination of greenhouse tomatoes by bees. Acta Horticulturae, 288, 194-197.

Chittka L., Ings T.C, Raine N.E., 2004. Chance and adaptation in the evolution of island bumblebee behaviour. Population Ecology, 46, 243–251.

Dafni A., Kevan P., Gross C.L., Goka K., 2010. *Bombus terrestris*, pollinator, invasive, and pest: An assessment of problems associated with its widespread introductions for commercial purposes. Applied Entomology and Zoology, 45(1), 101–113.

Dasgan H.Y., Özdoğan A.O., Kaftanoğlu O., Abak K., 2004. Effectiveness of bumblebee pollination in anti-frost heated tomato greenhouses in the Mediterranean Basin. Turkish Journal of Agriculture and Forestry, 28, 73-82.

Dimou M., Taraza S., Thrasyvoulou A., Vasilakakis M., 2008. Effect of bumblebee pollination on greenhouse strawberry production. Journal of Apicultural Research and Bee World, 47(2), 99-101.

Ercan N., Onus A.N. 2003. The effects of bumblebees (*Bombus terrestris* L.) on fruit quality and yield of pepper (*Capsicum annuum* L.) grown in an unheated greenhouse. Israel Journal of Plant Sciences, 51(4), 275-283.

Ersoy N., Yilmaz S., Gumus E., 2017. The importance of good agricultural practices in EU membership process. Mediterranean Agricultural Sciences, 30(2), 133–136.

FAO., 2003. Development of a framework for Good Agricultural Practices. FAO, Committee on Agriculture, Seventeen Session, 31 March-4 April, Roma, 35p.

Goka K., Okabe K., Yoneda M., Nivas S., 2001. Bumblebee commercialization will cause worldwide migration of parasitic mites. Molecular Ecology, 10, 2095-2099.

Gosterit A., Gurel F., 2014. Basic necessities for commercial rearing of bumblebee, *Bombus terrestris* L. Süleyman Demirel University Journal of the Faculty of Agriculture, 9(2), 102-111.

Gosterit A., Baskar V.C., 2016. Impacts of commercialization on the developmental characteristics of native *Bombus terrestris* (L.) colonies. Insectes Sociaux, 63(4), 609-614.

Goulson D., 2003. Effects of introduced bees on native ecosystems. Annual Review of Ecology Evolution and Systematics, 34, 1–26.

Hingston A., Marsden-Smedley J., Driscoll D., Corbett S., Fenton J., Anderson R., Plowman C., Mowling F., Jenkin M., Matsui K., Bonham K., Ilowski M., McQuillan P., Yaxley B., Reid T., Storey D., Poole L., Mallick S., Fitzgerald N., Kirkpatrick J., Febey J., Harwood A., Michaels K., Russell M., Black P., Emmerson L., Visoiu M., Morgan J., Breen S., Gates S., Bantich M., Desmarchelier J., 2002. Extent of invasion of Tasmanian native vegetation by the exotic bumblebee *Bombus terrestris* (Apoidea: Apidae). Austral Ecology, 27, 162–172.

Inoue M.N., Yokoyama J., Washitani I., 2008. Displacement of Japanese native bumblebees by the recently introduced *Bombus terrestris* (L.) (Hymenoptera: Apidae). Journal of Insect Conservation, 12, 135–146.

Kraus F.B., Szentgyorgyi H., Rozej E., Rhode M., Moron D., Woyciechowski M., Moritz R.F.A., 2011. Greenhouse bumblebees (*Bombus terrestris*) spread their genes into the wild. Conservation Genetics, 12, 187–192.

MacFarlane R.P.,Gurr L., 1995. Distribution of bumblebees in New Zealand. New Zealand Entomologist, 18, 29–36.

Morandin L.A., Laverty T.M., Kevan P.G., 2001. Bumblebee (Hymenoptera: Apidae) activity and pollination levels in commercial tomato greenhouses. Journal of Economic Entomology, 94(2), 462–467.

Rasmont P., Coppee A., Michez D., de Meulemeester T., 2008. An overview of the *Bombus terrestris* (L.1758) subspecies (Hymenoptera: Apidae). Annals de la Societe Entomologique de France, 44, 243–250.

Ruz L., Herrera R., 2001. Preliminary observations on foraging activities of *Bombus dahlbomii* and *Bombus terrestris* (Hym: Apidae) on native and non-native vegetation in Chile. ActaHorticulturae, 561, 165–169.

Sladen F.W.L., 1912. The humble-bee, MacMillan, London

van der Velden N.J.A., Suay R., Urbaneja A., Giorgini M., Ruocco M., Poncet C., Lefevre A., 2012. Recent developments and market opportunities for IPM in greenhouse tomatoes in southern Europe; Consequences for advanced IPM toolboxes and greenhouse engineering. Research report, LEI Memorandum 12-077, 41p.

Velthuis H.H.W., van Doorn A., 2006. A century of advances in bumblebee domestication and the economic and environmental aspects of its comercialization for pollination. Apidologie, 37, 421-451.

Williams P.H., 1998. An annotated checklist of bumblebees with an analysis of patterns of description. Bulletin of Natural History Museum, Entomology Series, 67, 79–152.

Yilmaz I., Ozkan B., Gurel F., Karaman S., 2002. The use of bumblebee in greenhouse vegetable production and bumblebee marketing in Turkey. Tanta University Journal of Agricultural Research, 28(3), 524–534.

Scientific Papers. Series D. Animal Science. Vol. LXI, Number 1, 2018
ISSN 2285-5750; ISSN CD-ROM 2285-5769; ISSN Online 2393-2260; ISSN-L 2285-5750

STUDY ABOUT CANNON BONE PERIMETER AVERAGE PERFORMANCES IN ROMANIAN HUCUL HORSE BREED – PRISLOP BLOODLINE

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Abstract

Study of average performances in a population have a huge importance because, regarding a population, the average of phenotypic value is equal with average of genotypic value. So, the studies of the average value of characters offer us an idea about the population genetic level. The biological material is represented by 93 Hucul horse from Prislop bloodline divided in 3 stallion families analyzed at 18, 30 and 42 months old, owned by Lucina Hucul stood farm. The average performances for cannon bone perimeter were 17.25 cm at 18 months, 18 cm at 30 months old and 18.57 cm at 42 months old. We can observe a good growth rate from one age to another and significant differences between sexes. The average performances of the character are between characteristic limits of the breed.

Key words*: bloodline, horse, Hucul, Lucina, Prislop.*

INTRODUCTION

The study of average performances for different characters in a population, have a great importance because, at the population level, the average of phenotypic values are equal with the average of genotypics values (Mărginean, 1997). That's mind that the study of average performances give us an ideea about the genetic level of population (Mărginean et al., 2005).

MATERIALS AND METHODS

For realising the purposed objectives, biological material became from Lucina Stood Farm, Suceava county, represented by a sample of 93 horses (males and females) divided at 3 stallion familys, presented in Table 1.

The sample was studied at three different ages: first grading at 1.5 years old, second grading at 2.5 years old and the third grading at 3.5 years old. After the third grade, the individuals support a performances testing for energetic capacity. The sample was extracted from population in according with registered performances for all three ages to have one balanced experimental plan (Harper, 2006). The analyzed statistics are: average, variance, standard deviation, average error and variability coefficient.

RESULTS AND DISCUSSIONS

The average performances for cannon bone perimeter, are presented in Table 2.

The character dynamic is presented in Figure 1.

Analyzing Table 2 and Figure 1, we can observe an important growth from one grading to another, in both sexes. Also we distinguish significant difference between sexes for mentioned character.

Calculated values for Fisher test does not reveal significant differences, from statistical point of view, between halfsibs families (F=0.03 at 18 months old, F=0.24 at 30 months old and F=0.62 at 42 months old).

The differences between sexes, for all three ages are insignificant to put in discussion some differences in energetic capacity between sexes (Saastamoinen, 1990).

Table 1. The biological material

Bloodline	Family size	Males	Females
PRISLOP	93	44	49
- Prislop VIII	26	14	12
- Prislop IX	62	27	35
- Prislop X	5	3	2

Table 2**.** The cannon bone perimeter average performances in Prislop bloodline

Family	Sex	Age (years)											
		1.5				2.5				3.5			
		n	$\overline{X} \pm S_{\overline{X}}$	**s**	**v%**	**n**	$\overline{X} \pm S_{\overline{X}}$	**s**	**v%**	**n**	$\overline{X} \pm S_{\overline{X}}$	**s**	**v%**
Pr VIII	M	14	17.39 ± 0.21	0.79	4.54	14	18.29 ± 0.13	0.47	2.57	14	18.71 ± 0.13	0.47	2.51
Pr IX		27	17.54 ± 0.15	0.80	4.56	27	18.41 ± 0.18	0.92	5	27	19 ± 0,14	0.75	3.95
Pr X		3	17	0	0	3	18.17 ± 0,17	0.29	1.6	3	19.17 ± 0.44	0.76	3.96
Total M		**44**	**17.45 ± 0.12**	**0.77**	**4.41**	**44**	**18.35 ± 0.12**	**0.77**	**4.2**	**44**	**18.92 ± 0,1**	**0.67**	**3.54**
Pr VIII	F	12	17.04 ± 0,2	0.69	4.05	12	17.83 ± 0,11	0.39	2.19	12	18.13 ± 0.16	0.57	3.14
Pr IX		35	17.04 ± 0.09	0.51	2.99	35	17.64 ± 0.08	0.48	2.72	35	18.31 ± 0,1	0.58	3.17
Pr X		2	17.5	0	0	2	17.5 ± 0,5	0.71	4.06	2	18 ± 0.5	0.71	3.94
Total F		**49**	**17.06 ± 0.08**	**0.55**	**3.22**	**49**	**17.68 ± 0.07**	**0.46**	**2.6**	**49**	**18.26 ± 0.08**	**0.58**	**3,18**
Total family		**93**	**17.25 ± 0.07**	**0.69**	**4**	**93**	**18 ± 0,07**	**0.71**	**3.94**	**93**	**18.57 ± 0.07**	**0.71**	**3.82**
Significance of observed differences between sexes (Student)		3.00 **				5.15 ***				5.50 ***			

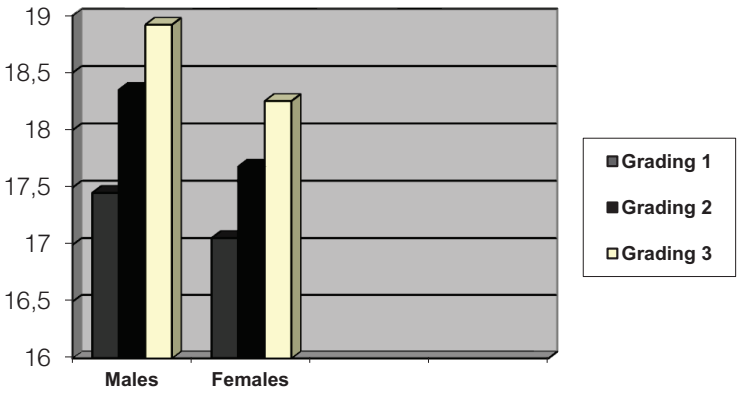

Figure 1. Cannon bone perimeter dynamic in Prislop bloodline

CONCLUSIONS

We reveal an important growth of character, from one age to another, especially in stallion case (14 cm between first and second grade of stallions).

Significant differences was recorded between the individuals from both sexes.

The calculated F value does not reveal significant differences, from statistical point of view, between half sibs families (F=0.03 at 18 months old, F=0.24 at 30 months old and F=0.62 at 42 months old). For the other two gradings the differences are not signifficant from statistical point of view (F = 2 at 2.5 years old, and F = 0.49 at 3.5 years old).

To see between which families are significant differences, we applied Tuckey test. The test does not succeed to show between which families are significant differences, probably as a result of sample error associated with any statistical analysis.

The cannon bone perimeter evolution vary in postutherin period in correlation with age, with an decreasing trend of values due to this factor.

REFERENCES

Mărginean Gh., 1997. Tratat de hipologie, Editura Orion, Bucuresti.

Mărginean Gh., Georgescu Gh., Maftei M., 2005. Indrumator de lucrări practice pentru exploatarea cabalinelor, Editura Agrotehnica, Bucuresti.

Harper F., 2006. Growth in horses. Horse Express, 25(3).

Saastamoinen M., 1990. Factors affecting growth and development of foals and young horses. Acta Agriculturae Scandinavica, 40, 387-396.

Scientific Papers. Series D. Animal Science. Vol. LXI, Number 1, 2018
ISSN 2285-5750; ISSN CD-ROM 2285-5769; ISSN Online 2393-2260; ISSN-L 2285-5750

STUDY ABOUT SLAUGHTERING RESULTS BY SEX AT ADULT QUAILS FROM BOBWHITE SPECIES (*Colinus virginianus*)

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Abstract

In a study conducted on a total of 30 males and females of Bobwhite quail, Colinus virginianus species, was monitored to determine the average results at slaughter at 70 weeks of age. As a result of the research carried out was determined an average body weight similar in the two sexes, of 245.00 ± 2.35 g/head in the female, while in male was of 250.00 ± 2.65 g/head, and the weight of the carcase after evisceration was 170.00 ± 2.35 g for females and of 167.00 ± 2.05 g in males. The yield was of 68.16 ± 1.75 % in females and of 68.00 ± 1.25% in males, the difference was not significant. The average breast weight was of 87.00 ± 1.05 g in females and less with 2.3% in males, of 85.00 ± 1.13 g, the difference being statistically not ensured. The average proportion of the chest was 52.10 ± 1.55% in females and of 50.00 ± 1.05% in males.

Key words: *Bobwhite quail, slaughtering, carcass, yield.*

INTRODUCTION

Raising Bobwhite quail, called Virginia quail too (*Colinus virginianus*) in specialized establishments is less widespread, being the most common in the USA, where this quail lives in a natural way, in the wild too. In Pennsylvania are produced annually approximately 250,000 Bobwhite quails in private farms (www.extension.psu.edu/ bobwhite-quail-production), and in Georgia are produced annually approximately 5,000,000 heads annually for hunting lands and trading (Dozier and Bramwell, 2009). Bobwhite quails were introduced as game birds in some countries in the European Union and in New Zealand.

From the point of view of the taxonomic, Bobwhite make part of the kingdom Animalia, Chordata phylum, class Aves, order Galliformes, Odontophoridae family, genus Colinus, species *Colinus virginianus* (Linnaeus, 1758) (https://en.wikipedia.org/wiki/Northern_bobwhite).

The live medium weight in wild quails is between 170 and 255 g/head. The average weight of the egg is 9.5 - 11 g and the colour of the egg shells is white. The duration of incubation period at Bobwhite quails is 23 - 24 days, and the females begin to lay eggs at the age of 22 weeks from their hatchability (Dozier and Bramwell, 2009) (https://en.wikipedia.org/wiki/Northern_bobwhite).

It is considered that Bobwhite quails are easily raised and reproduced. Adult quails ensures good production even at an ambient temperature between very wide limits, 10°C/50°F to 33°C/85°F while providing at the same time a duration of at least 17 hours light per day (Skewes and Wilson, 2003). You can feed them with a mixture of a commercial small seeds, supplemented with green feed or with compound feed for meat chicken in the form of crumbles.

The viability of the adult Bobwhite quails is very high considering that in the wild state they are more sedentary and adjusts easily to the winter conditions (C. Clark, http://www.birdwatchersgeneralstore.com/quails.htm; www.sdakotabirds.com/species/northern-quail).

The rate of sexual dimorphism at Bobwhite quail species is between 50.85% and 51.20% at young quails (in the first year of life) and between 56.70% and 62.90% on adult quails (Leopold, 1945, 1951; Kabat and Thompson, 1963; Sinn, 1978, quoted by Brown and Guttierez, 1980). Sexual dimorphism less

pronounced in these quail requires the age of slaughter to be greater for a good differentiation between male and female when the females shall be retained for the eggs production.
Raising Bobwhite quails represents a perspective activity and a very good opportunity considering that are less widespread in specialized poultry farms in Romania and the market price is very high. This quail can be raised for the décor, either for the population of hunting lands or for meat. Rising for meat production is a prospective activity for these quails in our country too, in view of the fact that it is a product with great taste, new and rare.
The literature is very limited as regarding farmed Bobwhite quails, and the present paper is also intended to be a starting point for research into the productivity of these quails, especially in our country.

MATERIALS AND METHODS

Research has taken place within the holding of quails Ionita T. Lucian Individual Enterprise located in the village Gherghița, Prahova County, Romania.
Has been analysed a number of 30 adult Bobwhite quails, of which 15 females and 15 males, have been slaughtered in average age of 70 weeks from the hatching (20 weeks of growth and 50 weeks of laying).

Figure 1. Male (left) and female (right) of Bobwhite quail

The quails were housed in battery cages of wire mesh and have been fed with compound feed. The nutritional value of the recipe of the used compound feed was the following: 2720 kcal metabolisable energy/kg of compound feed, 21% crude protein, 3.24% calcium, 0.66% phosphorus, 1.14% lysine, 0.56% methionine, 3,6% crude fat and 4.8% crude fibre. In the structure of the recipe of compound feed entered maize, wheat, soybean meal, sunflower meal, calcium carbonate, dicalcium phosphate, and vitamin-mineral premix.
The primary data have been obtained by individual weighing, before and after slaughter, and then, on the basis of them have been established the proportions of the various parts of the carcase and was done their statistical processing. The differences were tested by the Student test.

RESULTS AND DISCUSSIONS

The body weight in females was of 245.00 ± 2.35 g/head, while in males was of 250.00 ± 2.65 g/head, the difference between the sexes being of only 2%, insignificant.
The weight of the carcase after bleeding has been with 2.45% higher in males (245.00 ± 2.15 g/head) compared with that of the females (239.00 ± 2.25 g), the difference being statistically uninsured.
The carcass weight after plucking was of 214.00 ± 2.15 g in females and of 217.00 ± 2.55 g to males, difference being insignificant.
The weight of the carcase after evisceration was of 170.00 ± 2.35 g in females and of 167.00 ± 2.05 g to males, the difference being insignificant between the two sexes.
The yield of the carcase was of 68.16 ± 1.75% in females and 68.00 ± 1.25% in males, the difference being insignificant.
The average weight of blood was of 5.00 ± 0.75 g in females and by 16.67% higher, of 6.00 ± 0.35 g in males, the difference being statistically significant. The average proportion of blood was of 2.00 ± 0.15% to females and of 2.45 ± 0.25% to males.
The average weight of flakes was of 28.00 ± 1.35 g in females and less with 10.71 %, of 25.00 ± 1.65 g in males, the difference being statistically significant. The average proportion of flakes was of 11.42% ± 0.95 in females and of 10.46% ± 0.65 to males.

Table 1. Slaughter results in females and males from the Bobwhite quail population at the age of 70 weeks

Specification	Females	Males
Body weight (BW) (g)	245.00 ± 2.35ns	250.00 ± 2.65ns
Carcase weight after bleeding (CWB) (g)	239.00 ± 2.25ns	245.00 ± 2.15ns
Carcase weight after plucking (CWP) (g)	214.00 ± 2.15ns	217.00 ± 2.55ns
Carcase weight after evisceration (CWE) (g)	170.00 ± 2.35ns	167.00 ± 2.05ns
The carcase yield (carcase eviscerated/body weight) (%)	68.16 ± 1.75ns	68.00 ± 1.25ns
Blood weight (g)	5.00 ± 0.75*	6.00 ± 0.35*
Flakes weight (g)	28.00 ± 1.85*	25.00 ± 1.65*
Organs and intestines weight (g)	31.00 ± 1.35ns	32.00 ± 1.45ns
Blood proportion (%)	2.00 ± 0.15*	2.45 ± 0.25*
Flakes proportion (%)	11.42 ± 0.95*	10.46 ± 0.65*
Organs and intestines proportion (%)	14.74 ± 1.05ns	14.95 ± 1.35ns

Note: ns – insignificant difference; * - significant difference

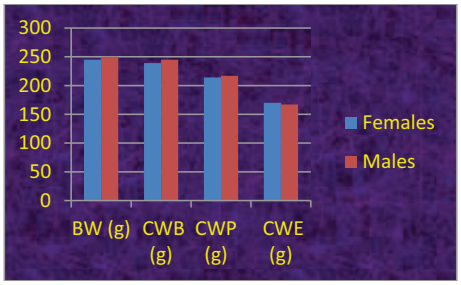

Figure 2. Comparative results in the two sexes of a Bobwhite quail population slaughtered at 70 weeks

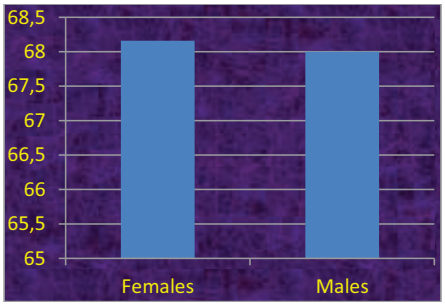

Figure 3. The yield of the carcase at the Bobwhite females and males slaughtered at 70 weeks

The average weight of the organs and intestines was of 31.00 ± 1.35 g to females and of 32.00 ± 1.45 g to males, the difference being statistically insignificant. The average proportion of organs and intestines was of 14.74% ± 1.05 to females and of 14.95 ± 1.35% to males.

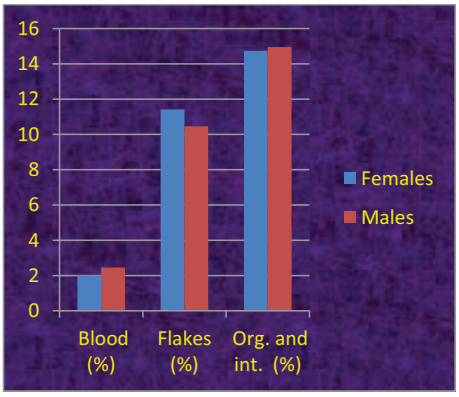

Figure 4. The average proportion of blood, flakes, organs and intestines to the studied females and males of Bobwhite quail at the age of 70 weeks

Table 2. The weight of the component parts of the carcase at the males and females of the Bobwhite population at the age of 70 weeks

Specification	Females	Males
Chest weight (g)	87.00 ± 1.05ns	85.00 ± 1.13ns
Thighs weight (g)	47.00 ± 0.85ns	46.00 ± 0.66ns
Back weight (g)	27.00 ± 0.37*	32.00 ± 0.44*
Wings weight (g)	10.00 ± 0.14*	12.00 ± 0.10*
Chest proportion (%)	52.10 ± 1.55ns	50.00 ± 1.05ns
Back proportion (%)	16.17 ± 0.87*	18.82 ± 0.19*
Thighs proportion (%)	27.64 ± 0.38ns	27.54 ± 0.47ns
Wings proportion (%)	6.00 ± 0.38*	7.05 ± 0.47*

Note: ns – insignificant difference; * - significant difference

The average weight of the chest was of 87.00 ± 1.05 g to females and of 85.00 ± 1.13 g to males, the difference being statistically insignificant. The average proportion of the chest was of 52.10 ± 1.55% to females and of 50.00 % ± 1.05 to males.

The average weight of the thighs was of 47.00 ± 0.85 g for females and 46.00 ± 0.66 g for the males, the difference being statistically insignificant. The average proportion of the thighs was of 27.64% ± 0.38 in females and of 27.54% ± 0.47 in males.

The average weight of the back was of 27.00 ± 0.37 g to females and 32.00 ± 0.44 g to males, the difference being statistically significant. The average proportion of the back was of 16.17 ± 0.87% in females and of 18.82% ± 0.19 to males.

The average weight of the wings was of 10.00 ± 0.14 g in females and of 12.00 ± 0.10 g to males, the difference being statistically significant. The average proportion of the wings was of 6.00 ± 0.38% in females and of 7.05% ± 0.47 to males.

CONCLUSSIONS

The average body weight at the age of 70 weeks at the analysed Bobwhite quails (*Colinus virginianus*) was of 245.00 ± 2.35 g/head in females, while in the males has been with only 2% higher, of 250.00 ± 2.65 g/head, the difference between the two sexes being insignificant. The average weight of the carcase after evisceration was of 170.00 ± 2.35 g in females and of 167.00 ± 2.05 g to males, the difference being insignificant. The average weight of blood was significantly higher in males, as well as the proportion thereof, and the weight of flakes has been significantly less with 10.71%, in males, as well as their average proportion.

The mean breast size proportion was similar in both sexes, of 52.10 ± 1.55% in females and of 50.00 ± 1.05% in males. The average proportion of the thighs was of 27.64 ± 0.38% in females and of 27.54 ± 0.47% to males, without that the difference between females and males being significant. The mean proportion of the wings was of 6.00% ± 0.38 in females and significantly higher, of 7.05% ± 0.47 to males.

The yield of the carcase was of 68.16% ± 1.75 in females and of 68.00% ± 1.25 to males, the difference being insignificant.

As a general conclusion, the weight and the yield of the carcase are alike at the two sexes, in the same way as the weight and the proportion of the chest and thighs. Only the weight and the proportion of the wings and back are significantly higher in males.

Researches on the performance of Bobwhite quails production are pretty rare, wild Bobwhite quails being studied more frequently. However, for spreading in specialized farms new research is needed on the growth and production parameters, especially on the youth and the egg production of these quails.

REFERENCES

Bennit R., 1951. Some aspects of Missouri quail and quail hunting: 1938 – 1948. Mo. Conserv., Comm., Tech., Bull. 2, 51.

Brown D.E., Guttierez R.J., 1980. Sexual ratios, sexual selection and sexual dimorphism in quail, J. Wild Manage., 44(1), 1980.

Clauer Phillip J., George L. Greaser, R. Michael Hulet, and Jayson K. Harper, 2012. Bobwhite Quail Production, www.extension.psu. edu/bobwhite-quail-production.

Clark Catherine, Bird Watcher's General Store * 36 Rt. 6A, Orleans, MA 02653. http://www.bird watchersgeneralstore.com/quails.htm.

Dozier W.A., Bramwell K., 2009. Bobwhite Quail Production and Management Guide, The University of Georgia, Cooperative Extension, Colleges of Agricultural and Environmental Sciences & Family and Consumer Sciences, Poultry Sciences Departments.

Ferket P.R. Feeding Bobwhite Quail, https://www.ces.ncsu.edu/depts/poulsci/tech_manuals/feeding_quail.html#

Kabat C., Thompson D.R., 1963. Wisconsin quail, 1834 - 1962: population dynamics and habitat management. Wis. Conserv. Dep. Tech. Bull., 30, 135.

Leopold A.S., 1945. Sex and age ratios among Bobwhite quail in Southern Misouri. J.Wildl. Manage., 9, 30 – 34.

Sinn J., 1978. Surveys and management of bobwhite quail. Nebr. Game and Parks Comm., Fed. Aid. Proj. W-15-R. Rep. 13 pp.

Skewes P.A., Wilson H.R., 2003: Bobwhite quail production, Institute of Food and Agricultural Sciences, University of Florida, CIR 879, august 2003.

***Northern bobwhite, https://en.wikipedia.org/wiki/Northern_bobwhite

***Northern bobwhite quail, www.sdakotabirds.com/species/northern_quail_info.htm

Scientific Papers. Series D. Animal Science. Vol. LXI, Number 1, 2018
ISSN 2285-5750; ISSN CD-ROM 2285-5769; ISSN Online 2393-2260; ISSN-L 2285-5750

PARTIAL RESULTS REGARDING GOAT MILK CHARACTERISTICS IN MURCIANA GRANADINA BREED, AND MODALITIES TO IMPROVE MILK QUALITY

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Abstract

Improving of goat milk quality is the last trend in goat breeding. Productivity is allready at a high level so we must do the price difference only by the quality parameters. More than that, the market demand high quality products certified as „bio" or „organic". We analyze, in this case goat milk production, from qualitative point of view, in Murciana Granadina breed, exploited in Romania but imported from Spain. The breeder choice was explained by the fact that this goat breed have maybe the best qualitative paramethers in Spain, so it is possible to obtain high quality dairy products, solving a part of the market demands. Results show us a high conten of fats (5.28%), protein (2.98%) and lact. (4.41%). This paramethers give an important advantage in production of dairy specialties, and of course in increasing of economical value.

Key words*: Murciana Granadina, milk, dairy, goat*

INTRODUCTION

This study is just a part for an ample research regarding the modalities to improove goat milk quality by enriching it in polyunsaturated fatty acids. The milk quality became the first indicator from the economical point of view („Chifre cles 2017"). Dairy productivity is now at a high level, so the price of regular milk has decreasing. But the dairy products market demand high quality products, especially certified as „bio" or „organic". In this conjucture, the goat milk became a very important raw material for dairy industry, because of his suitability for obtaining high quality cheese production, and not only (Maathuis et al., 2017).

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MATERIALS AND METHODS

For realising the purposed objectives, biological material became from a farm from Contesti village, Dambovita County, owned by a romanian company. All the individuals from this farm belongs to Murciana Granadina breed and was imported from Spain (Vlad et al., 2017). We analyze 48 individuals divided in three groups, on three periods: A (10.11.2017-19.11.2017); B (20.11.2017-27.11.2017); C (28.11.2017-09.12.2017). Each group had a particular nutrition. The feeding ratio composition, at this moment, is confidential. We had a basic feeding ratio (based on hay and cereals), and an addition of 0.7 kg oleaginous feed such as sunflower groats, linseed and rapeseed. We analysed $\sum X$, $\overline{X}$, ΣX^2, Σx^2, Cx, S^2 and Sx. Ranking of analysed characters was made using average performances (Figure 1).

RESULTS AND DISCUSSIONS

The best performance for group 1, for fat character was recorded in period A, being almost double compared to period B. Individuals of period C are characterized by an average of performances with only 0.856% extra compared to the lowest performance recorded in this batch (Table 1).

Regarding SNF (solid non fat), the average of performances, for analyzed individuals, is quite close, the greatest difference in performance averages being only 0.962%.

For density, after processing the results, it was observed that performance averages from periods B and C are quite close.

The lowest performance was recorded in period A, with 7.12% smaller compared to the average recorded in period B.

On the second place, in terms of performances for lactose, are individuals in period C with only 0.019% less compared to the individuals from period B.

On the last place, with a difference of 0.553% compared to the average of performances from the first position, is the group of individuals from period A.

Individuals from group 3 perform an average performance of protein at 0.327% in addition to the average of performances recorded by individuals in the period A.

Average performance of protein in period A occupies the last place, respectively 0.389% compared to the group of individuals who occupy the best position for analyzed character.

The average performances for analyzed characters, in these three periods, for group 2 are presented in Table 2. The average performances recorded in this three periods, for fat, are very close, between the best and the worst performing being a difference of only 0.805%. Analyzing the data presented in Table 2, it can be noticed that the individuals of period C are noted by the highest average of performances. Thus, they have a bigger value, with 0.567% higher than the average performance of individuals from group 1, and with only 0.054% higher than the individuals analyzed in period B. Analyzing the average performances obtained for the three study periods, it can be observed that the highest density was determined by the group of individuals in the period C. The second place is occupied by the group from period B, with only 0.282% less than the average performances from period C.

From the data presented in Table 2, it can be observed that, for the lactose character, the differences between the average performances for these three groups of individuals are very small, up to 0.314%.

Table 1. Average performances (%) for group 1

Period		Fat $\overline{X}\pm S_{\overline{X}}$	SNF $\overline{X}\pm S_{\overline{X}}$	Density (g/cm^3) $\overline{X}\pm S_{\overline{X}}$	Lactose $\overline{X}\pm S_{\overline{X}}$	Salts $\overline{X}\pm S_{\overline{X}}$	Protein $\overline{X}\pm S_{\overline{X}}$
A	**10-19.11.2017**	7.88±0.375	7.826±0.154	23.999±0.746	4.297±0.084	0.633±0.013	2.835±0.057
B	**20-27.11.2017**	3.927±0.278	8.820±0.148	31.119±0.743	4.850±0.081	0.723±0.012	3.224±0.055
C	**28.11.-09.12.2017**	4.783±0.418	8.788±0.133	30.29±0.660	4.831±0.074	0.717±0.011	3.207±0.049

Table 2. Average performances (%) for group 2

Period		Fat $\overline{X}\pm S_{\overline{X}}$	SNF $\overline{X}\pm S_{\overline{X}}$	Density (g/cm^3) $\overline{X}\pm S_{\overline{X}}$	Lactose $\overline{X}\pm S_{\overline{X}}$	Salts $\overline{X}\pm S_{\overline{X}}$	Protein $\overline{X}\pm S_{\overline{X}}$
A	**10-19.11.2017**	6.765±0.409	8.163±0.096	26.227±0.626	4.482±0.053	0.663±0.008	2.963±0.037
B	**20-27.11.2017**	6.048±0.596	8.676±0.094	28.815±0.681	4.766±0.052	0.709±0.008	3.157±0.035
C	**28.11.-09.12.2017**	5.960±0.465	8.730±0.124	29.097±0.644	4.796±0.068	0.712±0.010	3.179±0.046

Table 3. Average performances (%) for group 3

Period		Fat $\overline{X}\pm S_{\overline{X}}$	SNF $\overline{X}\pm S_{\overline{X}}$	Density (g/cm^3) $\overline{X}\pm S_{\overline{X}}$	Lactose $\overline{X}\pm S_{\overline{X}}$	Salts $\overline{X}\pm S_{\overline{X}}$	Protein $\overline{X}\pm S_{\overline{X}}$
A	**10-19.11.2017**	5.458±1.299	6.650±1.367	21.410±4.319	3.653±0.750	0.540±0.110	2.416±0.496
B	**20-27.11.2017**	4.300±1.160	6.983±1.473	23.657±4.891	3.837±0.809	0.572±0.120	2.545±0.535
C	**28.11.-09.12.2017**	4.465±1.143	6.949±1.457	23.391±4.856	3.820±0.800	0.567±0.118	2.531±0.530

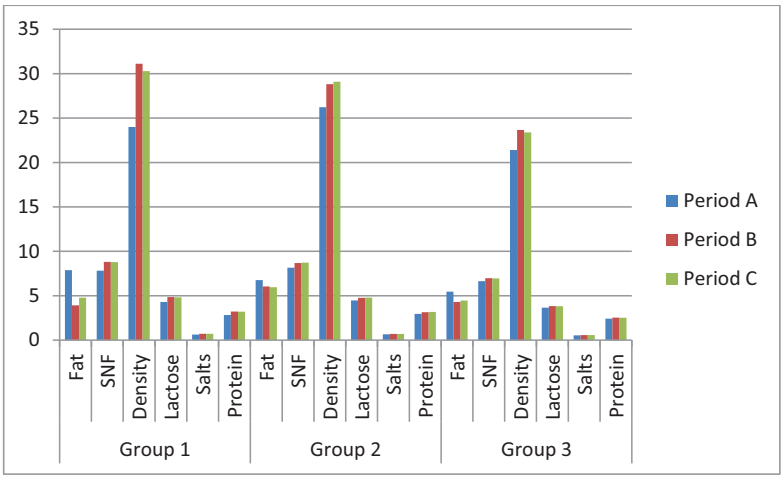

Figure 1. Graphical representation of average performances (%)

The situation is similar for protein. Thus, from the data presented in Table 2, we can see that, during the period C has recorded the best average performance for analyzed character, with a plus of 0.216%, compared to the average performance from period A. Between the average performance established in the periods B and C, the difference is very small, at only 0.022%. If we analyze simultaneously all the six characters, we can conclude that the best performances are achieved during the period C.

In Table 3 are presented the average performances for group 3, for all six analyzed characters in all three periods. From the data presented in Table 3, it can be seen that for the fat character, the best performance is recorded in period A, with a plus of 1.158%, compared to the determined performance for the individuals analyzed in the period B. Compared with the average of the performances established for the period C there is a difference of only 0.993%.

CONCLUSIONS

The differences between performances for SNF do not exceed 0.333%. An average performance with a plus at only 0.034%, was determined during period B, in comparation with the average performance for individuals analyzed during C period. For density, the data processing indicated that between averages calculated in periods B and C there is a difference of only 0,266%. Even the comparison with the average performances determined for the other group shows that the averages calculated for this character are very close. The situation is similar if we compare the other three elements: lactose, salts and protein. For lactose, the difference between the highest and lowest averages was only 0.184%, 0.032% for salts and 0.129% for protein. For any of the items presented, the lowest average performance was determined in period A (start period) and the best performance in period B.

Another very important and obvious fact is that the increasing of protein levels is associated with the decreasing of fat levels in milk. Even if the datas regarding the feeding ratio composition of feed ratios are confidential, at this time, we must say that the results from B are caused by the feeding from A period with a singular exception: fat. The differences between B and C period are smaller in comparat because the feeding has remain the same (from quantitative point of view).

ACKNOWLDGEMENT

This research work is part of Project 26 PTE/2016: PNIII-P2-2.1-PTE-0196.

REFERENCES

Maathuis Annet et al., 2017. Protein digestion and quality of goat and cow milk infant formula and human milk under simulated infant codition. Journal

of Pediatric Gastroenterology and nutrition, 65(6), 661-666.

Vlad I, Maftei M, Cărătuș Stanciu M, Pogurschi E., 2017. Study on Murciana Granadina goats phenotype remarks on productive performances in Agri-Valahia farm from Contești Dîmbovita County, Romania. Scientific Papers Series Management, Economic Engineering in Agriculture and Rural Development, 17(4), ISSN 2285-3952 .

„Chifre cles 2017". Institut de L`elevages „Production caprine - Lait et Viande – Confederation Nationale de l`elevage" C.N.E.

Scientific Papers. Series D. Animal Science. Vol. LXI, Number 1, 2018
ISSN 2285-5750; ISSN CD-ROM 2285-5769; ISSN Online 2393-2260; ISSN-L 2285-5750

AN EXPECTATION OF BIORESOURCE FUNCTION AGAINST PARASITE INFECTION ON ANIMAL HEALTH

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Abstract

The studies of the utilization of bio-resources substance has an increasing to be applied in preventing parasitic infections in livestock related both to endo-parasite and ecto-parasite. The use of bio-resource could be extracted from a substance originated plants and animals. The immunoglobulins in colostrum are well known as important bio-resource for young neonate individual to protect against parasites even to the pathogenic microbes. In other part plants have a lot of bio-actives compound that are useful to conserve the animal health. This article is to present our study by using colostrum immunoglobulins and curcuma on endo-parasite infection treated in young experimental animals. The study was conducted by using seven-teen mice with four treatments (T). T1 signified an infection with parasite only, T2 represented a treatment of curcuma in animal infected with parasite, T3 was the treatment of colostrum immunoglobulin in animal infected with the parasite larva, and T4 showed a treatment of curcuma mixed with colostrum immunoglobulin in infected mice. The distribution of the colostrum Ig and curcuma substances was realized through a drinking water, given ad libitum. The data collection of larva in gut was realized at 7^{th} days post infection while the EPG data collection was taken at 5^{th} to 7^{th} days post infection. The results revealed that there has a significant response between the treatment ($P<0.01$) which a combination between colostrum immunoglobulin (T4) caused a highest number of larva observed, while There has a non-significant difference of EPG number ($P>0.05$) between all treatment (T1, T2, T3 and T4). We concluded that curcuma and colostrum immunoglobulin were useful to suppress the parasite of Strongyloides venezuelensis in the gut rather than to suppress the number of EPG in the fecal matter of experiment mice.

Key words*: parasites, livestock, curcuma, colostrums.*

INTRODUCTION

Animal and human health can be affected seriously by parasite infection. Parasites that found in the skin are categorized as ecto-parasites while the parasites that take place in digestive tract were called endo-parasites. Some arthropods are well known their infestation in animals and human skin, otherwise some helminths preferred live inside in the host body.

The use of bio-resources to overcome the parasite threat has been reported by Jolles et al. (2015), Toar et al. (2013) and Rumokoy et al. (2017[a]). Morais et al. (2013) used curcumin to evaluate its effect on the parasite *Schistosoma mansoni* infection in mice. Sanatombi and Sanatombi (2017) discussed about curcuma as nutritionally rich food products, which interest in medicinal properties and its bioactive compounds possessing wide bioactivities such as antioxidant, antiviral, antimicrobial, and anti-inflammation activities. Those authors mentioned six-teen edible curcuma included: *Curcuma zanthorrhiza*, *Curcuma aeruginosa, Curcuma amada, Curcuma longa, Curcuma angustifolia, Curcuma aromatic, Curcuma australasica, Curcuma caesia, Curcuma caulina.*

Rumokoy et al. (2017[b]) showed that thoraxial protein could be used to enhance young horse immunity. Colostrum has been used as an

important substance for new born animal's defense against pathogenic agents (Rasmussen et al., 2016; Rumokoy and Toar, 2014).

MATERIALS AND METHODS

The study of the role of bio-resource substance on the development of parasite was realized by using a level of curcuma extract 20 mg/L and a colostrum immunoglobulin 2g/L of drinking water distributed to mice infected with *Strongyloides venezuelensis.* This drink water was distributed *ad libitum.*

Several parameters were evaluated: eggs per gram, and the number of female larva in gut of experiment animals.

In this investigation we used 17 female mice housed in Centre of Animal Research, Salamanca University.

All experiment animals were maintained in this research centre. The colostrum IgG was obtained through the laboratory of Parasitology USAL. The larvae suspended at a concentration of 3 x 10^5/mL in PBS.

The animals were divided in four group according to the treatment (T). T1 was the control treatment, the animals were infected only with the parasite. T2 was the group of animals infected and received curcuma. T3 was the treatment of immunoglobulin substance applied to the infected animals. T4 was the treatment of the curcuma mixed with colostrum immunoglobulin. The data collection of larva numbers was made at 7th days post infection, and the EPG was observed at 5th, 6th and 7th days post infection (DPI).

The data of eggs number in fecal matter were statistically analyzed by ANOVA. The development of female adult in the gut, for each group, were performed by using the SPSS software.

RESULTS AND DISCUSSIONS

The Table 1 and the Figure 1 represented that there has a significant response between the treatment ($P<0.01$) which the treatment of a combination between colostrum immune-globulin (T4) caused a highest number of larva observed while the single treatment of colostrum immunoglobulin substance was able to decrease the number of parasite in the gut.

Tabel 1. Treatment response on parthenogecetic females in gut on day 7th post infection

Treatment group	# mouse	Number of parasite found
T1a_inf	1	30
T1b_inf	2	45
T1c_inf	3	30
T1d_inf	4	35
T1e_inf	5	15
T2a_cur+inf	1	30
T2b_cur+inf	2	35
T2c_cur+inf	3	90
T2d_cur+inf	4	20
T3a_ig+inf	1	15
T3b_ig+inf	2	5
T3c_ig+inf	3	5
T3d_ig+inf	4	5
T4a_cur+ig+inf	1	30
T4b_cur+ig+inf	2	15
T4c_cur+ig+inf	3	170
T4d_cur+ig+inf	4	60

Notes: T1a-e_inf represented the treatment of infection with parasite only, T2a-d_cur_inf represented to the treatment of curcuma in animal infected with parasite, T3a-d_ig_inf represented to the treatment of colostrum immunoglobulin in animal infected with the parasite larva, and T4a-d_cur_ig_inf showed a treatment of curcuma mixed with colostrum immunoglobulin in infected mice.

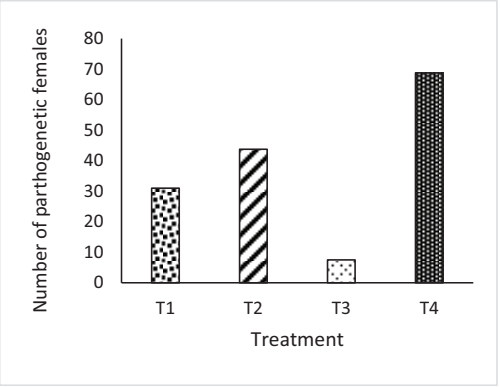

Figure 1. The comparison of each treatment respons on parasite number in the gut of experiment animals

The post hoc test by using the LSD analysis showed that the T3 and T4 has a significant different on parasite number in the gut. The response in T4 described that level curcuma (20 mg/L) mixed with colostrum immunoglobulin

in this experiment was not able to decrease the larva in gut compared to other treatment T2 and T3. This results showed that colostrum was more effective compared to the curcuma to overcome the parasite of *Strongyloides* in the gut. The positive effect of colostrum Ig as in treatment T3 on parasite infection in mice could be linked to the report of Rumokoy and Toar (2014) that colostrum IgG was important to protect the neonate from an infection with microbe or parasite *ex-utero* environment. According to Rasmussen et al. (2016) the neonates have a better chance to growth by receiving colostrum. Other possibility to overwhelm the problem of parasite infection is to empower the insect immunogenic substance as studied by Rumokoy et al. (2017[b]).

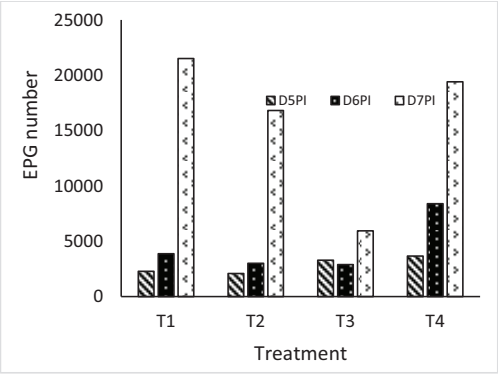

Figure 2. The treatments effect on EPG number. DPI corresponded to 'day post infection'

Figure 2 described a total of EPG of animal infected with parasite of *Strongyloides venezuelensis* after treating with curcuma and immunoglobulin colostrum to the animals. There has a non-significant difference of EPG number ($P>0.05$) between all treatment (T1, T2, T3 and T4). The highest number of EPG was in D7 post infection followed by D6 post infection. Otherwise in the T1 in the day 7thpost infection increased the EPG number compared to others treatment. The treatment T3 in all day of observation tended to reduce the number of EPG. Although the combination treatment between curcuma and colostrum immunoglobulin has no difference effect on parasite infection, but those substance has an expectation to be used in treating the other health problems as described by Maheshwari et al. (2006), Ruby et al. (1995). Gutiérrez-Gutiérrez et al. (2017) presented that curcumin disrupted the cytoskeletal structures of a protozoa (*Giardia lamblia*), it correlates with a reduction of tubulin expression and changes on its distribution. Furthermore, according to this authors that there are several drugs for treating giardiasis but they are often cause sides effect. Otherwise Morais et al., 2013, demonstrated that the *in vitro* incubation of *S. mansoni* with different doses of curcumin causes a reduction in viability of adult worms, a decrease in egg production and an increase in worm pairs separation.

CONCLUSIONS

Curcuma and colostrum immunoglobulin were useful to suppress the parthenogenetic female *Strongyloides venezuelensis* in the gut rather than to suppress the number of EPG in the fecal matter of experiment mice.

REFERENCES

Esam Almalki, Esam M. Al-Shaebi, Saleh Al-Quarishya, Mansour El-Matboulic, Abdel-Azeem, S.Abdel-Baki, 2017. *In vitro* effectiveness of *Curcuma longa* and *Zingiber officinale* extracts on *Echinococcus protoscoleces*. Saudi Journal of Biological Sciences, 24(1), 90-94.

Jolles A.E., Brianna R.B., Dolan B.P., 2015. Beyond mice and men: Environmental change, immunity and infections in wild ungulates. Parasite Immunol., 37(5), 255–266.

Maheshwari R.K., Singh A.K., Gaddipati J., Srimal R.C., 2006. Multiple biological activities of curcumin: a short review. Life Sciences, 78, 2081-2087.

Morais E.R., Oliveira K.C., Magalhães L.G., Moreira E.B.C., Verjovski-Almeida S., Rodrigues V. 2013. Effects of curcumin on the parasite *Schistosoma mansoni*: a transcriptomic approach. Mol. Biochem. Parasitol., 187, 91-97.

Rasmussen S.O., Martin L., Østergaard M.V., Rudloff S., Li Y., Roggenbuck M., Bering S.B., Sangild P.T., 2016. Bovine colostrum improves neonatal growth, digestive function, and gut immunity relative to donor human milk and infant formula in preterm pigs. Am. J. Physiol. Gastrointest Liver Physiol., 1, 311(3), G480-91. doi: 10.1152/ ajpgi.00139.2016. Epub 2016 Jul 21.

Ruby A.J., Kuttan G., Babu K.D., Rajasekharan K.N., Kuttan R., 1995. Anti-tumour and antioxidant activity of natural curcuminoids. Cancer Letters, 94, 79-83

Rumokoy L., Kaunang C., Toar W.L., 2017[a]. Efek ekstrak minyak citronella *Cymbopogon nardus* L. terhadap proporsi frekuensi kontak fisik *Musca domestica* L. (Diptera: Muscidae) padaransum dan performa broiler. Jurnal Entomologi Indonesia, 14(2), 89-96. DOI: 10.5994/jei.14.2.89.

Rumokoy L., Adiani S., Assa G.J.V., Toar W.L., Aban J.L., 2017[b]. Entomology contribution in animal immunity: Determination of the crude thoraxial glandular protein extract of *Stomoxys calcitrans* as an antibody production enhancer in young horses. Journal of Entomological and Acarological Research, 49 (3), 140-143. http://www.pagepressjournals.org/index.php/jear/article/view/7074/6924.

Rumokoy L.J.M., Toar W.L., 2014. The equine colostrums of milk treatment against pathogenic agent. Scientific Papers Series D. Animal Science, 52, 174-177.

Sanatombi R., Sanatombi K., 2017. Nutritional value, phytochemical composition, and biological activities of edible Curcuma species: A review. International Journal of Food Properties. 20(3). https://doi.org/10.1080/10942912.2017.1387556.

Toar W.L., Warouw J., Tulung M., Najoan M., Rumokoy L., 2013. The landing periodicity of *Stamoxys calcitrans* in ration, supplemented with citronella and papain on broiler health. Journal of Lucrări Ştiinţifice Seria Zootehnie, 59 (18). http://www.uaiasi.ro/zootehnie/Pdf/Pdf_Vol_59/Wisje_Lusia_Toar.pdf

Toar W.L., Kaunang C., Untu I.M., Rumokoy L., Kiroh H., 2017. The empowerment of crude extract antigen-G of insect on goats immunity enhancement. An entomology contribution in animal husbandry. Scientific Papers. Series D. Animal Science, Vol VX. http://animalsciencejournal.usamv.ro/pdf/2017/Art47.pdf

Scientific Papers. Series D. Animal Science. Vol. LXI, Number 1, 2018
ISSN 2285-5750; ISSN CD-ROM 2285-5769; ISSN Online 2393-2260; ISSN-L 2285-5750

THE PRESENCE OF INSECTS IN ANIMAL FARM IN NORTH SULAWESI

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Abstract

The existence of insects in the environment of animal farm has a big role in connection with the development of livestock production in tropical humid areas especially for those located in North Sulawesi, Indonesia. By understanding of the presence of insects in the animal farm environment could help to control the various roles of insect in transmitting pathogenic agents to livestock. Today this understanding is important because there are so many farms that are traditionally carried out which confronted with health control. This article aims to present some important insect order located within the livestock environment in North Sulawesi Indonesia as well as various achievements in detecting the existence of flies. Some of the important species of Diptera found in farms such as: Stomoxys calcitrans, Musca domestica, Musca bakeri, Chrysomyamega cephala, Haematobia exigua, Haematobia irritans and Sarcopaga spp. This scientific information is expected to be a technical information for farmers, observers, and researchers who are interested in this domain.

Key words*: insect, Diptera, livestock, environment.*

INTRODUCTION

Clean environmental conditions of livestock in tropical humid areas can support maximum livestock production. This situation depends on various factors, such as climate, sanitation level, quality aspects of cattle house construction, control of disease, and the commitment of farmers to maintain quality livestock management (Baldacchino et al., 2013).

The insects have a big role in the development of livestock. They can spread pathogenic agent to the animals (Rumokoy et al., 2017[a]) and therefore the presence of some orders of insect in North Sulawesi may interfere to the health of livestock even to its house building quality.

A farm which has traditionally maintained could get a higher risk than in modern livestock with more stringent health control standards (Toar et al., 2017).Up to now, in tropical humid region, exist many farms which are traditionally managed. For that reason, it is necessary to recognize the presence of various insect orders that can affect the livestock improvement.

This article specifically discusses the presence of insects, especially flies, in farming environments conducted in several locations in North Sulawesi Province, on the other hand to be linked with the development of cattle and goat farms located in this province.

MATERIALS AND METHODS

The presence of insect in animal livestock was detected through a field observation. The part of this study to evaluate the insect orders in an environment of livestock was realized in an animal house. An observation has been done in „Sentrum Agraris Lotta" (SAL) located in the village of Pineleng, Minahasa regency during a week in July of 2017. The animals reared in the animal houses were goats, pigs and cows. In this part, three objects were applied to detect the insects: animal feed, floor, and wood part of house construction.

To obtain the data of the species of flies (Diptera) in the livestock area then an observation was realized during seven days in cattle farms which were traditionally maintained from three regions in the North Sulawesi province in Indonesia: Minahasa, Minahasa Utara and Tomohon. The adhesive trap was used to collect the insects.

The livestock improvement achievement was evaluated by using the data about goats and cow's quantity evolution in North Sulawesi during a period of year 2011 to 2016. This data obtained from a data source of BPS North Sulawesi Indonesia (BPS, 2018).

RESULTS AND DISCUSSIONS

The data of the insects found around in farm sites is presented in Table 1 above. The order of Diptera was the most common type of insect found on the floor and in the remaining animal feed material in the cage.

Table 1. Some orders of insect found in animal husbandry

Insect order	Feed	Floor	Wood material
Diptera	+++	+++++	+
Coleoptera	++	+	++
Hymenoptera	+	++++	+++
Phtiraptera		+	+
Blattodea		++	++
Isoptera			+++

The Muscoid Diptera were an important insect in this order, and almost of them recognized as animal pest as reported by Almeida et al. (2014). Baldacchino et al. (2013) mentioned a member in *Muscidae* family was *Stomoxys calcitrans* as a pathogen agent transmitter in livestock. Rumokoy et al. (2017[a]) and Haselton et al. (2015) underlined that *Musca domestica* included also as an important pathogen transmitter to animals. This study confirmed that the presence of Coleoptera in animal feed could led a nutrition quality reducing.

Darsilawati (2015) has been studied five species of Coleoptera as pest in row material of animal feed: *Tribolium castaneum, Cryptolestes ferrugineus, Sitophilus zeamays, Alphitobius diaperinus, Oryzae philus surinamensis.* We found also that Formicidae as one of the insect family in Hymenoptera, was the most frequent insect found at the location of animal house during the observation. This insect acted as decomposer by feeding the organic matter in livestock area (Evans et al., 2011). We have found also another orders Phtiraptera and Blattodea. During the observation we have got these insects in the goat's pen although the activities of the parasite in general directly in the skin of the animals.

These insects could be a responsible organism for spreading of various pathogen micro-organism and parasites to animal husbandry. The Anoplura and Mallophaga were classified as suborder in this order (Ward, 1977). Almost of these insect found were grouped as ectoparasites. Seyoum et al. (2015) reported that some important ecto-parasites in small ruminant and by its infestation could lead to considerable economic losses to farmers due to loss of productivity, mortality, and skin diseases.

The abundance of flies in these region as described in Figure 1, showed that the highest values of the flies was *Haematobia irritans,* followed by *Musca domestica, Stomoxys calcitrans* while the others species still existed in almost areas, excepted *Haematobia exigua* was identified by our flies-trap the region of Minahasa Utara.

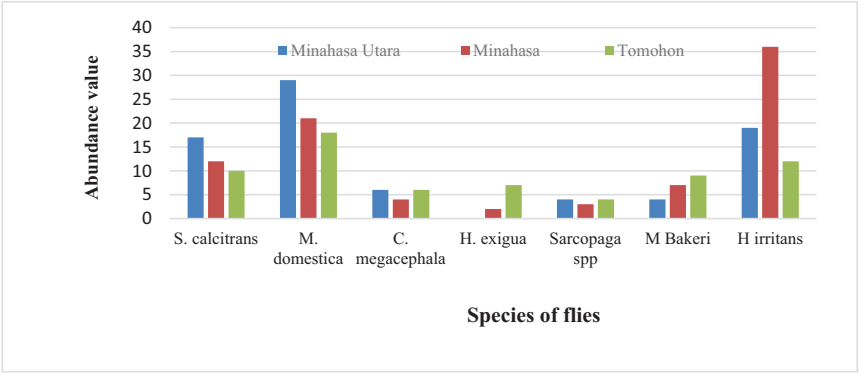

Figure 1. Graph of flies found cattle farm in Minahasa, Minahasa Utara and Tomohon

Table 2. Cows quantity evolution in North Sulawesi during a period of year 2011 to 2016

Areas	2011	2012	2013	2014	2015	2016
Bolaang Mongondow	22670	23433	21011	22406	23078	24693
Minahasa	17821	26113	17291	20559	21499	24238
Kepulauan Sangihe	1808	1802	1658	2017	1813	1843
Kepulauan Talaud	1702	1787	1386	1518	1603	1698
Minahasa Selatan	15431	17438	15541	16192	16439	17345
Minahasa Utara	13436	14271	14802	15341	16064	16718
Bolaang Mongondow Utara	12490	13072	12847	13738	14690	16392
Kepulauan Sitaro	60	4253	83	39	30	30
Minahasa Tenggara	3716	107	3935	4375	4392	4392
Bolaang Mongondow Selatan	3775	4050	3879	4214	4667	5442
Bolaang MongondowTimur	3108	3847	3098	4128	4595	4825
Kota Manado	2343	2462	2678	2692	2695	2964
Bitung	2226	2361	2564	2615	2668	2721
Kota Tomohon	2941	3110	3056	3202	3294	3619
Kota Kotamobagu	1733	1999	2118	2161	2217	2295
Sulawesi Utara	105260	120105	105947	115197	119744	129215

Source: BPS Indonesia

Table 3. Goats quantity evolution in North Sulawesi during a period of year 2011 to 2016

Areas	2011	2012	2013	2014	2015	2016
Bolaang Mongondow	8716	8654	8010	8034	8112	8176
Minahasa	3023	3059	3202	2682	2601	2601
Kepulauan Sangihe	3643	3703	3792	3856	5224	5284
Kepulauan Talaud	2161	2946	3012	3162	3034	2799
Minahasa Selatan	2394	3309	3329	3387	4046	4248
Minahasa Utara	3053	3475	3508	3542	3585	3680
Bolaang Mongondow Utara	6294	6483	6742	7972	9426	10634
Kepulauan Sitaro	549	1826	738	832	936	949
Minahasa Tenggara	1691	636	1646	2007	2487	1936
Bolaang Mongondow Selatan	3662	3793	4550	2680	2975	4267
Bolaang MongondowTimur	3250	3966	4110	2612	3678	4000
Kota Manado	1532	1538	1554	1552	1667	1883
Bitung	1701	1852	1873	1929	1989	2047
Kota Tomohon	895	951	895	860	812	570
Kota Kotamobagu	2199	1257	1220	1092	1120	1165
Sulawesi Utara	8716	8654	8010	8034	8112	8176

Source: BPS Indonesia

The legs and proboscis of flies used as carriage instruments to transport the pathogenic microbes to other animals (Barro et al., 2006; Toar et al., 2013)

The evolution of livestock quantity as presented in Table 2 and Table 3 describes the condition of animal husbandry production, particularly connected to the quantity of cows and goats in North Sulawesi during the years 2011 up to 2016. In this province, the increasing of cow's quantity occurred sharply from the year of 2013 (105,947 heads) until to the year of 2016 (129,215 heads). This reality was different if compared to the quantity of the goats: the quantity of goat's number dropped sharply from 8,716 goats in 2011, reduced to 8,010 in 2013, after that tended to be increased slightly in 2014 (8,034 heads), and then in 2016 raised up to be 8,176 heads. It has been recorded that in 2008 has reached up to about 16,000 heads (BPS, 2018), which meant that in this intervals of time about 50% declined. Although the goat's production was decreased but according to Ditjenak (2015) reported that in the GRDP (*gross regional domestic product*) of livestock in North Sulawesi province Indonesia started at 996 billion rupiahs in the year of 2010 attained 1.305 billion rupiahs in the year of 2014.

The decrease in production in terms of amounts in goat farms has a relation with disease caused by microbes or parasites agents such as insects. Some scientific efforts to control the adverse insects have been reported by Rumokoy et al. (2017[b]), Baldacchino et al. (2013) Barro et al. (2006). It becomes a challenge in overcoming the impact of insect both as ecto-parasites and as organisms that degrade the quality of feed or pen house especially that made of wood. However, there are always other factors, intercepting each other as a challenge in developing livestock production in North Sulawesi in the zone of the tropical humid climate.

CONCLUSIONS

We concluded that various insect orders were capable to spread pathogens in livestock in North Sulawesi Province located in tropical humid climates. To overcome the negative role problems of some insects in livestock development, it should also necessary to consider other factors contributing to the livestock improvement.

ACKNOWLEDGEMENTS

We address our thankful to all parties for those who have contributed to this study, specifically addressed to the Director of „Sentrum Agraris Lotta" (SAL) who has provided facilities support for conducting some part of this research.

REFERENCES

Almeida J.L., Giufrida R., Andrade R.A.P., Chave M.P., 2014. Muscoid Díptera as potential vectors of bacterial agents on dairy farms in the northern region of Paranà, Brazil. Semina: Ciências Agrariárias (Londrina), 35 (6), 3127-3137. http://dx.doi.org/10.5433/1679-0359.2014v35n6p 3127.

Baldacchino F., Muenworn V., Desquesnes M., Desoli F., Charoenviriyaphap T., Duvallet G., 2013. Transmission of pathogens by Stomoxys flies (Diptera, Muscidae): a review. Parasite, 20 (26), 1–13.

Barro N., Aly S., Tidiane O.C., Sababénédjo T.A., 2006. Carriage of bacteria by proboscises, legs, and feces of two species of flies in street food vending sites in Ouagadougou, Burkina Faso. J. Food Prot., 69(8), 2007-2010.

BPS (BadanPusatStatistik) Indonesia. www.bps.go.id. Accessed 14 April 2018.

Darsilawati I., 2015. Hama gudangordo Coleoptera pada bahan baku pakan ternak impor dan status resistens in yaterhadapfosfin. Tesis. Sekolah Pascasarjana Institut Pertanian Bogor.

Ditjenak (Direktorat Jendral Peternakan dan Kesehatan Hewan), 2015. Livestock and animal health statistic. 238 p.http://perpustakaan.bappenas.go.id/lontar/file? file=digital/156517-[_Konten_]-Konten%20D782. pdf. Accessed 25 April 2018.

Evans T.A., Dawes T.Z., Ward P.R., Lo N., 2011. Ants and termites increase crop yield in a dry climate. Nature Communications, 2, 262. http://doi.org/ 10.1038/ncomms1257.

Forrester D.J., Mclaughlin G.S., Telford Jr S.R., Foster G.W., Mccown J.W., 1996. Ectoparasites (Acari, Mallophaga, Anoplura, Diptera) of White-Tailed Deer, *Odocoileus virginianus* from Southern Florida. Journal of Medical Entomology, 33(1), 96–101.

Haselton A.T., Acevedo A., Kuruvilla J., Werner E., Kiernan J., Dhar P., 2015. Repellency of α-pinene against the house fly, *Musca domestica*. Phytochemistry, 117, 469-75. doi: 10.1016/j.phytochem

Rumokoy L., Toar W.L., 2015. The Paradox of Nutrient Fulfilement and Immunity Challenge on Chicken Livestock Development in Tropical Humid Regions. Journal of Agriculture and Agricultural Science Procedia, 6, 259-264. DOI 10.1016/j.aaspro. 2015.08.069

Rumokoy L., Kaunang C., Toar W., 2017[a]. Effects of citronella oil extract of *Cymbopogon nardus* L. on contact frequency proportion of *Musca domestica* L. (Diptera: Muscidae) in ration and broilers performance. Indonesian Journal of Entomology (JEI), 14(2), 89–96. DOI: 10.5994/jei.14.2.89.

Rumokoy L., Adiani S., Assa G.J.V., Toar W.L., Aban J.L., 2017[b]. Entomology contribution in animal immunity: Determination of the crudethoraxial glandular protein extract of *Stomoxys calcitrans* as antibody production enhancer in young horses. Journal of Entomological and Acarological Research, 49(3), 140–143. http://www.pagepressjournals.org/index.php/jear/article/view/7074/6924

Seyoum Z., Tadesse T., Addisu A., 2015. Ectoparasites Prevalence in Small Ruminants in and around Sekela, Amhara Regional State, Northwest Ethiopia. Journal of Veterinary Medicine, article ID 216085, 6 pages. http://dx.doi.org/10.1155/2015/216085.

Toar W.L., Kaunang C., Untu I.M., Rumokoy L., Kiroh H., 2017. The empowerment of crude extract antigen-G of insect on goats immunity enhancement: An entomology contribution in animal husbandry. Scientific Papers, Series D, Animal Science, vol. LX, 271-273.

Toar W.L., Warouw J., Tulung M., Najoan M., Rumokoy L., 2013. The Landing Periodicity of *Stomoxys calcitrans* in rations, supplemented with citronella and papain on broiler health. Lucrări Ştiinţifice-Universitatea de Ştiinţe Agricole şi Medicină Veterinară, Seria Zootehnie, 53, 325-328.

Ward R.A., 1977. Pathogens of Anoplura and Mallophaga. Bull World Health Organ., 55(1), 305–309.https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2366774/.

Scientific Papers. Series D. Animal Science. Vol. LXI, Number 1, 2018
ISSN 2285-5750; ISSN CD-ROM 2285-5769; ISSN Online 2393-2260; ISSN-L 2285-5750

EFFECT OF COMBINATION CHITOSAN AND TURMERIC POWDER (*Curcuma domestica* Val.) FOR IMPROVING BLOOD LIPID PROFILE IN BROILERS

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Abstract

Chicken's meat is commonly eaten by most of the world's population. Besides, it has a good source of dietary protein and can provide high biological value, the meat also has a source of fat including saturated fatty acids (SFA), unsaturated fatty acids (USFA), cholesterol, triacylglycerol and phospholipids. But lipids categories can be the risk factors or the initiator of metabolic disease. The study aimed to know a potential and the best level of combination chitosan and turmeric powder for improving composition of blood lipid profile such as cholesterol and triacylglycerol, so it can increasing meat quality. The experimental was held for 30 days and the blood samples was investigated in the last days. The experimental design was used completely randomized design with 5 treatment (R1 = Chitosan 0% + Turmeric Powder 0%; R2 = C 1% + T.P 1%; R3 = C 2% + T.P 1%; R4 = C 1% + T.P 2%; R2 = C 2% + T.P 2%) and 4 replicated, in total using 100 DOC. The result is significantly (P<0,05) decreasing cholesterol and triacylglycerol levels. The best level recommendation of combination chitosan and turmeric powder is 1-2%.

Key words*: chitosan, turmeric powder, cholesterol, triacylglycerol, broiler.*

INTRODUCTION

Chitosan is a chitin derivative, a natural polymer compound which was isolated from aquaculture waste, such as shrimp shells and crab shells with a chitin content of between 65-70%. This compound was processedby chemical using sodium hidroxide or an enzymatic using a chitin deacetylase. Chitosan is a multipurpose fiber-shaped chemical and is a white, or yellow copolymer with a particle size, less than 30 μm, with specific gravity is 1.35 - 1.40 g/cm^3.

Chitosan has the ability to decrease triglyceride levels by reducing the absorption of triglycerides, as well as other lipidslike cholesterol. Reducing triglyceride absorption will affect to binding fat molecules from feed and then absorbed by the intestinal mucosa. Chitosan is capable to bindfats such as triglycerides through hydrophobic bonds as a fat absorber (Martati and Lestari, 2008).

The other function of Chitosan is an antioxidant that prevent apperances of free radicals. The activity as an antioxidant relates to amino and hydroxyl groups in the reversed position of C-2, C-3 and C-6, with unstable free radicals forming stable macromolecular radicals. Chitosan action can be assisted by turmeric powder.

Turmeric is an herbal plant that contains active compounds such as essential oils, curcumin and flavonoids. The chemical composition of turmeric is water 6.0%, protein 8.0%, carbohydrate 57.0%, crude fiber 7.0%, mineral 6.8%, volatile oil 3.0%, curcuma 3.2%, non-volatile materials 9.0% (Bintang and Nataamijaya, 2005). Chemical compounds in turmeric can reduce fat in the body through the process of bile secretion and released by feces.

According to Riyadi (2009), curcumin can improve the poultry digestive activity by preventing chain reaction, forming lipid peroxidation, and taking free hydrogen atoms, andalso reducing and arresting free radicals. Moreover, it also stimulates the gallbladder wall to secrete bile and pancreatic to release of amylases, lipases and proteases that are useful for improving digestion carbohydrates, fats, proteins, and expedite bile secretion (Agustina and Sri, 2009).

Essential oils can reduce triglyceride levels by inhibiting the action of forming triglyceride, i.e glycerol-3-phosphate derived from glycerol and dihydroxy acetone phosphate and reduced synthesize of Glycerol-3-Phosphate

dehydrogenase (GPDH) assynthesis triglyceride enzyme. This substance is also capable to decrease the activity of Glycerol-3-Phosphate dehydrogenase (GPDH), which is an enzyme in the biosynthesis of triglycerides. Increased free radical also decreases lipoprotein lipase (LPL) enzyme activity, which is causing accumulative triglyceride in liver cells and liver cell will degeneration (Goldberg, 2001). Flavonoids in turmeric can increase the activity of LPL enzymes so it can lower triglyceride levels by inhibiting the occurrence of free radicals.

According to Amic (2003), flavonoids act as antidote to free radicals, which is has hydroxyl (OH-) groups in aromatic rings and stopping lipid peroxidation chain reactions by protecting cells. The antioxidant mechanism can occured by administration of hydrogen atoms and slowrate of autoxidation.

Research on chitosan by Amrullah et al. (2010), stated that giving chitosan 1 - 1.5% can give positive effect to decrease triglyceride level and raise HDL level of duck blood. Another study by Ardi et al. (2013) demonstrated that administration of turmeric starch mixtures in rations at 1.5% level was the most efficient to increase HDL levels and lower blood triglyceride levels of broiler chickens.

Sundari et al. (2014) states that turmeric extracts of powdered dosage encapsulated with chitosan in nanoparticle size can increase the digestibility of curcumin from 46 to 70.64%. Provision of broiler chicken with a level of 0.4% significantly improves the body performance, intestinal performance, digestibility, antibiotic free meat residue, high protein, contains fatty acid eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) as well as lower abdominal fat, subcutaneous and cholesterol.

Based on the description in the above, hypothesis can be withdrawn that with the addition of 1% dosage chitosan and turmeric dose 2% of the total ration can lower levels of triglycerides and blood cholesterol.

MATERIALS AND METHODS

A total of 100 DOC's Broiler was raised during 28 days. The treatment started from second weeks until the broiler's age 28 days. The ratio from commercial with content is sufficient for broiler's maintenance and production. Energy metabolism and protein in the ratio is 3025-3125 Kcal/kg and 21.5-23.8%, respectively.

The experimental design used Completely Randomized Design (CRD), within 5 dietary treatment groups. The replicates were designated as the experimental units and were randomized with respect to the dietary treatments. The five dietary treatments were: (1) Basal ratio + 0% chitosan + 0% turmeric powder; (2) Basal ratio + 1% chitosan + 1% turmeric powder; (3) Basal ratio + 1% chitosan + 2% turmeric powder; (4) Basal ratio + 2% chitosan + 1% turmeric powder; (5) Basal ratio + 2% chitosan + 2% turmeric powder. Data will be analyzed by statistical method using Anova and continued by multiple test Duncan.

RESULTS AND DISCUSSIONS

The results in table 1 and figure 1 showed that triglyceride levels of treatment R5 were significantly different ($P<0.05$) lower than R1, but R4, R3, and R2 were significantly different ($P<0.05$) compared to R1. Levels of triglycerides between R2 and R3 were not significantly different ($P>0.05$), whereas at R5 and R4 were significantly different ($P<0.05$) lower than R2 and R3. The results can show a decrease in triglyceride levels. The above explains that the addition of a 2% chitosan and 2% turmeric mixture of the total ration reduced triglycerides, i.e 7.13 µL/mL, lower than broiler with triglyceride levels without chitosan and turmeric, i.e 14.16 µL/mL.

It can be said that giving high chitosan level on R5 (2%) can decrease blood triglyceride of broiler chicken if accompanied by giving turmeric with high level (2%). However, there is a tendency to give chitosan and turmeric with unbalanced levels (R3 and R4) and with a balanced but low level (1% each) in R2 actually increases blood triglyceride levels of broiler chickens.

Based on the results of this study can be explained that under normal conditions (without giving chitosan and turmeric), the fat contained in the diet will be decomposed into cholesterol, triglycerides, phospholipids and free fatty acids when digested and then transported by kilomicron (Linder, 2006).

Table 1. Triacylglycerol and cholesterol levels with treatment of combination chitosan and turmeric powder in broiler blood lipid profile

Parameter	R1	R2	R3	R4	R5
Triacyl-glycerol (µL/mL)	14.16 ± 2.69[b]	35.90 ± 1.56[d]	35.86 ± 2.18[d]	19.75 ± 3.94[c]	7.13 ± 0.53[a]
Cholesterol (µL/mL)	35.09± 2.42[e]	22.07± 0.57[c]	12.81± 0.71[a]	25.14± 0.91[d]	14.89± 0.46[b]

Kilomicron will bring triglycerides and cholesterol into the bloodstream, then triglycerides in kilomicrons are decomposed by lipoprotein lipase enzymes to form free fatty acids and kilomicron remains. Kilomicron will enter the lymphatic system and will eventually lead to blood flow, which ishydrolyzed by lipoprotein lipase into free fatty acids. The free fatty acids will be absorbed by the vascular endothelium and partially stored in the adipose tissue in the form of triglycerides. Some triglycerides will be taken by the liver to form liver triglycerides if they are in large quantities. Liver triglycerides are secreted into the blood circulation in the form of VLDL (Very Low Density Lipoprotein) (Linder, 2006). Based on these facts that cause blood triglyceride levels without chitosan and turmeric treatment (R1) is quite high compared to R5.

High triglyceride levels with chitosan on R4, R3 and R2 may be due to increased feed digestibility by chitosan (Huang et al., 2005). Xu et al. (2013) also reported that giving chitosan at a certain level actually causes improving the rhythm or metabolic rate. This means that chitosan causes anabolic metabolism was increased, so the synthesis of triglycerides from fatty acids also increased. This condition is also stimulated by the action of turmeric powder, as Bengmark et al. (2009) reported that curcumin with a level that is not too high can improve the efficiency of rations, stimulate anabolism, causing adipose tissue to increase. This is because the content of the substance curcuminoids and essential oils in turmeric is effectively absorbed by intestinal epithelial cells, thus affecting metabolism.

According to the results of this study, the combination of chitosan and turmeric flour with a certain level (R2, R3, and R4) actually increase the formation of triglycerides. The decrease of chicken blood triglyceride level drastically by treatment of R5 (2% chitosan + 2% turmeric flour) is 7.13 µL/mL because of the ability of essential oil to decrease Glycerol-3-Phosphate Dehydrogenase (GPDH) activity, that is enzyme that play a role in triglyceride synthesis. Inhibition of triglyceride synthesis in the liver and small intestine will result in a decrease in blood triglyceride levels (He et al., 2009).

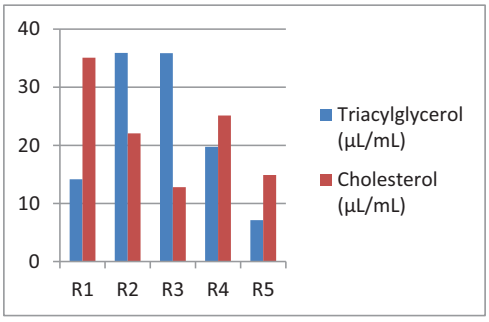

Figure 1. Level of triacylglicerol and cholesterol in 5 dietary treatment of combination chitosan and turmeric powder

The activity of this enzyme (GPDH) increases because it was supported by the giving of chitosan with high level (2%). Huang et al. (2005) showed that chitosan can improve the condition of intestine acidity as the impact of oligosaccharide reform from chitosan by lactate bacteria in intestine. Xu et al. (2013) showed that the morphology of intestine looked better with chitosan and increased absorption of macro and micro minerals as a result of increased acidity in the intestine. It was further reported that the activity of hormones and enzymes increased as a simulated effect of minerals absorbed passed the intestine

The presence of tannins in turmeric can also lead to the occurrence of coatingsintestinal membrane to inhibit the absorption of nutrients. The tannins in the body bind proteins and will coat the intestinal wall, forming a layer of mucus in the digestive tract and inhibiting the absorption of nutrients, including triglycerides (Adriani, 2014). Based on that the combination of chitosan and turmeric flour with the right dose decreases triglyceride levels. Conversely, an incorrect combination of doses actually can increases triglycerides.

Chitosan and turmeric powder can basically lower blood cholesterol levels by different mechanisms. Chitosan as a natural fiber can

lower cholesterol levels that work in the intestine, because the fiber was hard absorbed by the intestine. The role of dietary fiber is to increase the production of bile acids for excretion.

Wolever (1997) states the mechanisms of cholesterol reduction by fiber caused by binding of bile acids in the small intestine which can lead to increased excretion of fecal bile acids, decreased absorption of fat and cholesterol. Thus, can decreased carbohydrate absorption with resulting in decreased serum insulin levels and decreasing stimulation of cholesterol synthesis and lipropotein. Decreased insulin can slow the activity of HMG-CoA reductase so that cholesterol formation can be slowed too (Murray, 2014).

The role of turmeric as a cholesterol-lowering mechanism can be understood considering turmeric contains substances that can lower cholesterol levels.

The content of these substances such as curcumin and essential oils. Curcumin is a phenol compound that can increase the secretion of bile acids. A decrease in cholesterol levels through bile acid secretion is a major route in cholesterol excretion, a pathway called enterohepatic circulation. Bile acids are formed from cholesterol and synthesized in the liver. Most of the released bile acids will be reabsorbed by the small intestine in the ileum (98-99%) and the rest will be excreted by the feces (Murray, 2014).

Essential oils can lower blood cholesterol levels of broiler chickens. This decrease resulted from HMG-CoA reductase inhibited its performance. The inhibitory properties of essential oils are similar to the use of statin substances such as simvastatin (Murray, 2014). The results showed that the treatment of R3 (1% chitosan + 2% turmeric flour) was the most optimal treatment to lower blood cholesterol levels compared with other treatments.

Related to this can be explained that R3 is the best level in lowering blood cholesterol, this is in accordance with the results of research Zhou et al. (2009), that giving chitosan with higher levels can lower total cholesterol but increase LDL (Low Density Lipoprotein) cholesterol. It is known that LDL plays a role in transporting cholesterol from the liver tissue to all body cells. This means that chitosan with a high level of 2% (R4 and R5) is the cause of increased blood cholesterol levels. Similar results were also reported by Lim et al. (2006), higher cholesterol levels with increased chitosan levels in broiler chickens.

Giving of 2% turmeric with 2% chitosan combination still shows higher cholesterol level compared to R3 because turmeric also stimulates the increase of HDL (High Density Lipoprotein) cholesterol (Claeson et al., 1993). However, the dominance effect of chitosan is higher with increasing LDL levels than HDL (Lee et al., 2001).

CONCLUSIONS

In conclusion, there was decreased in cholesterol and blood triglyceride levels which significantly increased the mixture of chitosan and turmeric supplementation. The mixture of chitosan supplementation 1% and 2% turmeric starch (R3) showed the lowest blood cholesterol level (12,81 µL/mL), however the mixture of chitosan supplementation 2% and 2% turmeric flour (R5) showed the lowest tryacilglycerol level (7.13 ± 0.53 µL/mL) compared with other treatments.

ACKNOWLEDGEMENTS

This research work was carried out with the support of college faculty students who had assisted in this research.

REFERENCES

Adriani L., 2014. Decreasing Cholesterol And Triglyceride Level On Blood By Adding Orange (Citrus Sinensis) Waste On Padjajaran I Sheep. Fakultas peternakan. Universitas Padjadjaran. Sumedang.

Agustina Laily, Sri Purwanti, 2009. Ilmu Nutrisi Unggas. Lembaga Pengembangan Sumberdaya Peternakan (IDICUS): Makassar.

Amic D., Beslo D., 2003. Structure-radical Scavenging Activity Relationships of Flavonoids. CCACCA 76(1), 55-61.

Amrullah M.P., Indriyani Nur, 2010. Pengaruh Kitosan Asal Cangkang Udang Terhadap Kadar Lemak dan Kolesterol Darah Itik, Vol: 18, ISSN 0854-0667.

Bengmark S., Mesa M.D., Gil A., 2009. Plant-derived health - the effects of turmeric and curcuminoids Nutr. Hosp., 24(3), 273-281

Bintang I.A.K, Nataamijaya A.G., 2005. Pengaruh penambahan tepung kunyit (*Curcuma domestica* val) dalam ransum broiler. http://balitnak.litbang.deptan.go.id/index2.php?option=com_content&do_pdf=1&id=129. (access web at 10 October 2017, 19.30 WIB).

Claeson P., Panthong A., Tuchinda P., Reutrakul V., Kanjanapothi D., Taylor W.C., Santisuk T., 1993. Three Non Phenolic Diarylheptanoids with anti-inflammatory activity from *Curcuma xanthorrhiza*. Planta Medica, 59(5):451-454.

Goldberg I.J., Merkel M., 2001. Lipoprotein Lipase, physiology, biochemistry and molecular biology. Front Biosci, 6, D388.

Goldberg I.J., Merkel M., 2001. Lipoprotein Lipase, Physiology, biochemistry and molecular biology. Front Biosci, 6:D388.

He M.L., Yang W.Z., You J.S., Chaves A.V., Mir P.S., Benchaar C., McAllister T.A., 2009. Effect of garlic oil on fatty acid accumulation and glycerol-3-phosphate dehydrogenase activity in defferentiating adipocytes. Asian-Aust. J. Anim. Sci., 22, 1686-1689.

Huang R.L., Yin Y.L., Li M.X., Wu G.Y., Li T.J., Li L.L., Yang C.B., Zhang J., Wang B., Deng Z.Y., Zhang Y.G., Tang Z.R., Kang P., Guo Y.M., 2005. Effect of dietary oligochitosan supplementation on ileal digestibility of nutrients and performance in broilers. China Agricultural Univ. Press, Beijing, China.

Lee Y.K., Paik I.K., Jang M.B., Son T.I., 2001. Preparation of chitosan mineral chelate and applecation of feed additives. J. Food Resources Institute, Chungang University, 13(1), 61-68.

Lim H.S., Paik I.K., Sohn T.I., Kim W.Y., 2006. Effects of supplementary copper chelates in the form of methionine, chitosan and yeast on the performance of broilers. Asian-Aust. J. Anim. Sci., 19(9), 1322–1327.

Linder M.C., 2006. Biokimia Nutrisi dan Metabolisme. Jakarta: UI Press.

Martati E., Lestari L.A., 2008. Pengaruh Pemberian Kitosan terhadap Profil Lipid Serum Darah Tikus Sprague Dawley. Jurnal Teknologi Pertanian, Universitas Brawijaya, 9(3), 157-164.

Murray R.K., Bender D.A., Botham K.M., 2014. Biokimia Harper, Edisi 29. Buku Kedokteran EGC, Jakarta, ISBN 978-0-07-176576-3.

Riyadi S. 2009. Kunyit dan Jahe Baik untuk Ayam Broiler. http://slamet-riyadi03.blogspot.com/2009/04/kunyit-dan-jahe-baik-untuk-ayam-broiler.html. (access web at 10 October 2017, 19.30 WIB).

Sundari Zuprizal, Tri-Yuwanta, Ronny Martien, 2014. Pengaruh Nanokapsul Ekstrak Kunyit dalam Ransum terhadap Kualitas Sensori Daging Ayam Broiler. Universitas Gadjah Mada, Yogyakarta.

Wolever T.M.S., Hegele R.A., Connelly P.W., Ransom T.P.P., Furumoto E.J., 1997. Long-term effect of soluble-fiber foods on postprandial fat metabolism in dyslipidemic with E3 and apo E4 genotypes. Am.J. Nutr., 66, 584-590.

Xu Yuanqing, Binlin Shi, Sumei Yan, Tiyu Li, Yiwei Guo, Junliang Li, 2013. Effects of Chitosan on Body Weight Gain, Growth Hormone and Intestinal Morphology in Weaned Pigs. College Of Animal Science, Inner Mongolia Agricultural University, Huhhot 010018, China.

Zhou T.X., Chen Y.J., Yoo J.S., Huang Y., Lee J.H., Jang H.D., Shin S.O., Kim H.J., Cho J.H., Kim I.H., 2009. Effects of chitooligosaccharide supplementation on performance, blood characteristics, relative organ weight, and meat quality in broiler chickens. Poultry Science, 88, 593–60.

Scientific Papers. Series D. Animal Science. Vol. LXI, Number 1, 2018
ISSN 2285-5750; ISSN CD-ROM 2285-5769; ISSN Online 2393-2260; ISSN-L 2285-5750

STUDY OF THE VARIATION OF BODY PARTS AND CARCASSES AT THE SHEEP YOUTH OF THE TSIGAI BREED AND CROSSBRED (GERMAN BLACKHEADED X TSIGAI) FATTENED IN INTENSIVE SYSTEM

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Abstract

The paper aim to present the variation of body parts and the main characteristics of carcasses obtained from sheep youth of the Tsigai breed and crossbred (German Blackheaded breed x Tsigai breed) fattened in intensive system. The intensive fattening experiment developed over a 100-day time period was performed on Tsigai young male sheep (lot 1), and young male sheep crossbred German Blackheaded (GBH) x Tsigai (lot 2). The two batches of animals subjected under fattening were made up of 12 heads each. At the end of experiment were slaughtered 5 animals from each lot. The subjective assessment of the carcasses through the SEUROP classification system after conformation and the fattening status led to framing in a superior classification of carcasses provided from the crossbreds compared to those obtained from Tsigai breed. The warm carcass weight in crossbred and Tsigai breed recorded average values of 19.04 kg at GBH x Tsigai and 16.95 kg at Tsigai breed, with distinctly significant difference to the advantage of the GBH x Tsigai (+2.09 kg, p <0.01). Also, distinctly significant differences in favor of the batch GBH x Tsigai (+2.10 kg) were recorded regarding the cold carcass weights compared with the Tsigai breed batch. Regarding the main cut sections of carcass from young sheep submitted to intensive fattening, the crossbred lot recorded greater average values (in particular in the cuts of first quality - pulp and cutlet) compared to the Tsigai breed lot. In conclusion, the assessment of the carcasses according to subjective and objective methods highlights a higher potential of meat production and of better quality at GBH x Tsigai lot, compared with the Tsigai breed lot.

Key words*: assessment, carcasses, crossbreds, German Blackheaded breed, Tsigai breed.*

INTRODUCTION

Worldwide, especially in countries with a developed animal production, the improving activity for meat production at sheep has held and continues to play a leading role. The interest in quantitative and qualitative improving of meat production at sheep has led to the creation of specialized breeds for meat production and the development of methods for the production of meat lambs and new technologies for rearing and fattening of sheep youth (Taftă, 2010).

In Romania, up to 1990, sustained efforts have been made to improve local sheep breeds in meat production direction along with the applying of advanced technologies for fattening of sheep youth (in intensive and semi-intensive system). After 1990 year the improvement of local sheep breeds for meat production is a activity limited as concern in scientific research and almost non-existent in the practice of sheep rearing.

At present, the annual consumption of sheep meat in our country is low (2.6 kg/capita), being polarized on the milk lamb during the Easter holidays and of the young sheep and the reformed adult sheep during the autumn period (MARD, 2018). The categories mentioned above in annual consumption per capita are exploited in extensive system based on cheap feed (hay and pasture grass, depending on the season) without the use of any fattening technology.

The main way to rapidly improve the growth rate and meat quality is the crossbreeding of the local sheep breeds with specialized sheep breeds for meat production (Taftă et al., 1997; Răducuță, 2010; Pădeanu, 2011).

During the last 10 years, for this purpose, the professional associations of sheep breeders from Romania, as well as the specialized universities and research centres, imported animals from specialized meat breeds from different European countries (Duman et al., 2017).

The paper aim to present the variation of body parts and the main characteristics of carcasses obtained from sheep youth of the Tsigai breed and crossbreds (German Blackheaded breed x Tsigai breed) fattened in intensive system.

MATERIALS AND METHODS

The intensive fattening experiment developed over a 100-day time period was performed on Tsigai young male sheep (lot 1), and young male sheep crossbred German Blackheaded (GBH) x Tsigai (lot 2), obtained at the Reghin Research and Development Station for Sheep and Goats in Mures County in 2016. The two batches of animals subjected under fattening were made up of 12 heads each. At the end of experiment were slaughtered 5 animals from each lot (Duman et al., 2017).

The following determinations were recorded in slaughtered young sheep: variation of body parts, live weight before slaughter, warm carcass weight, cold carcass weight and cold slaughter yield, subjective assessment of the carcasses through the SEUROP classification system after conformation and the fattening status, the main cut sections of carcass and chemical composition of meat.

The main statistical parameters were calculated (average, standard deviation, coefficient of variation, average error etc.) and the significance of differences between groups was performed by applying Student test.

RESULTS AND DISCUSSIONS

The weight of parts of the body is important in the conditions in which it provides information about phenotypic correlations, the physiological and metabolic relationships that can be established between the development and the proportion of certain parts of the body and the abilities of the sheep breeds for meat production (Răducuță and Custură, 2010).

The variation of constituent parts of body at young sheep from two batches of animals subjected under fattening are presented in Table 1. From this data it can be note that are distinctly significant differences (P<0.01) between crossbred GBH x Tsigai batch and control group (M) on live weight at slaughter, hot carcass weight and full gastrointestinal tractus, fact that highlights the superior abilities for meat production in favor of crossbreds lot.

Table 1.The variation of constituent parts of body at young sheep

Specification	Breed/Crossbred (n = 5)	$\overline{X} \pm s_{\overline{x}}$	%
Live weight at slaughter (kg)	Tsigai GBH x Tsigai	36.80 ± 0.29 39.68 ± 0.63**	100 100
Hot carcass weight (kg)	Tsigai GBH x Tsigai	16.95 ± 0.41 19.04 ± 0.36**	46.06 47.98
Full gastro-intestinal tractus (kg)	Tsigai GBH x Tsigai	8.26 ± 0.21 9.54 ± 0.24**	22.45 24.04
Empty gastro-intestinal tractus (kg)	Tsigai GBH x Tsigai	3.94 ± 0.08 4.01 ± 0.08NS	10.71 10.11
Skin weight (kg)	Tsigai GBH x Tsigai	3.01 ± 0.13 3.42 ± 0.17NS	8.18 8.62
Head weight (kg)	Tsigai GBH x Tsigai	1.37 ± 0.01 1.40 ± 0.01*	3.72 3.53
Spleen weight (g)	Tsigai GBH x Tsigai	74.33 ± 3.14 78.60 ± 3.95NS	0.20 0.20
Pulmon weight (g)	Tsigai GBH x Tsigai	623.33 ± 23.84 670.45 ±17.18NS	1.69 1.69
Liver weight (g)	Tsigai GBH x Tsigai	842.00 ± 14.75 866.67 ± 14.12NS	2.29 2.18
Heart weight (g)	Tsigai GBH x Tsigai	227.50±5.24 236.33±6.08NS	0.62 0.60
Kidney weight (g)	Tsigai GBH x Tsigai	111.30±4.16 116.00±6.12NS	0.30 0.29
Extreme weight (kg)	Tsigai GBH x Tsigai	1.21 ± 0.02 1.33 ± 0.03**	3.92 3.35

Note: Student test: NS=not significant (p > 0.05);*=significant (p < 0.05); ** = distinctly significant (p < 0.01);***= very significant (p < 0.001).

It can also be noted that there are significant differences (P<0.05) regarding the head weight and distinctly significant differences in the weight of the extremities (P<0.01) in favor of the animals from crossbreds group, while for the other body components the differences are not significant (P>0.05).

The average values and differences concerning body weight before slaughter, warm and cold carcass weight and cold slaughter yield are presented in Table 2. Thus, the average of body weight before slaughter is for GBH x Tsigai 39.68 kg, while for Tsigai young sheep is 36.80 kg, being with about 8% higher (distinctly significant difference) in favor of crossbred lot.

The warm carcass weight in crossbreed and Tsigai breed recorded average values of 19.04 kg at GBH x Tsigai and 16.95 kg at Tsigai breed, with distinctly significant difference to

the advantage of the GBH x Tsigai lot (+2.09 kg, p<0.01).

Table 2.The mean values of the carcass weight and slaughtering yield

Specification	Tsigai lot (n = 5)	GBH x Tsigai lot (n = 5)
Weight before slaughtering (kg)	36.80	39.68**
Warm carcass weight (kg)	16.95	19.04**
Cold carcass weight (kg)	16.56	18.66**
Cold slaughter yield (%)	45.00	47.03**

Note: Student test: NS=not significant (p > 0.05);*=significant (p < 0.05); ** =distinctly significant (p < 0.01);***= very significant (p < 0.001).

Also, distinctly significant differences between a group of GBH x Tsigai (+2.10 kg) were recorded regarding the cold carcass weights compared with the Tsigai breed lot. Regarding cold slaughter yield, distinctly significant differences (p<0.01) were recorded between crossbred lot and the Tsigai breed lot, being with 2 procentual points higher in the advantage of crossbred lambs.

Concerning the pulp, cutlet, shoulder + arm and carcass rest, the results are presented in Table 3.

Table 3.The main cut sections of carcass from young sheep submitted to intensive fattening

Specification	Tsigai lot (n = 5)	GBH x Tsigai lot (n = 5)
Cold carcass weight (kg)	16.56 ± 0.44	18.66 ± 0.38**
Pulp (kg)	5.16 ± 0.25	6.29 ± 0.22**
Cutlet (kg)	2.72 ± 0.09	3.08 ± 0.03**
Shoulder + arm (kg)	2.94 ± 0.13	3.43 ± 0.12*
Carcass rest (kg)	5.74 ± 0.15	5.86 ± 0.08 NS

Note: Student test: NS=not significant (p > 0.05);*=significant (p < 0.05); ** = distinctly significant (p < 0.01);***= very significant (p < 0.001).

The crossbred lot recorded greater average values compared to the Tsigai pure breed lot. The values obtained for the GBH x Tsigai lot are distinctly significant (p<0.01) compared to Tsigai breed lot in the case of pulp and cutlet parts, while for shoulder+arm part the differences recorded were significant (p< 0.05) and for the carcass rest differences are not significant (p > 0.05).

The same conclusion is released from Figure 1, where are presented the percentages held by the main cut sections from the total of carcass. Thus, for the crossbred lot GBH x Tsigai the pulp section holds 33.71% of the total carcass while for the Tsigai breed lot only 31.16%.

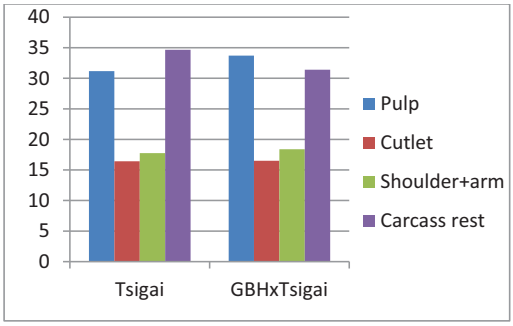

Figure 1. The main cut sections of carcass (%)

It is also worth to be mention the share held by the carcass rest section at the two lots, namely from these data it can be noticed that the inferior quality meat has a lower weight at the crossbred group (31.4%), compared to the Tsigai breed lot (34.66 %).

In Table 4 is presented the assessment of the carcasses through the SEUROP classification system after conformation and the fattening status.

Table 4. Assessment of the carcasses through the SEUROP classification system after conformation and the fattening status

Specification		Genotype			
		GBH x Tsigai		Tsigai	
		Number of carcasses	%	Number of carcasses	%
Class after conformation	S	-	-		
	E	-	-		
	U	-	-		
	R	5	100	2	40.0
	O	-	-	3	60.0
	P	-	-	-	-
	Total	5	100	5	100
Class by degree of fattening	1	-	-	-	-
	2	-	-	-	-
	3	5	100	3	60.0
	4	-	-	2	40.0
	5	-	-	-	-
	Total	5	100	5	100

From the data provided in Table 4, at the crossbred group the assessment according to the carcass conformation reveals that all 5

carcasses are classified in class R (100%), while in the control group 40% of the carcasses are classified in the R class and 60% in class O. This fact leads to the conclusion that the carcasses obtained from the control group are inferior to those obtained from crossbreds.
The same conclusion is highlighted after the assessment according to the degree of fattening, respectively all 5 carcasses obtained from crossbreds batch are classified in class 3, while in the case of witness batch 60% are classified in class 3 and 40% in class 4, with more fat cover. These results confirm the data from other research papers met in speciality literature (Ilișiu, 2009).
In Table 5 and Figure 2 is presented the chemical composition of meat.

Table 5. The chemical composition of meat (%)

Specification	GBH x Tsigai lot	Tsigai lot
Dry matter	27.40 ± 0.52	26.07 ± 0.34
Water	73.60 ± 0.80	74.00 ± 0.52
Protein	20.15 ± 0.40	19.00 ± 0.72
Fat	6.10 ± 0.17	5.80 ± 0.44
Ash	1.17 ± 0.38	1.13 ± 0.17

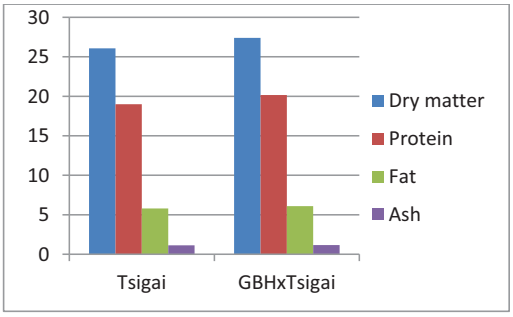

Figure 2. The chemical composition of the meat (%)

From this data can note that meat obtained from crossbred lot has the dry matter and protein content with 5.1% respectively 6.1% higher than those from meat obtained from Tsigai lot, fact which lead at the conclusion that meat provided by crossbred batch has a superior quality.

CONCLUSIONS

The variation of constituent parts of body reveals distinctly significant differences ($P<0.01$) between crossbreds GBH x Tsigai batch and control group on live weight at slaughter, hot carcass weight and full gastrointestinal tractus, fact that highlights the superior abilities for meat production in favor of crossbreds lot.
The share of cuts meat of best quality is higher in the case of the crossbred lot and also the share of cuts meat of inferior quality is lower compared with Tsigai lot.
Assessment of the carcasses through the SEUROP classification system leads to the conclusion that the carcasses obtained from the crossbred lot are superior to those obtained from control group.

REFERENCES

Duman L., Răducuță I., Ilișiu E., Marin A., Ciurea A.M., Dreve V., Bucătaru T., Călin I., 2017. Improvement of meat lamb production in Mures county by crossbreeding of local Tsigai breed with German Blackheaded breed. Scientific Papers. Series D. Animal Science. Vol. LX, 226-230.

Ilișiu E., 2009. Contribuții la îmbunătățirea performanțelor pentru producția de carne prin încrucisarea industrială a rasei Țigaie cu berbeci din rasele specializate Suffolk si Germană de carne cu capul negru. Teză de doctorat, USAMV Cluj-Napoca.

Ministry of Agriculture and Rural Development (MARD), 2018 (www.madr.ro).

Pădeanu I., 2011. Creşterea ovinelor. Editura Mirton, 254-275.

Răducuță I., 2010. Sporirea producției de carne la ovine prin încrucișare industrială. Revista de Zootehnie, nr. 1, 25-28.

Răducuță I., Custură I., 2010. Factorii care influențează producția de carne la ovine. Revista de Zootehnie, nr. 3, 21-26.

Taftă V., Vintilă I., Zamfirescu S., 1997. Producția, ameliorarea și reproducția ovinelor. Editura Ceres, 150-185.

Taftă V., 2010. Creşterea ovinelor şi a caprinelor. Editura Ceres, 10-35.

Scientific Papers. Series D. Animal Science. Vol. LXI, Number 1, 2018
ISSN 2285-5750; ISSN CD-ROM 2285-5769; ISSN Online 2393-2260; ISSN-L 2285-5750

STUDIES ABOUT INFLUENCE OF BREEDING TECHNOLOGY ON EJACULATE VOLUME OF BROILER BREEDER ROOSTERS

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Abstract

Study aimed to analyze influence of some microclimate factors (light intensity, bird's density and sex percentage) and litter material on semen quality (ejaculation volume) in broiler breeding males. Researches are part of a large study to analyze semen material and breeding efficiency of broiler breeding hybrid parents. Researches were performed during a two years period on ROSS 308 commercial hybrids with 25 roosters and 250 laying hens and three control weeks (25, 35 and 45) during breeding period (19-64 weeks). Three experimental groups were formed as one for each trial (A - with analyze parameters sub-standard and litter made of chopped straws; B - with analyze parameters above standard and litter made of rice hulls and C - with analyze parameters at the level recommended by the manufacturer of biological material and litter made of wood shavings). Ejaculation volume has been between 0.74 ±0.04 ml in week 25 - trial A and 1.20 ± 0.06 ml in week 45 - trial C. Results of trial B experiments are above the other ambient conditions with the exception of ejaculation volume in week 45. Results would seem to support usage of technological parameter values above standard recommendations and a litter made of rice hulls.

Key words: *litter, roosters, density, light intensity, ejaculation volume.*

INTRODUCTION

Bird's usual spermatogram is varying according to some factors among whom the most significant are: specie, race, age, management, feed, breeding usage regime (Vacaru Opriș, 2002; Dumitrescu, 1978, Jarinkovicova L. et al., 2012).

Ejaculation volume is having a slight rise at the beginning of breeding season in all bird species and races at it is decreasing afterwards according to organism's aging and physiological resources depleting process curb. Decrease is different for each individual (Bunaciu, 2009; Peters S.O. et al., 2008).

Average ejaculation volume in roosters is varying between 0.5 and 1.0 ml, but volumes under and above these values are constantly obtained (Parker et al., 1940; Sturkie and Opel, 1976; Orunmuyi Modupe et al., 2013; Almahdi A.B. et al., 2014)). Average ejaculation volume in main bird species (Lake, 1978) is being as following: Cornish roosters 0.35 ml (values between 0.1 and 0.9 ml), Leghorn roosters 0.15 ml (values between 0.05 and 0.3 ml), mixed races roosters 0.2 ml (values between 0.08 and 0.5 ml).

Roosters estimated sperm quality is related with individual fertilization capacity (Wishart and Palmer, 1986, Hani N. Hermiz et al., 2016).

MATERIALS AND METHODS

Roosters fecundity is directly depending by seminal material's qualities (volume, concentration, mobility etc. - Bunaciu, 1978). Technological factors (temperature, humidity, density, light intensity and period, litter quality etc.) might affect rooster's fecundity. There is a significant decrease of seminal material parameters in roosters in some stress conditions due to microclimatic factors similar to female fecundity dropping.

Researches were performed during a two years period on ROSS 308 commercial hybrids to study influence of some microclimate factors (light intensity, bird density and sex

proportion) on semen quality in broiler breeders (hen). Studied parameter was analyzed in three different experimental situations (three experiment series):

- trial A with some microclimate factors at sub-standard values and litter made of chopped straws;
- trial B with analyze parameters above standard and litter made of rice hulls;
- trial C with analyze parameters at standard values and litter made of wood shavings.

Work was done in three houses, one for each experimental trials: Avicola Călăraşi, S.C. Agrafood S.A. and Avicola Focşani and observations and records were performed in three control weeks (25, 35 and 45) during production period (19-64 weeks) during two years on 25 males and 250 females from each experimental group.

Microclimate parameters of trial A experiments considered have been:

- litter: chopped straws;
- sub-standard light intensity: 30 lux;
- sub-standard bird density: 3 males/m^2;
- sex proportion substandard: 25 weeks - 8 birds, 35 weeks - 7.5 birds, 45 de weeks - 6.5 birds.

For trial B experiments microclimate parameters considered have been:

- litter: rice hulls;
- light intensity above standard: 70 lux;
- bird density over standard: 5 males /m^2;
- sex proportion above standard: 25 weeks - 9 birds, 35 weeks - 8.5 birds, 45 weeks - 7.5 birds.

Trial C had following microclimate parameters:

- litter: wood shavings;
- light intensity standard: 40 lux;
- bird density standard: 4 males/m^2;
- sex proportion standard: 25 weeks - 8.5 birds, 35 weeks - 8 birds, 45 weeks -7 birds.

Poultry were kept in uniform conditions in the three houses (corresponding to the three experimental groups), on permanent litter (large captivity), in upgraded houses, with feed and water delivered according to the technical book of the hybrid. Birds analyzed in the three trials had the same feeding conditions to assure compatibility of results.

During production period was analyzed *semen quality* (ejaculation volume) by direct assessment in the collecting bowl.

Phonotypical characterization of groups was performed by classical statistical methods (Sandu, 1995) and study of parameters variation which has a normal repartition was performed using *Student* test to compare average homogeneities of two samples (Sandu, 1995; Dragomirescu, 1999).

RESULTS AND DISCUSSIONS

We are about to point to average value of characters analyzed in the three trials and statistical significance of differences observed between averages to emphasize the possible influence of microclimate factors (birds density, light intensity and sexes percentage) and of litter used on quantitative and qualitative parameters of semen material (ejaculation volume). Observations and records were performed in three control weeks (25, 35 and 45) during the production period (19-64 weeks).

In Table 1 and figure 1 are shown values for ejaculation volume from individuals in trial A during the three control weeks. These values are inside normal limits for species concerned and a high variability is noticed during all three control weeks.

Table 1. Average values of ejaculate volume for first experience series (trial A)

Week	n	$\bar{X} \pm s_{\bar{X}}$ (ml)	s	c.v.%
25	25	0.74 ± 0.04	0.21	29.46
35	25	1.00 ± 0.05	0.27	26.69
45	25	1.08 ± 0.05	0.25	23.13

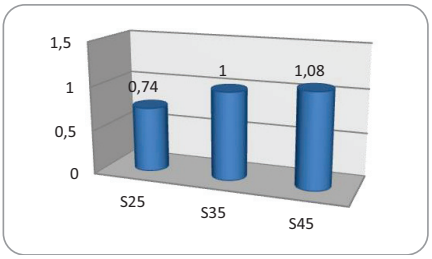

Figure 1. Average values of ejaculate volume for first experience series (trial A)

Data analyze are revealing that ROSS 308 roosters had biggest ejaculation volume (1.08 ±

0.05 ml) in week 45 compared with weeks 35 (1.00 ± 0.05 ml) and 25 (0.74 ± 0.04 ml).
It was tested the statistical significance of differences observed between average values of the character and in Table 2 are shown values calculated by Student test.

Table 2. Testing the significance of differences observed between the three control weeks in terms of volume of ejaculat, first series (trial A)

Specification	S25	S35	S45
S25	-	6.08^{***}	8.07^{***}
S35		-	0.87^{NS}

Calculated values of Student statistics point to highly statistical significant differences between average values of ejaculation volume obtained during the three control weeks excerpting last combination which are showing that between weeks 35 and 45 differences are not significant. As groups had same environmental conditions during whole trial observed differences between weekly averages in trial A could be explain most probable by physiological processes during incomplete spermatogenesis in first weeks of adult period.
Values for ejaculation volume from individuals in trial B from adult period are shown in Table 3 and graph from figure 2.

Table 3. Average values of ejaculate volume for second experience series (trial B)

Week	n	$\bar{X} \pm s_{\bar{X}}$ (ml)	s	c.v.%
25	25	0.82 ± 0.06	0.28	34.56
35	25	1.12 ± 0.05	0.26	22.88
45	25	1.11 ± 0.05	0.26	23.81

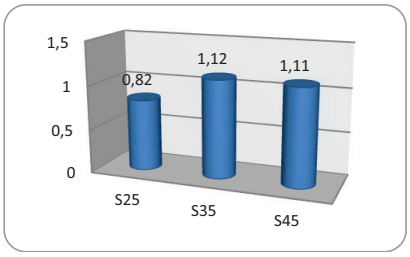

Figure 2. Average values of ejaculate volume for second experience series (trial B)

Ejaculation volume during the three control weeks stays inside normal limits of specie however a high variability is noticed throughout the controlled period and higher in week 25. This heterogeneity of observations might be due to human error because collecting semen from roosters is somehow tricky.
In trial B compared to trial A hierarchy of control weeks is changed. Highest ejaculation volume was obtained in week 35 (1.12 ± 0.05 ml) and the lowest in week 25 (0.82 ± 0.06 ml). Observed differences between averages of analyzed character were tested and found statistically significant (Table 4); there were found differences with different degrees of signifycance between average values of ejaculation volume in the three control weeks of trial B most probable due to physiological processes and with human error not excluded. There are also noticed higher values of ejaculation volume in trial B. Considering the uniformity of feeding condition and usage of the same genetic type higher values could be due to microclimate parameters above standard and a litter of rice hulls.

Table 4. Testing the significance of differences observed between the three control weeks in terms of volume of ejaculat, second series (trial B)

Specification	S25	S35	S45
S25	-	8.56^{***}	11.02^{***}
S35		-	0.76^{NS}

Ejaculation volume values obtained from individuals in trial C in adult period (Table 5, figure 3) has been inside normal species limits with a high variability throughout the production period.

Table 5. Average values of ejaculate volume for third experience series (trial C)

Week	n	$\bar{X} \pm s_{\bar{X}}$ (ml)	s	c.v.%
25	25	0.80 ± 0.06	0.2765	34.563
35	25	1.10 ± 0.05	0.2517	22.884
45	25	1.20 ± 0.06	0.2857	23.806

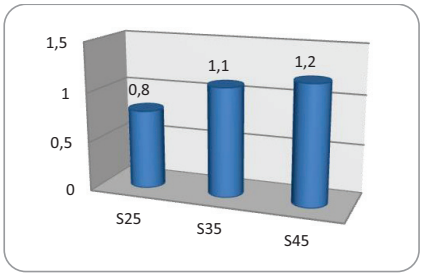

Figure 3. Average values of ejaculate volume for third experience series (trial C)

Hierarchy by control weeks is similar to trial A because ejaculation volume was higher in week 45 (1.20 ± 0.06 ml) and lower in week 25 (0.80 ± 0.06 ml).

There are noticed differences between average values of ejaculation volume with different degrees of statistical significance in the three control weeks of trial C most probable due to physiological picture of each individual plus the human factor (Table 6). There are also noticed higher values of ejaculation volume in trial C in week 45 compared to the other trials and considering the uniformity of feeding condition and usage of the same genetic type higher values could be due to microclimate parameters at standard values and a classical wood shavings litter. We notice however that this superiority might be also obtained by chance (sampling error).

Differences observed between ejaculation volume averages in the three trials (A, B, C) (Figure 4) throughout the control period are tested for statistical significance to validate influence of microclimate parameters, sex proportion and litter type on quality of semen from ROSS 30 roosters.

Table 6. Testing the significance of differences observed between the three control weeks in terms of volume of ejaculat, third series (trial C)

Specification	S25	S35	S45
S25	-	10.27^{***}	11.98^{***}
S35		-	1.86^{*}

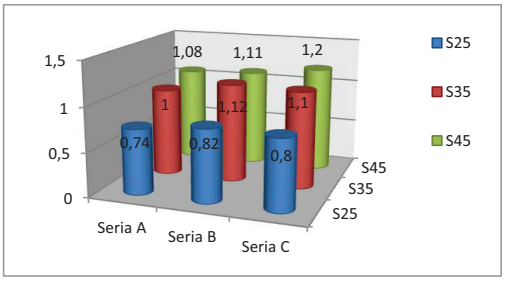

Figure 4. Comparative between the three expreimental series (A, B, C) on ejaculate volume

Calculated values of Student test shown in Tables 7-9 are revealing statistical significant differences between averages of ejaculation volume. There are noticed very significant differences between averages inside trial A and the other two trials excepting week 45.

Results obtained during trial B are superior to the other environmental condition with only one exception of ejaculation volume in control week 45 de. Student test value for this situation (1.96^{*}) although significant is not relevant because sample is highly heterogeneous probable due to human error.

Table 7. Testing of significance for differences between experimental series, 25th week, for ejaculate volume

Specification	t test value
A-B	6.37^{***}
A-C	6.94^{***}
B-C	1.43^{NS}
$t_{49;0.05} = 1.68$; $t_{49;0.01} = 2,40$; $t_{49;0.001} = 3,50$	

Table 8. Testing of significance for differences between experimental series, 35th week, for ejaculate volume

Specification	t test value
A-B	11.73^{***}
A-C	9.67^{***}
B-C	0.79^{NS}
$t_{49;0.05} = 1.68$; $t_{49;0.01} = 2,40$; $t_{49;0.001} = 3,50$	

Table 9. Testing of significance for differences between experimental series, 45th week, for ejaculate volume

Specification	t test value
A-B	1.21^{NS}
A-C	8.21^{***}
B-C	1.96^{*}
$t_{49;0.05} = 1.68$; $t_{49;0.01} = 2,40$; $t_{49;0.001} = 3,50$	

Results seem to plead for usage of values of technological parameters higher that those recommended by standard and a litter of rice hulls.

We notice however that ejaculation volume although important, is not crucial to describe semen. The other characters concerning spermatozoa mobility, concentration, morphological anomalies etc., with critical role in obtaining a good fertility and finally in assuring biological and economical efficiency of reproduction are especially important in describing semen fecundity. So environmental condition in trial B although better are not recommendable yet in practice and more investigations are necessary.

CONCLUSIONS

1. In trial A ROSS 308 roosters have had biggest ejaculation volume in week 45 (1.08 ± 0.05 ml) compared to weeks 35 (1.00 ± 0.05

ml) and 25 (0.74 ± 0.04 ml) and differences are highly significant statistical excepting the combination week 35-week 45.

2. In trial B highest ejaculation volume was obtained in week 35 (1.12 ± 0.05 ml) and the lowest in week 25 (0.82 ± 0.06 ml) with differences with different degrees of significance most probable due to physiological processes and with human error not excluded.

3. In trial C highest ejaculation volume was obtained in week 45 (1.20 ± 0.06 ml) and the lowest in week 25 (0.80 ± 0.06 ml) with differences with different degrees of statistical significance.

4. Superiority of ejaculation volume noticed in roosters from trial B might be assigned to microclimate parameters at values above standard and a litter of rice hulls.

REFERENCES

Almahdi A.B. et al., 2014. Comparative studies of semen quality on different breed of chicken in poultry breeding center temanggung central Java, International Refereed Journal of Engineering and Science, 3(2).

Bunaciu P., Şlefănescu M., Dima Viorica, 1978b. Folosirea raţională a cocoşilor din rasa Comish pentru însămânţări artificiale, Revista Creşterea Animalelor, 6, 15-18.

Bunaciu P., Maria Bunaciu, Dojană N., 2009. Reproducţia păsărilor, Ed. Printech, Bucureşti.

Dragomirescu L., 1999. Lucrări practice de biostatistică, Editura Ars Docendi, Bucureşti.

Dumitrescu I., 1978. Însămâmţările artificiale la animale. Ed. Ceres, Bucureşti.

Hani N. Hermiz et al., 2016. Evaluation semen caracterization of roosters resulted from different local lines and their crosses with ISA Brown, International Journal of Agricultural Science, Vol. 1.

Jarinkovicova L. et al., 2012. Relationship oj ejaculate quality and selected biochemical parameters of blood in cockerels of three layng lines. Chech Journal Animal Science, No. 57.

Lake P., Stewart J.M., 1978. Artificial Insemination in Poultry. Buletin 213, Her Majesty's Stationery Oftice, London.

Orunmuyi Modupe et al., 2013. Semen quality characteristics and effect of mating ratio on reproductive performance of Hubbard broiler breeders, Journal of Agricultural Science, 5(1).

Parker J.E., McKenzie F.F., Kempster H.L., 1940. Observations an the sexual behavior of New Hampshire males, Poult.Science, 19, 191.

Peters S.O. et al., 2008. Semen quality traits of seven strain of chickens raised in the humid tropics, International Journal of Poultry Science, no. 7.

Sandu Gh., 1995. Modele experimentale în zootehnie, Ed. Coral Sanivet.

Sturkie P.D., Opel H., 1976. Reproduction in the male, fertilization, and early embryonic development. In: Avian Reproduction (3rd ed.) (P. D. Sturkie, Ed.). New York: Springer-Verlag, Cap. 17.

Wishart G.J., Palmer F.H., 1986. The effect of cryopreservation at year 1968 on the viability of fowl and turkey spermatozoa assessed in vitro, Anim. Reprod. Sci., 10, 317–324.

Vacaru-Opriş I. şi colab., 2002. Tratat de Avicultură, vol II, Ed. Ceres, Bucureşti.

Scientific Papers. Series D. Animal Science. Vol. LXI, Number 1, 2018
ISSN 2285-5750; ISSN CD-ROM 2285-5769; ISSN Online 2393-2260; ISSN-L 2285-5750

FROM CONVENTIONAL TO ORGANIC AGRICULTURE – ROMANIAN PAST AND FUTURE PERSPECTIVES –

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Abstract

Organic farming is a sector of great perspective for Romania, due to the fact that it enjoys appropriate conditions for the development of such a system of agriculture, fertile soils and low level of pollution of the countryside, by comparison with the economically developed countries, where super intensive agricultural technologies are used extensively, based largely on chemical fertilizers and pesticides. According to some data from the Ministry of Agriculture and Rural Development, organic products market in Romania is in the full process of development and diversification. The aim of the present study was to analyse and interpret statistical data on the main crops, the evolution of cultivated areas, environment and ecological operators, the main genetically modified plants and the evolution of the areas cultivated with genetically modified organisms in the period 2008-2016 in Romania. Based on the statistical data of the plant sector, for the period 2010-2016, it is now taking shape that ecological agriculture was not a stable sector in Romania, having a constant evolution, in the years of study 2014-2016. Romania has great opportunities for promotion and development of organic agriculture due to the utilised agricultural area of 14.8 million ha and of the soils of unpolluted groundwater.

Key words*: organic farming, genetically modified organisms, certified operators.*

INTRODUCTION

The studies, research and the forecasts show that there is a need for higher production plant and animal both for food humanity in the following centuries (Tăpăloagă, 2017). These productions can be done only by applying technologies well investigated and regulated, based on scientific findings, but with respect to the issues of ethics. Green products have undergone significant development in the past two decades (Ilie, 2018). Care of the nature, the delicacy of natural balance, the multiplicity of diseases with which it faced more often, food without taste, industrial, all of which have led to the formation of a current of the stronger, current who wishes to restore a respect for nature and its protection. The genetically modified organisms may represent a solution to ensure the safety of the food supply, humanity, at the opposite pole being green products on food safety and consumer protection.

MATERIALS AND METHODS

On the basis of the major considerations in the field of organic agriculture, the present work proposed by the theme of study, the pursuit of the two planes, respectively the pros and cons of eco-friendly products and genetically modified organisms, using the technique of analysis and interpretation of official statistics provided by the recognized bodies and institutions (INFOMG, Eurostat, FiBL, FAO, MARD etc.) as well as the information in the magazines, articles, specialized sites, relating to the current situation in the country.

To achieve the aim and objectives of the work, the study was conducted and oriented in different periods of time, following several approaches and objectives at national level, as follows:

- the query, analysis and interpretation of statistical data on the main crops, the evolution of cultivated areas, environment and ecological operators in Romania, in the period 2010-2016;
- the query, analysis and interpretation of statistical data on the main genetically modified plants and the evolution of the areas cultivated with genetically modified organisms in the period 2008-2016 in Romania.

The statistical information on ecological agriculture, processed and presented in this

work has been extracted from the European statistics.

RESULTS AND DISCUSSIONS

The statistical data in Romania shows that, in the range of 2010 - 2016, the main ecological crops were represented by: cereals, meadows and permanent pastures, plants grown for industrial purposes, green plants, crops of orchards and vineyards, dry pulses and protein crops for the production of grain, vegetables, leafy and tuberculiferes (Table 1).

The most spectacular extensions of the cultivated areas (in percentage terms) in the period 2010 -2016, there have been permanent crops to orchards and vineyards (over 250%), followed by the permanent crops of pastures and meadows with 140% and vegetables with about 65%. It should be noted that the only culture where there has been a drop (-200%) of the area cultivated was represented by dry pulses and protein crops for the production of grain tank.

It has been on the rise, and the remaining green area of agricultural land set aside, from 580 ha in 2010, to 356 ha in 2015 (reduction of the surface being this time a positive indicator). Even if we found that, in the range of 2010 - 2016, in general it has recorded an increase of the cultivated areas for all major ecological crops, according to data provided by the MARD, in 2015 the organic cultivated area at national level (245,924 ha) decreased by more than 55,000 hectares in total (Figure 1), as compared to the maximum level recorded in 2013 (301,148 ha).

As regards the number of producers certified in the system of organic agriculture in Romania, has been an ascendant trend during the period 2010-2012, when it has recorded an increase of 123% people in 2012, compared with 2010 (over 350%), after which the evolution was descending throughout the period 2013-2015, amounting to 12,231 certified agricultural producers at the end of the year 2015 (Figure 2).

From the point of view of the counties which worked over 10,000 hectares of land in the organic system in the year 2014, in the top of the first 10 counties, Tulcea County is remarked with the highest agricultural area cultivated, recorded at 49,458 hectares, on the place of the two being Timis County with 19,455 hectares, and in the third place, Suceava with 16,567 hectares, followed, that the major organic areas Galati (14,731 ha), White (14,686 ha), Iasi (13,557 ha), Ialomita (13,205 ha), Bistrita (13,065 ha), Brasov (12,192 ha) and Cluj (12,015 hectares). At the opposite pole, the Valcea County (70 ha) and the Gorj County (91 ha) were the counties with the lowest areas cultivated in green.

By the analysis of the statistical data, in the plant field that is now taking shape, organic farming has not been a stable sector in Romania, with a production progeny, constant in the past few years (Tăpăloagă, 2017).

Special practices for organic farming - subsidies granted by the Ministry of Agriculture during 2017 in Romania, comprise six special packages: agricultural crops on arable land, including fodder - 293 euros/ha/year, vegetables - 500 euros/ha/year, orchards - 620 euros/ha/year, vine - 530 euros/ha/year, herbs and aromatic - 365 euros/ha/year, permanent meadows - 111 euros/ha/year.

The farmers must comply with the special practices for organic agriculture, to implement the commitments by consulting services relating to the identification of the agricultural parcels, to deposit at the time of payment applications.

However, in Romania, although the trend of the consumer by organic products begins to gain ever more land, according to the studies carried out, the consumption of organic products in our country is still very low, less than 2% of the total of foods while in Western Europe media is 3-5%. According to the Association of operators of Organic Agriculture "Bio Romania", approximately 80% of the annual bio products arrive on export, the value of their being of about 200 million euros, these being exported, particularly in Germany, Austria and Belgium (http://www.europabio.org).

The genetically modified organisms have been introduced in Romania in 1998, several varieties of genetically modified soya being cultivated. As regards the Romanian legislation in the field of GMOs, this is the result of the hurried harmonization of the national rules with European Directives.

Table 1. Dynamics of certified operators and cultivated in organic agriculture in Romania (http://www.madr.ro/agricultura-ecologica)

Indicator	2010	2011	2012	2013	2014	2015	2016
Number of certified operators in organic farming	3155	9703	15544	15194	14470	12231	10562
Total area of organic farming (ha)	182706	229946	288261	301148	289251.79	245923.9	226309
Cereals (ha)	72297.8	79167	105149	109105	102531.47	81439.5	75198.31
Dry pulses and protein crops (ha)	5560.22	3147.36	2764.04	2397.34	2314.43	1834.352	2203.781
Tuberous and root plants total (ha)	504.36	1074.98	1124.92	740.75	626.99	667.554	707.026
Industrial plants (ha)	47815.1	47879.7	44788.7	51770.8	54145.17	52583.11	53396.86
Plants harvested green (ha)	10325.4	4788.49	11082.9	13184.1	13493.53	13636.48	14280.55
Other crops on arable land (ha)	579.61	851.44	27.77	263.95	29.87	356.22	258.47
Vegetables (ha)	734.32	914.08	896.32	1067.67	1928.36	1210.08	1175.334
Permanent crops (ha) orchards, vineyards	3093.04	4166.62	7781.33	9400.31	9438.53	11117.26	12019.81
Permanent crops (ha) grasslands and pastures	31579.1	78197.5	105836	103702	95684.78	75853.57	57611.65
Fallow land (ha)	10216.8	9758.55	8810.73	9516.33	9058.66	7225.852	9457.2

Romania is member of most laws in this field - over 25 to the number - governing GMOs in a complicated manner which leaves room for interpretation. Many of these laws have not been the subject of public debate. A more serious aspect and linked to the legislation of the GMO is its implementation.

A concrete example is the way in which Romania has implemented a ban on the genetic modified soya cultivation (Gonciarov, 2014). The National Register of GMOs in 2006 shows that there are numerous cases of missing data. In Romania the legislation allows the cultivation of MON810 hybrid, resistant to the European corn borer. The condition is to obtain an opinion from the Ministry of Agriculture and the observance of a minimum distance of 200 meters from the neighboring conventional crops. Romania has the longest history in the cultivation of genetically modified organisms (GMOs) in European geographic location.

In Romania, the cultivation of genetically modified soya was banned in 2007, when it became EU member state, in the sense that the genetically modified crops aligned with what is allowed in the European space, and at present it is legal to the cultivation of a variety of genetically modified maize.

First commercial crops of genetically modified plants (GM) were introduced in Romania in 1998 (europabio.org).

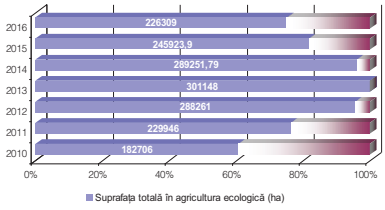

Figure 1. Dynamics of Romanian organic farming areas during 2010-2015 (sources: processed data, after MARD)

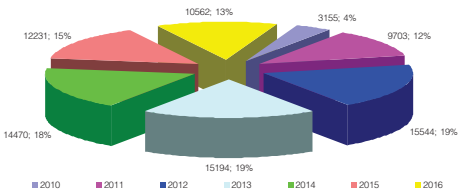

Figure 2. Dynamics of certified operators of Romanian farming during 2010-2015 (sources: processed data, after MARD)

Romania was the first member grower of genetically modified plants in Europe. In the period 1998 to 2007, Romania had the largest crops of genetically modified plants in Europe. It is genetically modified soya what belonged to Monsanto Company and official figures show that in the year 2004 have been grown 5,523 ha with soybean GM, in 2005, 87,600 hectares were cultivated with soybean GM and in 2006 have been grown 137,275 hectares (http://www.ecolife.ro/articole/stiin). Yet, at the same year (2007), Romania has approved tacitly the cultivation for a variety of GM maize with the name of the M0N810

(belonging to Monsanto Company). There have never been carried out evaluation studies in Romania of genetically modified maize in order to see which the effects on the environment or to health are. This genetically modified maize contains (by techniques of genetic engineering) gene from a bacterium from the ground (*Thuringiensis bacillus*) which produce a toxin acting as a pesticide giving the maize resistant to the worm shrilly maize. In Romania maize crops have become a tradition, holding a valuable heritage of traditional varieties of maize. Over 2.5 million hectares cultivated annually with unaltered genetically modified maize are exposed to contamination with genetically modified maize in the future.The evolution of the number of maize growers (companies / farmers) during the post accession shows that they have scaut from 58 in 2008, to 5 in the year 2014. In 2015 the only one authorised grower was a research resort. The evolution of the cultivated with genetically modified maize M0N810 areas between 2007 - 2014, was one swinging drawbar, so, after the top of the year 2008, when Romania is growing approximately 6000 ha, this has been in continuous decrease in the period 2008 to 2012, and then to register again an increase in the years 2013 and 2014 (over 700 ha), and at the level of 2015 it was noted that the cultivated area was of 2.5 ha. A map of the counties which worked the GM corn in 2008 (6,130 ha), shows that there were outlined 4 large areas in the country, concentrates in 17 counties, in the top of the cultivator counties being Calarasi county (2,278 ha), Braila (1,512 ha), Timis (867 ha) and Arad (318 ha) (www.madr.ro/culturi-de-camp).

Progress is favorable, so that at the level of the year 2014, the cultivated area has dropped to 770 ha in total, being cultivated only in 4 counties: Timis, Calarași, Arad and Neamt, the trend being maintained in the following period, therefore, in the year 2015 it was recorded an area of 2.5 ha, only in Neamt county, and the grower was a research resort, not a farmer (Figure 3). Scientists have said with much conviction that there is an imminent risk that the genetically modified crops cultivated on a large scale, to contaminate organic crops on large area. The campaign „Area without genetically modified organisms" started in Romania in 2006. It is a concept developed at world level by many non-governmental organizations, local authorities and institutions. What means areas without genetically modified organisms? The concept of "Area without genetically modified organisms" refers to a public statement which shows the position of the various decision-makers in the society with regard to the use of GMOs for cultivation, either for consumption (http://asociatia.bio.)

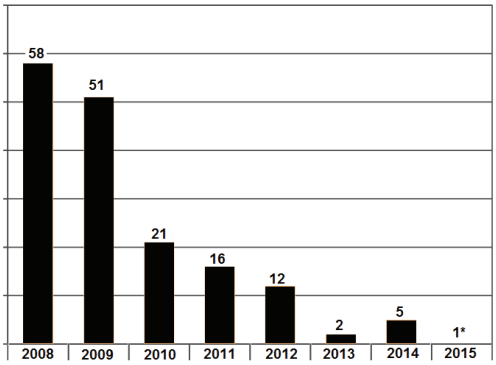

Figure 3. Situation of MON810 Romanian operators (https://infomg.ro/web/ro)

By declaring a zone without GMOs, they may not be prohibited de facto, this statement is a democraticall way to put pressure on the authorities at the central level, for a ban on the use of the genetically modified organisms in agriculture (cultivation) and in the food chain (the placing on the market of genetically modified organisms for human and animal consumption). Although local authorities benefit from local autonomy, they may not prohibit legal cultivation or consumption of genetically modified organisms due to the fact that the European legislation does not permit the implementation of statutory audits of such decisions. Also, the concept of zones without GMOs, promote sustainable agriculture, respecting the precautionary principle covered by the Protocol biosecurity levels of Cartagena, environment and health of consumers.

In Romania there are at present 72 local authorities (Bistrita Nasaud, Cluj, Sibiu, Brasov and Valcea County) and 24 restaurants (in Bucharest) who have declared public areas without GMOs, which have signed a declaration of intention, these statements do not have the force of law but represents the

positions taken by which it is requested the government to prohibit the journal cultivation and use of genetically modified products in Romania. An important role in the establishment of new areas without GMOs is played by the local action groups (lags) which represent a local partnership with the members from different sectors of the socio-economic: local mayors, non governmental organizations and the private sector (www.infomg.ro /web).
The inclusion in the strategy of sustainable development, by declaring a micro regions as Area without genetically modified organisms direct and indirect benefits of the area under the administration of Local Action Group, at the same time as the solution for sustainable agriculture for our generation and future generations. The quality of the products is always a proof for the food safety, meanwhile the health of the population (Ilie, 2017).

CONCLUSIONS

Organic farming should be regarded as an integral part of a sustainable way of agricultural production, and at the same time as a viable alternative to traditional agriculture, by the fact that focuses on the use of resources and the recycling of the unconventional restituind soil nutrients. The specific production techniques used in organic farming systems to which shall be added to the yields something less than in conventional systems are that the price of production to be something higher. As such, whether in developed countries they are accessible to the majority of the population and, in the least developed countries, where it is still important quantitatively appearance of food, green products are accessible to the segment of consumers with financial possibilities over the average population.
With the further development of ecological agriculture it is noted the increasing quality of its existence, and at present the specific activities of organic farming is a professional concern with well determined concrete boundaries and rules.
In the analysis of the statistical data of the plant sector, for the period 2010-2016, it is now taking shape that ecological agriculture was not a stable sector in Romania, having a constant evolution, in the years of study 2014-2016.
Romania has great opportunities for promotion and development of organic agriculture due to the utilised agricultural area of 14.8 million ha and of the soils of unpolluted groundwater.
The market of organic products has developed quickly, noting the annual rate of growth of level shall be worth about 20 billion euros which represents, forecast, a market share of 1.5% of the food market as a whole. The data on the share of the surface of the organic matter in the total utilised agricultural area, provided by the statistics (crops and the production of statistics on the use of land) shows that the indicator result, is one of the "sustainable development", whereas the total eco-area and the number of ecological operators continues to rise in both in Europe and worldwide.
In the past two decades, the genetically modified organisms have been one of the most controversial fields of science and the genetic engineering applied in agriculture is based on a simple understanding of the biological systems. Genetically modified is still not equivalent to genetically improved, the impact of the genetically modified organisms may be both positive and negative, both on humanity, as well as the environment. The genetic modification constitutes an attempt of humanity, science to develop products better, more resistant in time and against harmful factors, a look to the future and evolution, in order to improve the status of the social and economic problems. In order to feed the whole planet, about 9 billion people, the world population estimated by the UNO for the year 2050, the manner in which mankind farming should change and biotechnology may represent one of the mechanisms for this change, generating a higher production with a lower power consumption of resources, fertilizer, herbicides and pesticides. In Romania, the statistical data shows that, in the period 2007-2016, development in the cultivated with genetically modified maize MON810 areas, was one swinging drawbar, and after the peak in the year 2009, when there were grown about 6000 hectares, in 2015 it was registered 1 single grower with 2.5 hectares. The problem raised by the genetic engineering is particularly important, especially in the case of Romania, whose eco-tourism potential is based in particular on the conservation of

biodiversity of the country. In addition, the Romanian Government sustains the organic farming, by granting subsidies to farmers and organic farming and that based on the GMOs are incompatible.Supporters of biotechnologies and genetic engineering claim that genetically modified organisms "there are not more risky than conventional technologies to increase the plants' while the challengers claim a real ecologist movement. The quality of the products is a conclusive argument for the health of the mankind and in the category of "quality" may be included the green products, classified as "premium".

REFERENCES

Gonciarov Magdalena., Neagu Iuliana, Tăpăloagă Dana, 2014. Principles and standards of organic agriculture, Journal of Biotechnology, vol.185, S76, ISSN 0168-1656.

Ilie L.I., Tăpăloagă Dana. 2018. Conceptul de agricultură ecologică şi standardul IFOAM. Editura Ex Terra Aurum, Bucureşti, ISBN 978-606-8817-72-9.

Ilie L.I., and col. 2017. Assessment of the quality and safety of raw milk obtained in organic farming. Current Opinion in Biotechnology, Journal of Biotechnology, Volume 256, Supplement, S67-S68. doi.org/10.1016/j.jbiotec.2017.06.1029.

Ilie L., I. 2017. Organic agriculture - the guarantee of food safety and population health - Scientific Works. Series C. Veterinary Medicine, Vol. LXIII (2). PRINT ISSN 1222-5304, ISSN-L: 2065-1295, ISSN CD: 2343-9394, ISSN online 2067-3663, Bucharest.

Tăpăloagă Dana, Tăpălpagă P-R, 2017. Study regarding animal organic farming in Romania - current status and trends, Scientific Papers. Series D. Animal Science. Vol. LX, ISSN 2285-5750; ISSN CD-ROM 2285-5769; ISSN Online 2393-2260; ISSN-L 2285-5750.

http://www.madr.ro/culturi-de-camp.html.

https://infomg.ro/web.

http://www.europabio.org.

http://asociatia.bio.

http://www.ecolife.ro/articole.

Scientific Papers. Series D. Animal Science. Vol. LXI, Number 1, 2018
ISSN 2285-5750; ISSN CD-ROM 2285-5769; ISSN Online 2393-2260; ISSN-L 2285-5750

PIG WELFARE THROUGH BEHAVIOR LEARNING FROM CAMERA RECORDINGS

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Abstract

Animal Science students along with the farm staff have to monitor the behavior of pigs in order to assure their welfare. The video systems are used by our educational software and new methods of pig observation, evaluation and treatment are applied much faster and more efficient compared to the classical intervention. Each recording is stored as a media file and each frame taken at 0.1 seconds is stored as a Bitmap image. The Bitmap images are processed in parallel using the MapReduce programming model from Apache Hadoop. The contour of the image is automatically analyzed and based on it the presence of pigs is detected, as well as their location can be determined. The location is important because it can be denoting that the pig eats or that it stays aside. Pig limp was also detected. It was observed based on the recordings that 83% of the time the pigs spend it lying down, 7% is spent eating and 10% of the time they walk and sit. Video monitoring and automatic interpretation facilitates the learning of new intervention approaches and boosts the responsiveness among the students. The students can learn from the critical situations and benefit from these cases while learning.

Key words*: educational software, behavioral monitoring, video recordings, image filtering, MapReduce.*

INTRODUCTION

Image analysis starting from video recordings is a useful way of better understanding the monitored environment.

The use of camera recordings has been used while investigating the way students build their knowledge during the class while the teacher was presenting the lesson (Carvalho, 2004).

In vivo behavioral studies (Pasqualin et al., 2018) have been done in real time based on fast video capturing of dynamic phenomena like fast fluorescent calcium imaging and voltage mapping in cardiac myocytes and neurons.

Pigeons behavior consisting in moving, eating, returning to the box is observed without the need of watching the video (Madan et al., 2014). The algorithm finds out the bird's position at a specific moment of time, overlays this on the reference video frame and a summary of the activity is obtained. Based on this statistics about the length of the path and duration are computed.

Hen tracking based on image analysis processing (Kashiha et al., 2014) is used to determine the total time spent in each compartment, as well as their behavior when they are exposed to a certain ammonia concentration in the air. Ammonia results from the chemical decomposition of uric acid which is found in litter (Aziz et al., 2010). The experiment proved that hens avoid compartments with a level of ammonia higher than 22 ppm.

An automated monitoring system based on using depth sensors (Matthews et al., 2017) for monitoring the pig movement was created for having information about the pig standing, eating, drinking and moving activities.

The depth and infrared captures were done using the Kinect software development kit. The observations on the behavior have been stated manually.

The current article presents the automatic analysis done in parallel of camera recordings to investigate the presence, behavior and limping of pigs. The devices that are needed are just the video cameras. No other special and expensive devices are required. The solution allows the farm staff to assure welfare to pigs.

Students and the farm staff learn to prepare themselves to interfere during an emergency.
The article is organized as follows: in the next section are presented the algorithms for edge detection and the MapReduce parallel processing on images. The results and discussions section presents the impact of computation without and with MapReduce, along with the detection of pig body parts and behavior. The conclusions are drawn based on the obtained results.

MATERIALS AND METHODS

The camera recordings belonging to a pig farm are processed in parallel using the MapReduce programming model from Apache Hadoop. The retrieved media file is the input to be analyzed by the program. Each frame taken at 0.1 seconds is saved in the Bitmap format. The frames are processed in parallel using the C# language based on the MapReduce programming model from Apache Hadoop.
Edges present a significant change in the image intensity, marking the boundaries between the objects. The contours of the pigs can be detected using the Prewitt filter that calculates an estimation of the image intensity function gradient. This offers the direction of the largest possible increase from light to dark and the rate of change in that direction (Prewitt, 1970). The Canny edge detection (Canny, 1986) filter has multiple stages, where edges are the local maxima obtained after applying the horizontal and vertical masks.
The gradient for a slide of the radiography is a two dimensional vector, being composed of the derivatives in the horizontal and vertical directions. The vector points towards the direction of the biggest intensity increase, respectively from darker to brighter values.
There are two masks that are used for edge detection in the horizontal and vertical direction and which are convolved through the operator * with the original image A for which the approximations of the derivatives are done. The vertical mask, Gx, has a zero column and will retrieve the vertical edges of an image:

$$G_x = \begin{bmatrix} -1 & 0 & 1 \\ -1 & 0 & 1 \\ -1 & 0 & 1 \end{bmatrix} * A \quad (1)$$

The mask acts as a first order derivative and computed the difference between the intensities of pixels in an edge region. The central column is filled with zeros, indicating that the initial values of the image are not taken into consideration, but it enhances the calculation of the difference between the right and left pixel values placed around an edge.
The horizontal mask, Gy, has a zero row and works similarly to the previous mask, computing the difference of pixel intensities from above and below a particular edge, being equal to:

$$G_y = \begin{bmatrix} 1 & 1 & 1 \\ 0 & 0 & 0 \\ -1 & -1 & -1 \end{bmatrix} * A \quad (2)$$

The marks have opposite signs and their sum equals zero.
The resulting gradient is the combination of the previous two masks:

$$G = \sqrt{G_x^2 + G_y^2} \quad (3)$$

Another type of filter is the Canny edge detector which consists of multiple stages, being an improvement of the Sobel filter (Sobel, 1968). The application of the Gaussian filter to smooth the image in order to remove the noise which is done by blurring the image.
Finding the intensity gradients of the image due to the fact that a point may have a variety of directions. The horizontal and vertical masks are applied, such as for Prewitt which was previously explained.
The application of non-maximum suppression to get rid of response to edge detection. Only local maxima should be marked as edges.
The application of double threshold to determine potential edges. The suppression of all the other edges that are weak and not connected to strong edges. For the second step, the intensity of the gradient can be computed using a Sobel filter, where:

$$G_x = \begin{bmatrix} 1 & 0 & -1 \\ 1 & 0 & -1 \\ 1 & 0 & -1 \end{bmatrix} * A \quad (4)$$

and

$$G_y = \begin{bmatrix} 1 & 1 & 1 \\ 0 & 0 & 0 \\ -1 & -1 & -1 \end{bmatrix} * A \quad (5)$$

The gradient may be computed using equation 3 and its direction is determined by the arctangent function with two arguments:

$$\theta = \text{atan2}(G_y, G_x) \quad (6)$$

The MapReduce programming model consists of three steps: Map, Shuffle and Sort, Reduce (Dean, 2008) and was described by the engineers from Google, Jeffrey Dean and Sanjay Ghemawat. The Map step takes as input key-value pairs and outputs multiple pairs. These intermediate output is grouped by key at the Shuffle and Sort step, so that for each key is obtained a list of values and this will represent the input for the Reducer. The Reducer outputs a key-value pair.

MapReduce runs using Java as programming language, but it does not have a library for processing images such as the Bitmap, a raster image file format. Instead, the C# programming language of the .Net Framework contains the System.Drawing.Bitmap library, enhancing image processing. The code written in C# for processing the input data can be run in Java by transforming it into a .dll file which is used for the Map and Reduce functions, but the implementation complexity increases in the generation of the .dll file for the Prewitt and Canny filters.

MapReduce jobs can be run on Hadoop using HDInsight clusters and Microsoft Azure (Microsoft, 2018) that is a cloud computing platform employed to build, deploy and manage applications and services through a network of managed data centers. The Microsoft Azure cloud computing platform requires details about your own credit card and clients pay for the usage according to the pay-as-you-go pricing model.

There exists a MapReduce library called HIPI (Hadoop Image Processing Interface), created by Sean Arietta, Jason Lawrence, Liu Liu, and Chris Sweeney, from the University of Virginia Computer Graphics Lab. It is designed as a wrapper over the Apache's Hadoop MapReduce framework, specifically designed for image processing. It enables the storage of very large collections of images on the HDFS (Hadoop Distributed File System). HIPI integrates OpenCV, a popular open-source library for computer vision algorithms. A HIPI program is structured as in Figure 1.

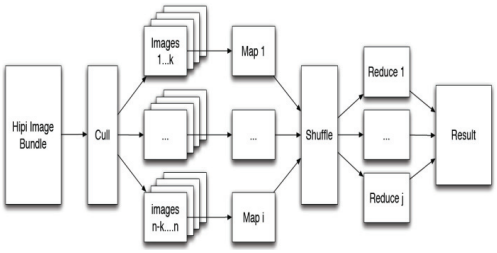

Figure 1. HIPI program design [http://hipi.cs.virginia.edu/images/hipi_pipeline.png, 2018]

A HIPI program takes a HIB (HIP Image Bundle) file as its main input. The custom format encapsulates a collection of images and is a single file on the HDFS.

Fortunately, the standard distribution of HIPI (HIPI, 2018) offers several tools for HIB creation, including a shell script that converts a directory of standard images (png or jpeg) into a HIB file.

The HIPI program's first step is culling, meaning that images from the HIB are filtered by user defined criteria. The remaining images are assigned to maptasks, maximizing data locality (like any Hadoop MapReduce program should). An image is sent to the Mapper as an object (various subclasses exist here, for ease of use). Along with the image, the Mapper is also given a Header object, to uniquely identify each image.

The built-in MapReduce shuffle algorithm minimizes network traffic, while transmitting all output from the Mappers to Reducers. The user can define custom reduce tasks, that provide the building blocks for the final output, stored in the HDFS.

The distribution provides several usage examples (Sweeney, 2018).

As previously mentioned, HIPI can be used with OpenCV. Once you have setup Hadoop and HIPI, the setup for OpenCV is very easy and is described at (Mayank, 2018).

The video recording files are converted into Bitmap images, where each frame is taken as 0.1 seconds. This supports lossless data compression through a program written in C#.

Each slide contains data patches and it is partitioned into four subsets that are further distributed to different map tasks (Fernandez, 2016; Yamamoto, 2012).

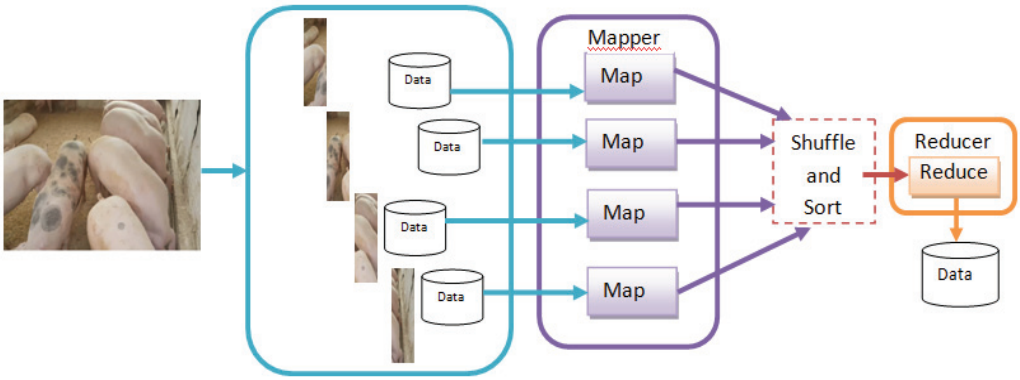

Figure 2. MapReduce processing flow

Parallel processing can be used for computing the performances (Marin, 2010), digestibility (Marin, 2017) of pigs based on forages (Marin, 2016), as well as that of fish (Nicolae, 2017), ducks (Marin, 2015).

The parallel distributed processing using MapReduce is illustrated in Figure 2. The MapReduce function takes as parameters the map function, reduce function and the list of inputs. Each input is transformed into a intermediate result of the type IntermediateResult. The reduce function starts only when all the concurrent transformations have been done. Moreover, it turns the intermediate results into final results. The map task starts by using Task.Factory.StartNew(() => map(input)). The reduce task waits until all the map tasks finished, after which is starts processing using Task.Factory.Continue WhenAll(mapTasks, tasks => PerformReduce (reduce, tasks)).

The input key-value pairs for the Mapper are stored inside a list of MyBitmap images. The list contains pairs of the type MyBitmap where the key is of the type String standing for the image name and the image split representation stored as System.Drawing.Bitmap. The output of the Map function are the intermediate key-value pairs where the key is the image name and the value is the processed subimage.

The Shuffle and Sort step emits key-value pairs where the key is the image name without containing the split number and the value is the list of processed subimages that belong to the same image.

Having this as input, the Reducer will generate MyBitmap key-value pairs where the key is the image name and the value if the final image obtained by merging the filtered subimages.

One important issue that appears is for the border pixels of the subimages, because edge detection algorithms imply convolution operations (Tesfamariam, 2011). According to this, each subimage data patches will be added another layer of pixels from the following subimage as in Figure 3.

Subimage A has an additional border of 2 pixels taken from the following image to its right hand side. The subimages B and C have a border of 2 pixels each taken from their neighbors added on both sides. Subimage D has the additional border of two pixels taken from the left image neighbor. The subimages are overlapping and the Reducer eliminates the added pixels and merges the filtered images according to their name that contains the split number.

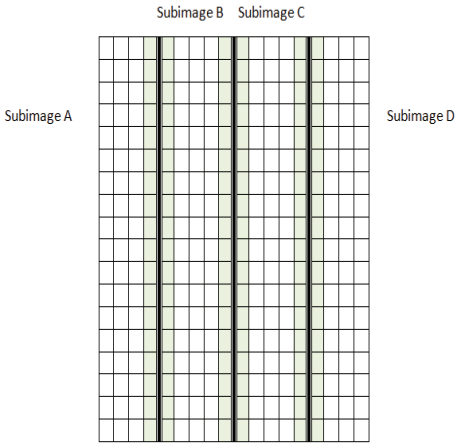

Figure 3. Input image splitting strategy

RESULTS AND DISCUSSIONS

The program runs both without the implemented MapReduce, as well as using it. The input data is represented by 20 folders, each folder containing video recordings. The total size is of 3 GB. The total execution time is illustrated in Table 1.

Table 1. Data execution time according to the input data amount

Number of files	Canny without MapReduce [s]	Prewitt without MapReduce [s]	Canny with MapReduce [s]	Prewitt with MapReduce [s]
840	470.670	315.067	312.810	280.965
630	358.772	237.025	238.874	207.840
420	259.375	152.734	163.102	138.346
210	157.015	68.526	88.293	71.853

Using the data from Table 1, the duration in seconds as function of total file number was displayed in Figure 4.

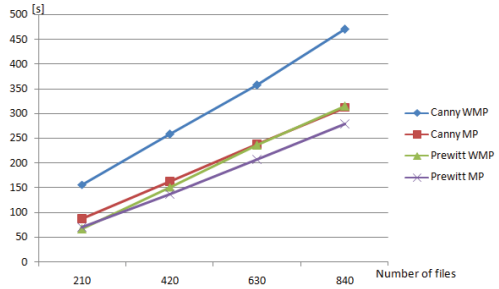

Figure 4. Duration in seconds as function of total file number

It can be observed that the dependency between the duration in seconds and the total number of files is linear.

The execution time without MapReduce data parallel processing lasted longer, as expected, compared to the version where MapReduce was applied.

Taking into consideration the quality of the output image, for the MapReduce filtered scan output Bitmap images of a video recording.

In Figure 5 is illustrated the output triggered by applying the Prewitt filter. For the same slide, the Canny filter was applied and the output looks as in Figure 6.

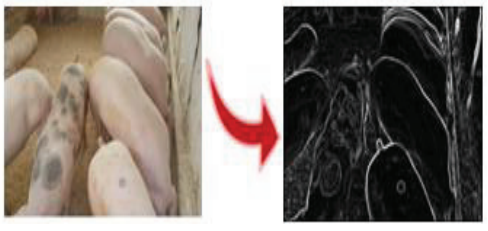

Figure 5. Application of the Prewitt filter

Figure 6. Application of the Canny filter

By applying the Canny filter, the pigs are even worse to be identified.

Based on the contour which is present in the Prewitt filtered frame, pig recognition is done based on a fragment dictionary for each feature, such as the pig's body, head, legs, including limp.

If limp is detected or if the pigs lay too much down, meaning that they are ill, the image which lead to this diagnosis is sent to the program's user via email to facilitate a faster intervention. In such a situation, the students can learn how to mitigate the problems.

The pigs are analyzed using the recording frame images, where each region is detected by performing a set of transformations, respectively scaling, rotation and translation. A classifier is used in order to trigger which is the detected body part.

Each camera has its own positioning and based on its orientation it is known the location where the pigs eat. The previous detected contours are known and behavioral inferences are triggered based on the positions occupied by the pigs, leading to the fact that 83% of the pigs lay down, 7% eat and 10% change their position frequently.

CONCLUSIONS

The implemented MapReduce accepts Bitmap images as input in order to process them in comparison with the option of using the code written in C# for running it in Java through the obtained .dll file that can be used for the Map and Reduce functions increases the complexity in the generation of the Prewitt and Canny filters.

Another option was that of Microsoft Azure cloud computing platform that asks for your personal information and it uses the pay-as-you-go pricing model.

Large data amounts processing requires the use of parallelization for optimum runtime duration in order to detect illnesses and to analyze the behavior of pigs. This has been proven by the use of the implemented MapReduce, while for the version without it, the execution time was even double.

A better image contour detection and further evaluation is to be investigated in the future.

REFERENCES

Scarfe, W. C., Farman, A. G., 2008. What is cone-beam CT and how does it work? Dent Clin North Am., 52(4), 707-730.

Prewitt, J.M.S., 1970. Object Enhancement and Extraction. Picture processing and Psychopictorics, Academic Press.

Canny, J., 1986. A computational approach to edge detection. Pattern Analysis and Machine Intelligence. IEEE Transactions on PAMI.

Sobel, I., Feldman, G., 1968. A 3x3 Isotropic Gradient Operator for Image Processing. Stanford Artificial Intelligence Project (SAIL).

Dean, J., Ghemawat, S., 2008. MapReduce: Simplified data processing on large clusters. Commun Acm, 51(1), 107-113.

Microsoft, 2018. Microsoft Azure, https://azure.microsoft.com/en-us/?b=16.17 .

HIPI, 2018. HIPI, https://github.com/uvagfx/hipi .

Sweeney, C., Liu, L., Arietta, S., Lawrence, J., 2018. HIPI: A Hadoop Image Processing Interface for Image-based MapReduce Tasks, University of Virginia, http://homes.cs.washington.edu/~csweeney/papers/undergrad_thesis.pdf .

Mayank, S., 2018. How to use opencv with Hadoop or HIPI (Hadoop Image Processing Interface), https://techgimmick.wordpress.com/2015/06/24/how-to-use-opencv-with-hadoop-or-hipi-hadoop-image-processing-interface/ .

Fernandez, B. A., Kumar, S., 2018. Distributed Image Processing using Hadoop MapReduce framework, http://search.iiit.ac.in/cloud/presentations/26.pdf .

Yamamoto, M., Kaneko, K., 2012. Parallel image database processing with MapReduce and performance evaluation in pseudo distributed mode. International Journal of Electronic Commerce Studies, 3(2), 211-228.

Marin, M., Urdes, L., Pogurschi, E., Dragotoiu, D., 2010. Research Concerning the Influence of the Reducing Level of the Compound Feed on the Performances of the Pigs for Fattening. Scientific Papers Animal Science and Biotechnologies, 43 (1), 72-75.

Marin, M., Hodosan, C., Dinita, G., Nicolae, C. G., 2017. The Influence of the Chemical Composition of Maize, Barley and Peas Hybrids on the Digestibility of Compound Feed for Pigs. AgroLife Scientific Journal, 6 (2), 127-132.

Marin, M., Hodosan, C., Nicolae, C. G., Dinita, G., Dragotoiu, T., Nistor, L., 2016. Researches regarding the Chemical Composition and Gross Energy of Sorghum in Comparison to other Forages for Feeding Cattle and Pigs. Scientific Papers, 95.

Nicolae, C. G., Moga, L. M., Bahaciu, G. V., Marin, M. P., 2017. Traceability system structure design for fish and fish products based on supply chain actors needs. Scientific Papers: Series D, Animal Science-The International Session of Scientific Communications of the Faculty of Animal Science, 60.

Marin, M., Dragotoiu, D., Nicolae, C. G., Dinita, G., 2015. Research on the influence of the oregano oil use over the productive performances and quality of duck meat. AgroLife Scientific Journal, 4 (2), 48-51.

Tesfamariam, E. B., 2011. Distributed processing of large remote sensing images using MapReduce: A case of edge detection methods. LAP LAMBERT Academic Publishing.

Scientific Papers. Series D. Animal Science. Vol. LXI, Number 1, 2018
ISSN 2285-5750; ISSN CD-ROM 2285-5769; ISSN Online 2393-2260; ISSN-L 2285-5750

SERICICULTURE INDUSTRY IN ROMANIA - ANALYSIS ON CURRENT SITUATION AND PROSPECTS OF DEVELOPMENT

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Abstract

Sericulture is an industry with a long tradition in Romania, with a good development throughout the XIX-XXth century, and, despite facing adversities, it still presents a great economic and technological potential for revival. The main objective of this article is to analyze the potential of the sericulture industry trough added value chain analysis, including sectorial analysis, constrain factor analysis and development directions. The research will use both quantitative and qualitative methods for data analysis, which is represented by statistical data from International Trade Center (ITC), EUROSTAT (Statistical Office of the European Union) and INS (International Institute of Statistics), obtained through and published scientific literature. The analysis concluded that, form an added value chain point of view, sericulture industry is facing constrains in most of its sector, from mulberry cultivation and silkworm egg production, to cocoon production and reeling.

Key words*: sericulture, silk, cocoons, potential, import, export.*

INTRODUCTION

Sericulture is an old industry, connected to agriculture and rural development, cultural part of the Asian regions that has extended, as industry, to a worldwide level, with over 40 countries involved in the sector.

Silk processing is the main objective of sericulture, but due to technological advancements, silk became wildly used in areas like biotechnology, nanotechnology, medicine, optical sciences and so forth. Research breakthroughs in silkworm research and industry have played and will play a major role in production and academic fields as well (Mărghitaș et al., 2013).

Concerning Romania, the sericulture, from a successful industry, due to a succession of downfalls, it has become fragmented, silk production becoming depended on imports.

As the potential of sericulture industry remains, strategies should be developed for its revival, taking into account the potential for rural economy diversification and possibility of workplaces generation (Pașca et al., 2008, Akram, 2015).

The analysis of the Global Value Chain (GVC), a series of activities, from imputes and production to marketing, is a tool used to help map the operations from an industry, to illustrate and analyze involved actors, involved interactions and the distribution of the benefits along the chain. It is also wildly used in the development of policies and intervention in different economic or industrial sectors (Kaplinski et al., 2002; Gerefky et al., 2006).

By using the value chain approach, the current review will attempt to show the current situation of the sericulture industry in Romania, in a global and local context, with its structure, involved actors and constrains the industry faces.

MATERIALS AND METHODS

The research strategy used was desk and literature review on the Romanian sericulture industry.

Primary and secondary data reviewed were given by specialty literature treating the

development of the sericulture industry and statistical data on the 2000-2016 time frames.
The Analysis of the Value chain followed the methodology recommended by Gerefky et al. (2006) and Kaplinski et al. (2002).
The main sectors analyzed are: market analysis with a focus on production, import and export aspects, current sericulture status analysis, from a value chain perspective, with a focus on production phases: moriculture (mulberry cultivation), silkworm egg production, silkworm rearing, cocoon reeling, silk and silk derived products processing, marketing and distribution.
The main focus is to highlight the current structure of the value chain, constrains present at sector level and proposed directions for development.

RESULTS AND DISCUSSIONS

World silk market overview

At a worldwide level, it can be stated that approximatively 30-40 countries, majority located in Asia, are involved in silkworm rearing and silk production. As a highly labor intensive industry, sericulture creates millions of workplaces world-wide (Pașca et al., 2008; Mărghitaș et al., 2013).

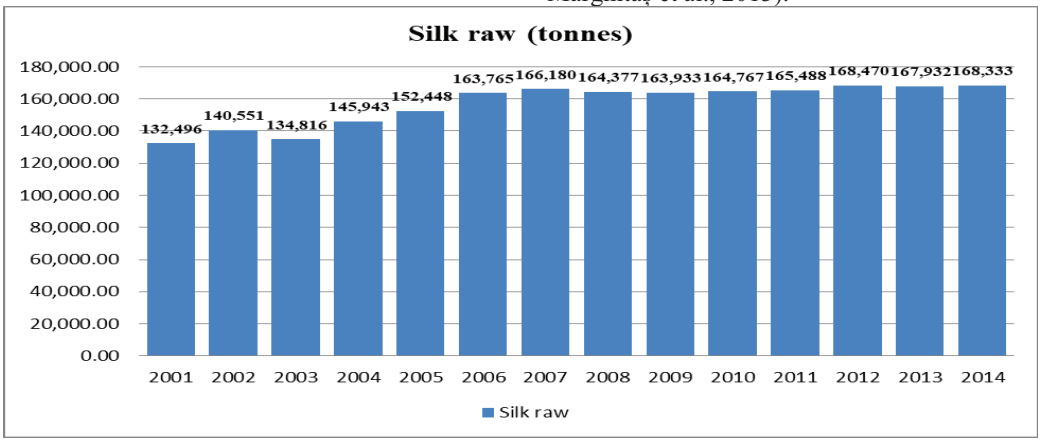

Figure 1. Global Production of raw silk(Source: FAOSTAT, 2017, data available until 2014)

In the present market, according to the International Sericultural Commission the main producers are China, with a silk production of 158,400 metric tons, followed by India with a silk production of 30,340 metric tons, Uzbekistan with a silk production of 1,256 metric tons, Thailand with silk production of 712 metric tons and Brazil with a silk production of 650 metric tons.
Silk production is affected, even in present day, by the production, at low cost, of synthetic fibers and low cost-low quality silk imports from China.
Another factor is the fact that affects the silk market, is the price that China sets, as lead producer and exporter, especially as it has taken measures to increase the silk price by reducing their mulberry plantations.
Sericulture is an industry that involves high level of labour force, and people being attracted by other, more profitable sectors, did affect the industry, as the figure above shows fluctuations in silk quantity produced. (Pașca et al., 2008; Mărghitaș et al., 2013). India is able to maintain its second place in the silk industry due to its efforts focused on silk reeling facilities modernization process and research focused on obtaining productive silkworm hybrids (Dezmirean et al., 2008; (Daltaet et al., 2005; Pasca et al., 2009; Dezmirean et al., 2008).
At a European level, the silk demand is mainly covered by imports from countries like China, India or Brazil. Up until 2008, the European sericulture industry was somehow protected by the World Trade Organization imposed measures and the import quotas implemented by the European Union to Non-EU countries, but once the restrictions were lifted, the whole textile sector felt the impact, not only the silk

industry. The high value-high cost input (fiber production at a farm level) production sector couldn't compete with the low cost imports from developing Asian countries like China and India. As a consequence, Europe remained specialized on producing high quality textile (including silk) with raw materials such as threads imported from Non-European Union countries. Other non-European countries that follow this trend are als Japan and Koreea (Daltaet et al., 2005; Dezmirean et al., 2008; Kipriotes, 2008; Pasca et al., 2009).

To be noted is the fact that the demand for natural fibers is still on the rise, at such a level that the European production sector cannot satisfy it, opening opportunities for European developing countries like Romania or Bulgaria, or developed countries like Italy to further explore the possibility of revitalizations and development of the textile sector, with a focus on sericulture (Kipriotes, 2008; Popescu, 2013).

Overview Sericulture Industry in Romania Silk, historically, first originated in China, more than 3000 years ago, and as cultures started to enter more into contact, the silk, as a fabric started to spread globally. Slowly, the occupation of silkworm rearing spread towards Korea and India, and during 500-552 e.n silkworms rearing entered the territory of Europe as well. Silk production, as an occupation, was first historically dated around 1496 in Transylvania area and 1797 in Romanian Country (Pașca et al., 2008; (Akram, 2015).

The sericulture develops throughout the decades, encouraged by implemented actions like organization of training schools (1904 - Saint Helen Church, Bucharest), establishment of reeling Institutions (1904- Lugoj Reeling Facility) and research and management institutions (1906 - Sericultural Station, Cotroceni 1916 - it gains a new location at Baneasa and is named SERICAROM BANEASA) (Pașca et al., 2008).

Before the year 1990, Romanian sericulture was a profitable industry with peak productions of 1,300 tons of silk cocoon in year of 1944, 1,300 tons in 1963 and 200 tons in 1989. Silkworm eggs production was also well established, with a high production of 2,469 kg in1989 (Pasca et al., 2009).

After the year 2000, the production continues to decline, due to continuous reduction of the mulberry plantations and the closure of the only silk cocoon reeling plant in 1995 (Pașca et al., 2008). The major decline of the industry started after the shift in political regime from social to democratic political regime. Other aspects that contributed to the decline of the sericulture industry are the lack of support legislation and the lack of reeling facilities (Mărghitaș et al., 2013).

One incentive initiative to encourage farmers to get involved in the production of silk and silk cocoons, was financial support from the European Union, under the form of subvention (136 euro per box of silkworm eggs), but as of late, starting with 2017, this form of financial support is no longer available, according to Agency for Payments and Intervention for Agriculture (APIA).

Sericulture market overview

The production of silk cocoons and raw silk has stopped completely, last crop of silk cocoons being obtained in the year of 2009 (National Institute for Statistics of Romania; Pasca et al., 2009).

The statistical data, presented in the table 1, presented below, containing data retrieved from Eurostat, office where the Romanian National Institute of Statists also reports, and the National Institute of Statistics, revealed, that, production wise, Romania focuses on production of silk textiles and silk fashion apparel, as only the silk yarn and derived products remains active in the silk value chain. From the table below, the demand for silk garments, allows for this sector to develop, as production in almost doubled in 2016, compared to 2001.

The industry can further develop into obtaining finished products like accessories (shoals or ties) or silk garments, as the economical returns proves to be quite high.

Table 1. Silk cocoon production (source: http://www.insse.ro/cms/)

Silk and silk products	Measure units	Years								
		2001	2003	2005	2007	2009	2011	2013	2015	2016
Silk cocoons, reelable	(Tons)	1.59	2.20	3.00	1.00	5.00				
Silk Fabric	(Thousand sqm)	31,831	34,732	22,916	19,773	10,472	9,443	12,085	8,379	8,267
Silk Knitted fabric	(Thousand units)	16,568	18,089	17,352	12,390	8,724	8,733	6,725	7,432	6,200

Imports and Export of silk and silk products on the Romanian market (table 2)

As production has decreased, to almost a non-existent sector, Romania relies heavily on importing silk and silk textiles, as reflected in data presented. According to United Nations Statistical data base, Romania imports silk and silk textiles mainly from Italy, China and Germany. As export markets, Romania had higher trade values with Italy, China, Bulgaria (especially silk yearn commodity), Germany and Greece.

Table 2. Import and export in silk and silk products (http://www.insse.ro/cms/)

Silk and silk products. Import.	Romania's Imports from the world								
	2001	2003	2005	2007	2009	2011	2013	2015	2016
	Thousands EURO								
Silkworm cocoons relabel	1	:	1	:	:	:	1	:	7
Raw silk (not twisted)	2,786	6,776	19,212	30,038	13,216	42,692	64,777	59,398	76,984
Silk waste	:	:	4	54	188	249	49	161	125
Silk yarn (other than silk waste yarn), not put up for retail sale	37	810	5,670	10,224	3,938	5,101	5,876	6,302	5,785
Yarn of silk waste, not put up for retail sale	:	1	12	108	60	288	494	1,382	971
Silk yarn or silk waste, put up for retail sale; hair of Messina (hair of Florence) Silk fabrics or silk waste	17	34	57	75	46	30	86	24	49
Silk or silk waste fabrics	3,105	5,757	12,842	17,363	13,938	19,229	20,069	17,202	19,925
Silk and silk products. Export	Romania's Exports to the world								
Raw silk (not thawn).	26	3,141	9,384	3,879	2,205	5,557	5,760	7,591	6,507
Silk waste	:	:	3	:	8	47	:	:	6
Silk yarn (other than silk waste yarn), not put up for retail sale	2,826	6,088	9,524	29,876	22,795	37,935	52,657	60,191	58,439
Yarn of silk waste, not put up for retail sale	:	:	2	:	3	22	39	856	420
Silk yarn or silk waste, put up for retail sale; hair of Messina (hair of Florence) Silk fabrics or silk waste	:	:	:	:	:	9	3	4	:
Silk or silk waste fabrics	231	1,422	7,856	12,940	5,743	7,487	11,417	11,957	12,207

Sericulture and biodiversity

The sericultural genetic patrimony concerning the silkworms is composed of 69 breeds and hybrids of mulberry silkworm (*Bombyx mori* L.) and of 4 breeds of ricin silkworm (*Salmya ricini*). The countries of origin are represented by Japan, China, Rusia, Bulgaria and India.

In terms of mulberry species diversity, Romania has about 10 local breeds and 49 foreign breeds and hybrids, originating from Japan, China, Russia, Bulgaria and India (Pau et al., 2008).

Sericulture and rural traditions

Sericulture in Romania originated as a rural based industry; proving to be a lucrative occupation for rural population, involving women in the production process through

activities like silkworm rearing, cocoon reeling and fabric weaving using manual handlooms. The thread obtained is known as "borangic" - silk, a thin filament obtained from rearing 5 to 10 cocoons at a time. Most known obtained products proved to be decorative objects and popular costumes rich in embroidery. Evidence of the silk handicraft industry can be located country wide, in museums dedicated to conservation of the crafts (Tzenov et al., 2006; Pau et al., 2006).

Sericulture and population's interest

In terms of future development, in the context of increased demand for silk products, there was a study conducted in 2012-2013, with its main focus on identifying interest level for sericulture and its development. Its conclusion revealed that sericulture as industry it is important for the Romanian farmer. Only 27% (out of 480 specialist and farmers interviewed) were interest in participation. Major reasons of concerns in terms of practicing sericulture were focused on interviewees being involved in other productive activities or being unable to invest at that moment (Matei et al., 2012).

Sectorial analysis of Romanian Sericulture Value Chain

Mulberry cultivation**,** also known as moriculture, is the first subsector of the input sector of the value chain, is a vital process as it provides food for silkworm rearing for sericulture farmers, but also provide samplings for establishing a new farms (Akram, 2015). The most wildly used breeds and hybrids of white mulberry (*Morus alba)* were Calafat, Galicea and Basarabi, with leafs with high protein content of up 24%. Most common plantation types were low bushes or plantations with trees with medium or high trunks (Pau et al., 2008).

Mulberry plantations have declined, not only in surface cultivated, from 3,550 ha in 1991, to 2,300 ha in 1995, to 321 ha in 2010, but the leaf quality has also declined (Pau et al., 2006) (Figure 2).

The last data concerning the number of farms that owned mulberry plantations was presented on the General Romanian Agricultural Census, 2010 and it was a total of 107, according to International Sericulture Commission.

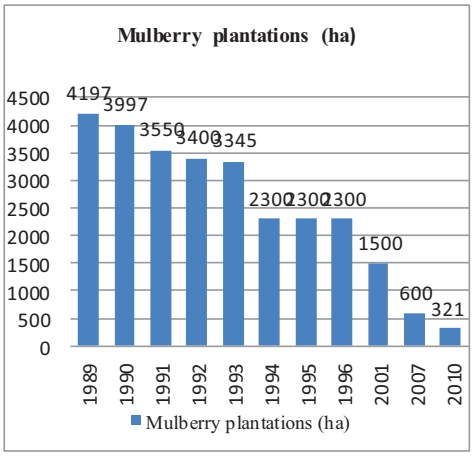

Figure 2. Evolution of mulberry plantations (Pasca et al. 2008)

Silkworm seed Production, the second subsector of the input sector of the value chain is responsible for providing disease free silkworm eggs from silkworm's productive breeds and hybrids (Akram, 2015).

Currently, no institution is involved in the commercialization of the silkworm egg production as the number of farmers involved in sericulture declined.

Romanian silkworm breeds and hybrids genetic stock is of upmost importance for the revival of the sericulture. According to conducted research, it can be stated that, overall, silkworm breeds and hybrids from the Romanian Sericultural Patrimony, proved to be quite productive: number of eggs/laying, cocoon weight and raw silk weight (and ratio), and last, but not least, fiber technological parameters (Matei et al., 2008).

Silkworm rearing is performed mostly at the University of Agricultural Sciences and Medicine Veterinary of Cluj-Napoca, with the purpose of conservation of the local genetic fund**.**

Silkworm rearing is usually done in the Sericulture Family Farm module type of exploittation, on mobile overlapped beds, and is system focused on providing rearing technologies for the small farmer. The technology was research and developed through the following project: „Organization and exploitation Model of Silkworm Rearing Family Farm in the Area of Transylvania", supported by the World Bank. This model is used to set a rearing

technology framework for future involved farmers (Mărghitaș et al., 2005; Dezmirean et al., 2008).

At a national level there are few to non reares left, but they conduct their activity independently, from procurement of inputs, to implementing the rearing of silkworm's technology, to traditionally rearing the silk cocoons and further processing them into fabrics and traditional garments for direct marketing (Mărghitaș et al., 2013; Ichim, 2013; Pau et al., 2006).

Silk production involves yarn and textile production from the silk cocoon. Raw silk is imported and textiles are fabricated.

There is no existing reeling factory for silkworm rearing. In this sector, Romania relies heavily on importing the raw material (Pașca et al., 2008).

Only traditional aspect of silk cocoon rearing left, and it is done manually using handlooms or using reeling facilities for processing high quality cocoons and pierced cocoons (waste from the production of biological Materials. Such a unit functions in the farm of one of the last silk farmers, Niculescu Family, from the village of Stoinesti, County of Valcea (Slădescu et al., 2012).

Marketing and Distribution

The sector is focused mostly on activities of import and export as cocoon and raw silk production has declined.

Support Institutions and Services

Sericulture value chain, like any other analyzed sector is influenced by different institutions through legislation, regulations, technological breakthrough, educational programs so forth (Kaplinski et al., 2002; Gerefky et al., 2006).

For Romania, there are 2 dimensions to consider when it comes to the support and research and development sector connected to the sericulture Value Chain, national and international institutions and organizations which can cooperate or support the Romanian sericulture industry revival process.

One the main bodies, at the national level, that overviews the development of agriculture and the rural industry, is the Minister of Agriculture and Rural Development. The possibility of sericulture industry revival could begin through a targeted plan designed to sustain the missing links from the silk value chain, starting from cocoon production to silk reeling and processing.

The reference Center for Advanced research in Sericulture and Silk Production, functioning within the University of Agricultural Sciences and Veterinary Medicine of Cluj-Napoca, center accredited and recognized by International Sericulture Commission, focuses its activities on germplasm conservation and its genetic characterization, managing the current bio-base it owns and is collaborating with students and didactic personnel in research activities (Mărghitaș et al., 2013; Dezmirean et al., 2008).

The Institute for Bioengineering, Biotechnology and Environmental Protection, a private research institute that was implicated in research projects concerning silkworm rearing waste, in collaboration with organizations from China and Bulgaria. (http://www.bioing.ro/en/; Ichim, 2013)

Universities are also implicated in the process of support and governance of the sericulture industry sector.

Most notably, The University of Agricultural Sciences and Veterinary Medicine, localized in the city of Cluj-Napoca, is involved in research activities and teaching activities (focused on aspects of silkworm biology, rearing technology, reproduction techniques, moriculture, and sericulture management and marketing) within the discipline of sericulture, which is managed by the Apiculture and Sericulture department. The institution was also involved in important research projects with focus on the following areas: Conservation of the sericultural germplasm, both in silkworm rearing and moriculture base; Research of technological and biological parameters of silkworm and silkworm cocoon breeds and hybrids under the influence of environmental conditions (Pașca et al., 2008) and under different diet additives (Dezmirean et al., 2008; Bojan et al., 2008; Bojan et al., 2008; Grigut et al., 2002).

The National Research and Development Institute for Textiles and Leather, located in Bucharest, also played an important role in silk industry, as it was a main partner in developing cocoon reeling technology suited for smaller farms. (http://www.certex.ro)

At an international level, Romania is part of the Organization with name Black, Caspian Seas and Central Asia Silk Association (BACSA, localized in Bulgaria) with main objectives focused on preservation of the sericultural fun of its member countries and production of biological material for cocoon production and cocoon processing and silk products marketing (Tzenov et al., 2006).
It is also a member of International Sericultural Commission, since 1959. Main objectives of the Commission focus on assistance in sericulture industry implementation and development, training programs and research with a focus on productivity of the silkworms. (http://www.inserco.org).

Development strategies

The main directions a development policy should focus on establishing an infrastructure for silkworm cocoons production and reeling, under associations as recommended form (Paşca et al., 2008) as this sector has been identified as the missing link the sericulture value chain in Romania.
In terms input sector development (mulberry plantations and silkworm egg production), it is imperative that some measures are taken:
Creation of a genetic fund in order to preserve the local mulberry breeds and hybrids and the local silkworm breeds and hybrids.
Implementation of advanced research in order to obtain high quality and highly productive silkworm eggs.
Mulberry plantations should be created for production of high quality samplings (Mărghitaş et al., 2013).
Research in this sector has led to the development of a Sericultural Family Farm Module designed for silk production for small size farms, as they represent a majority in the rural area of Romania (Mărghitaş et al., 2013).
Further actions in light of sericulture revival, at production level, should focus on modernization of silkworm rearing technology and silk processing at farm level; focus on silkworm rearing under the formation of a value chain with specialized sectors, from silkworm egg production, to young larvae rearing (instar 1 and 2), to adult larvae rearing and cocoon production and silk processing; on strategies developed to sustain and develop the silk handicraft cottage industry as traditional processed products are on demand. (Pau et al., 2008; Pau et al., 2006).
When referring to possibilities for the diversification of the sericultural farm activity with the purpose of increasing the profit, some recommended solutions focused on production through processing of secondary sericulture outcomes like pierced cocoons and remained pupas (Tzevnov et al., 2008).

CONCLUSIONS

Sericulture is an old and rural industry with high possibilities for revival. Although was a developed industry, the changes that came after 1989, including the reduction of mulberry plantations and downfall of the reeling plant have set back the industry.
In this context, Romania has turned from a producer of silk to importer of silk.
Active research is vastly conducted to preserve the genetic fund and to develop revival strategies that could relaunch the sericulture industry in Romania, an activity that will take quite a few years.
Currently Romania should focus on establishing a production infrastructure set to connect the silk cocoon production with the existing silk industry sectors.

REFERINCES

Akram S., 2015. Analysis of the Silk Value Chain in Pakistan. In: International Jurnal of Modern Trends in Engineering and Research (IJMTER), 2(8), 223-236.

Bojan C., Mărghtaş L.Al., Dezmirean D., Matei. A., Diniţă M., Paniti-Teleky O., 2008. Quantitative characteristics by silkworm larvae after topical application of methropene. Proceedings of the First International Conference „Sericulture – From Tradition to Modern Biotechnology", 146-150.

Bojan C., Mărghitaş L.Al., Dezmirean D., Diniţă G., Moise A., Maghear O., Coroian. C., 2008. The biological effects of potasium nitrate on the hybrids silkworm (*Bombyx mori* L.*)*. Proceedings of the First International Conference „Sericulture – From Tradition to Modern Biotechnology", 141-145.

Dalta R.K., Nanavaty M., 2005. Global Silk Industry, a Complete Source Book, Universal Publishing, USA, 1-78.

Dezmirean D.S., Mărghitaş L.Al., 2008. Sericulture. Present and Future at the University of Agricultural Sciences and Veterinary Medicine, Cluj Napoca. Proceedings of The First International Conference „Sericulture – From Tradition to Modern Biotechnology". Cluj-Napoca, 7-10.

Dezmirean D.S., Pașca I., Morar R., Matei A., coord. Mărghitaș L.Al., 2008. Sericicultură. Teoretică și practică. Ed Riso-Print Cluj Napoca, 1-12.

Gerefly G., Fernandez-Stark K., 2006. Global Value Chain Analysis. A primer. Ed. Duke (Center for Globalization, Governance & Competitiveness at the Social Science Research Institute).

Griguț A., Mărghitaș L.Al. et al., 2002. The use of some bio stimulants in feed administration to *Bombyx mori* L silk worms reared in Transylvania. Buletin USAMV-CN 57/2002, 223-225.

Ichim M., 2013. Sericulture status, problems and strategies in Romania. 6th International Conference "Building Value Chains in Sericulture - Biserica". Padua, Italy, 93-96.

Kaplisli R., Moris M., 2002. A handbook for Value Chain Research. from: Institute of Development Studies.

Kipriotis E., 2008. The silk product consumption in the European Union and the Sericulture's Future Potential. Proceedings of The First International Conference „Sericulture – From Tradition to Modern Biotechnology". Cluj-Napoca, 24-42.

Mărghitaș L.Al., Dezmirean D.S., 2013. Sericulture Status and Developing Strategies in Romania. Buletin USAVM Animal Sciences and Biotechnologies, 70(1), 1-8.

Mărghitaș L.Al, Dezmirean D., Pașca I., 2005. Practicum Sericicol. Ed Todesco. Cluj Napoca.

Matei A., Androne M. et al., 2008. The Description of The Silkworm Genetic Resources from Romania as A Source of Initial material For Breeding. In: Proceedings of the First International Conference „Sericulture – From Tradition to Modern Biotechnology". Cluj-Napoca, 62-68.

Matei A., Popescu A., 2012. Research concerning specialist's opinion on the development of integrated sericulture in family reproduction farms. Lucrări Științifice – seria Zootehnie, 59, 133-139.

Matei A., Diniță G., Chioveanu G., Ciulu M., Lenghel G., Constatinescu M.. 2010. Phenotypic characterrization silk moth races from the genetic stock of *Bombyx mori* L. SP in Romania. The 39th International Session of Scientific Communications of the Faculty of Animal Sciences, Bucharest, Romania, Scientific Papers, Animal Science, series D, Vol III, 21-26.

Najera J., 2017. Integration of small farmers into the Global Value Chains, Opportunities and Chalanges inside the current global demand. Tec Empresarial Aogosto-Octubre, 11(2), 7-16.

Nguyen T., Eiligmann A., 2010. Value Chain Study For Sericulture Inphu Tho, Hoa Binh, Thanh Hoa And Nghe An, Viet Nam. Joint Programme on Green Production and Trade to Increase Employment and Income Opportunities for the Rural Poor, 7-32.

Pașca I., Dezmirean D. et al., 2008. Sericicultură teoretică și practică. Ed Risoprint, Cluj-Napoca.

Pașca I., Șara A., Bențea M., Dezmirean D, coord. Mărghitaș Al.L., 2009. Producția unor hibrizi de viermi de mătase ai dudului. Ed. Academic Press, Cluj Napoca, 15-16.

Pașca I., Mărghtaș L.Al., Dezmirean D., Laslo L., Diniță G., Pusta D., Cîmpean A., Oroian R., Maghear O., Bagita C., 2008. Technological Features of PARENTAL Breeds Dry Cocoons and Hybrid Combination on the Mulberry Silkworm (*Bombyx mori* L.*).* Proceedings of the First International Conference „Sericulture – From Tradition to Modern Biotechnology", Cluj-Napoca, 119-132.

Pau E., Tănase D.B., 2006. Silk Handicraft Cottage Industries and Silk Industry Enterprises in Romania. In: International Workshop on Silk Handicrafts Cottage industries and Small Enterprises in Africa, Europe, Asia and Near East & The Second Executive Meeting of The Black, Caspian Seas and Central Asia Silk Association (BACSA), Turkey, 175-195.

Pau E., Constantinescu M., 2008. Solutions for Sericulture Reorganization in Romania. Proceedings of The First International Conference „Sericulture – From Tradition to Modern Biotechnology", Cluj-Napoca, 133-140.

Popescu A., 2013. The EU Textile and Clothing trade and it's impact on the silkworm rearing development. In: Scientific Papers. Series "Management, Economic Engineering in Agriculture and rural development", 13(1).

Popescu A. Matei A., Tzenov P., Radulescu R., 2008. The Increase profitability of a Family Sericultural Farm using the Silk Reeling Machine. Proceedings of The First International Conference „Sericulture – From Tradition to Modern Biotechnology", Cluj-Napoca, 76-82.

Slădescu V, Diniță G. et al., 2012. Researches concerning the organization of a craft manufactory for processing unreeling silk cocoons. Scientific Papers, Series D, Animal Science, Vol. LV, 239-245.

Tzenov P.I., Kipriotis E.A., 2008. Product diversification. An alternative for sericulture development in the Black, Caspian Seas and Central Asia Region Countries. 21st Intentional Sericultural Congress Proceedings, Greece, 233-246.

Tzenov P.I., Lece H.Z., 2006. Silk Handicraft Cottage Industries and Small Enterprises in Africa, Europe, Asia and Near East. International Workshop on Silk Handicrafts Cottage industries and Small Enterprises in Africa, Europe, Asia and Near East & The Second Executive Meeting of The Black, Caspian Seas and Central Asia Silk Association (BACSA), Turkey, 17-71.

TECHNOLOGIES OF THE AGRO FOOD PRODUCTS PROCESSING

Scientific Papers. Series D. Animal Science. Vol. LXI, Number 1, 2018
ISSN 2285-5750; ISSN CD-ROM 2285-5769; ISSN Online 2393-2260; ISSN-L 2285-5750

COMPARATIVE ANALYSIS OF MULTIGRAIN AND COMPOSITE FLOURS BASED ON WHEAT, RYE AND HULLED OAT

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Abstract

The chemical composition, functionality and thermo-mechanical properties of the composite flours obtained by blending wheat (80-60%), rye (10-20%) and hulled oat (10-20%) flours were compared with the multigrain flours obtained through milling the equivalent blends of wheat, rye and hulled oat. The flours were obtained using an experimental roller mill. The composite flours had higher ash, fat, crude fiber and protein contents compared to the corresponding multigrain flours. However, the quality of proteins from multigrain flour was better than of the composite flours. Differences in terms of particles size of flours were observed between composite and multigrain flours, the modules ranging from 2.50 to 2.52 and from 2.78 to 2.87, respectively. The Mixolab parameters defining starch gelatinization, gel stability during heating and starch retrogradation were lower for composite flours compared to the multigrain flour. Due to the disruption of gluten network formed by wheat, the specific volume of bread decrease with increasing the level of rye and hulled oat in wheat, from 1.84 to 1.63 cm^3 and from 1.93 to 1.71 cm^3 for composite and multigrain flours, respectively.

Key words*: composite flours, multigrain, roller mill, flours functionality, thermo-mechanical properties.*

INTRODUCTION

Cereals play a key role on food security due to their contribution to the daily energy requirements, ensuring about 19% of the caloric needs (Shiferaw et al., 2013). Moreover, about 21% of the daily dietary protein intake is delivered by wheat, what makes this cereal to be considered the main protein source at the global level.

It is therefore important for the cereal products to accumulate other nutrients in addition to proteins. In this respect, producers permanently provide on the market new products with improved nutritional value. The easiest method to accomplish it is to use whole cereal flours. Other method relies on the preparation of the composite flours in which the wheat flour is substituted with different levels of other cereals, pseudo-cereal, or legume flours.

The most advanced roller milling technology was developed over the years for wheat and rye. The roller milling systems is long-flow or short flow diagrammed, being based on different numbers of break rolls, reduction rolls, middlings divider, bran finisher or purifier machines, and the final flours can be formed by combining various mill streams.

In addition to wheat and rye, oat can be also milled by means of roller mills. Doehlert and Moore (1997) studied the composition of the milled products resulted by milling the oat by using three different mechanisms, namely roller mill, impact mill and pearling mill. They reported similar composition of the brans obtained through roller mill and impact mill, the bran having high amounts of β-glucan arising from the endosperm cell walls. Under the action of break rolls, the endosperm cell walls are ground into larges particles that are further separated into bran by sifting. Moreover, Doehlert and Moore (1997) showed that protein and mineral substances adhere to the cell walls and finally get into the bran. In another study, Aprodu and Banu (2017) reported that hulled oat milling with a roller mill resulted in low flour yields and oat bran containing large and flattened particles.

In this study wheat, rye and hulled oat were milled separately with an experimental roller mill and the resulting flours were blended to get three different composite flours having the wheat flour as base. Additionally, multigrain flours were obtained by milling with the same experimental roller mill the multigrain blends

consisting on cereals mixed in the same proportion as in case of the composite flours. All resulting flours were analysed in terms of physical chemical properties, functionality and thermo-mechanical properties.

MATERIALS AND METHODS

Wheat (Boema variety), rye (Suceveana variety) and hulled oat purchased from a specialized local market (Galaţi, Romania) were used in this study.

Flours preparation

In order to obtain wheat (W), rye (R) and hulled oat (O) flours, the cereals were milled with the SK experimental roller mill (Sadkiewicz Instruments, Poland) described by Aprodu and Banu (2017). The obtained flour yields were 61.2%, 59.4% and 57.7% for wheat, rye and hulled oat, respectively. Further composite flours were prepared by mixing wheat, rye and hulled oat flours in the following ratio: 80:10:10, 70:15:15 and 60:20:20.

The multigrain blends were obtained by first blending wheat, rye and hulled oat in the same ratio of 80:10:10, 70:15:15 and 60:20:20, followed by milling the blends with SK experimental roller mill. The yields of the multigrain flours were the following: 58.9%, 54.7% and 50.5% for the blends with different amounts of rye and oat, namely 10%, 15% and 20%, respectively.

Chemical composition

The following methods were used to determine the chemical compositions of the composite and multigrain flours: SR ISO 71:2005 for moisture, SR ISO 2171/2002 for ash, semimicro-Kjeldahl method for protein, Soxhlet extraction with ether for fat, and the Fibretherm Fibre Analyser for crude fiber. For each flour, the module was determined according to Godon and Willm (1994) using sieves with 250, 180, 160, 125 and 90 mm mesh.

Solvent retention capacity tests

Solvent retention capacity (SRC) of the composite and multigrain flours was determined according to the AACC Method 56-11.02. In order to evaluate the flour functionality, the water (W-SRC), sucrose (S-SRC), sodium carbonate (SC-SRC), and lactic acid (LA-SRC) were determined. The gluten performance index (GPI) was calculated according to Kweon et al. (2011). Sodium dodecyl sulphate (SDS) index was determined according to the method described by Seabourn et al. (2012), by using 2.5% (w/w) SDS solution and 0.005 g/ml lactic acid.

Thermo-mechanical properties of multigrain flours

The Chopin+ protocol (AACC Method 54-60) and the Chopin Mixolab device were used to estimate the thermo-mechanical properties of the composite and multigrain flours. The minimum torque C2 (Nm), starch gelatinization C3 (Nm), gel stability during heating C4 (Nm), starch retrogradation C5 (Nm), dough stability S (min) and amplitude A (Nm) were extracted from the Mixolab curve.

The bread-making procedure and bread analysis

The one-stage method for dough preparation described by Banu et al. (2010) was used for preparing the bread samples. Specific volume and crumb firmness were determined for bread samples, as indicated by Banu et al. (2010).

Statistical analysis

The results were reported as mean values together with standard deviation, the experiments being realized in triplicate. The correlation coefficients between different parameters ($p<0.05$) were calculated using Microsoft Excel Soft.

RESULTS AND DISCUSSIONS

The chemical composition of the composite and multigrain flours is presented in Table 1. The results showed that the increase of the substitution level of wheat flour by rye and hulled oat flours resulted in the increase of ash, crude fiber and fat contents in all flour samples. Comparing the chemical composition of the composite flours with the multigrain flours, it appears that for the same level of rye and hulled oat, the ash, fat and crude fiber and protein contents are higher in case of the multigrain flours (Table 1). Moreover, if in case of the composite flours the protein content decreased from 9.77 to 9.44%, with increasing the level of rye and hulled oat flours, in case of the multigrain flours the protein content increased from 7.87 to 8.64%. The decrease of

protein content in case of composite flours can be explained by the fact that the wheat flours had higher protein content compared to the rye and hulled oat flours used for substituting the wheat. Thus, according to Aprodu and Banu (2017) the protein content in flours used for obtained the composite flours were: 10.1, 7.4 and 9.5% for wheat, rye and hulled oat flours, respectively.

Table 1. Chemical compositions of composite and multigrain flours

Flours	Ash, %	Protein %	Fat, %	Crude fiber, %	Modu-les	SDS index, ml
Composite						
80W+ 10R+ 10O	0.48	9.77	1.62	1.73	2.50	36
70W+ 15R+ 15O	0.51	9.61	1.88	1.82	2.51	33
60W+ 20R+ 20O	0.54	9.44	2.14	1.90	2.52	31
Multigrain milling						
80W+ 10R+ 10O	0.62	7.87	1.67	1.66	2.78	44
70W+ 15R+ 15O	0.69	8.32	1.81	1.77	2.80	42
60W+ 20R+ 20O	0.71	8.64	2.52	2.89	2.87	38

The low protein content in the multigrain flours can be due to the endosperm breaking in large particles that are refused into bran. This assumption is supported by the values of the modules of multigrain flours that varied from 2.78 to 2.87, being higher than those corresponding to the composite flours that varied from 2.50 to 2.52. Although the multigrain flours contain low protein amounts, the SDS index values suggested that these proteins had higher quality than those from corresponding composite flours (Table 1). Analysing the trend of the SDS index values registered for the multigrain flours, one can see a decrease of this index with increasing level of rye and hulled oat. Similar observation was reported by Tulse et al. (2014) when studying the multigrain milling of blends consisting of wheat, green gram and barley.

The solvent retention capacity tests of composite and multigrain flours were also performed, and the results are presented in Table 2. The W-SRC, S-SRC and SC-SRC values of the composite flours were higher than those of multigrain flours. One explanation for this behaviour could be the higher module values given by the high content of particles with higher dimensions, which can suggest in the same time a low content of damaged starch in the multigrain flours. The LA-SRC values of the multigrain flours were higher than for composite flours, which suggest higher quality of gluten protein in the multigrain flours compared to the composite flours. Moreover, a significant positive correlation (0.93, $p<0.05$) was found between LA-SRC and SDS index. The GPI was calculated in order to estimate the global quality of gluten protein. According to Kweon et al. (2011) GPI describes the overall performance of the gluten. As can be seen from Table 2, the GPI values of multigrain flours varied from 0.52 to 0.45, and were higher than those corresponding to the composite flours, which varied from 0.48 to 0.43. A significant positive correlation (0.87, $p<0.05$) was registered between GPI and SDS index. The increase of the rye and hulled oat addition level resulted in the decrease of LA-SRC. This effect could be a consequence of the gluten dilution effect when reducing the amount of wheat within the blends.

Table 2. Solvent retention capacity profiles of composite and multigrain flours

Flours	W-SRC	S-SRC	SC-SRC	LA-SRC	GPI
Composite					
80W+10R+10O	68.2	79.2	80.7	77.0	0.48
70W+15R+15O	69.0	81.0	84.8	75.5	0.46
60W+20R+20O	70.1	89.7	86.5	75.1	0.43
Multigrain milling					
80W+10R+10O	66.5	79.8	76.1	81.3	0.52
70W+15R+15O	67.6	80.2	79.9	78.3	0.49
60W+20R+20O	68.5	86.4	85.9	76.7	0.45

The thermo-mechanical properties of composite and multigrain flours are presented in Table 3. In addition in Figure 1 are depicted the Mixolab curves for composite and multigrain flours.

The amplitude (A) decreased with increasing the addition level of rye and hulled oat, which means that the dough elasticity decreased (Dubat and Boinot, 2012). Moreover, the dough stability (S) increased, suggesting that the doughs were stronger and develop high resistance to kneading. When increasing the level of rye and hulled oat within wheat, the

minimum torque during dough kneading at 30°C (C2) decreased from 0.53 to 0.43 Nm and from 0.51 to 0.48 Nm for composite and multigrain flours, respectively (Table 3). The Mixolab parameters that define starch gelatinization (C3), gel stability during heating (C4) and starch retrogradation (C5) were lower for composite flours compared to the multigrain flour. These results can be a consequence of particles size of flours that most probably induced lower contents of damaged starch in the multigrain flours. The starch was therefore found to be more stable during heating.

Table 3. Thermo-mechanical properties of composite and multigrain flours

Flours	C2, Nm	C3, Nm	C4, Nm	C5, Nm	S, min	A, Nm
Composite						
80W+10R+10O	0.53	2.50	2.44	3.78	7.78	0.11
70W+15R+15O	0.46	2.49	2.44	3.70	7.52	0.09
60W+20R+20O	0.43	2.43	2.34	3.59	10.00	0.07
Multigrain milling						
80W+10R+10O	0.51	2.52	2.60	4.06	5.53	0.19
70W+15R+15O	0.51	2.54	2.58	3.95	9.03	0.08
60W+20R+20O	0.48	2.58	2.50	3.88	9.65	0.07

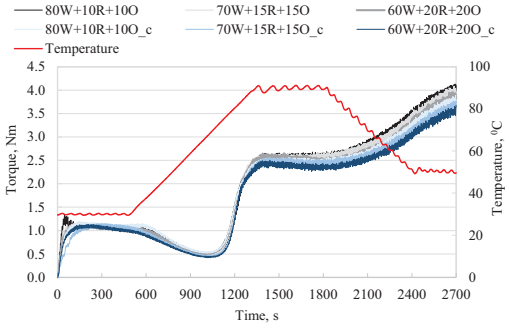

Figure 1. Mixolab curves of composite (80W+10R+10O_c, 70W+15R+15O_c, 60W+20R+20O_c – flours with 80, 70 and, 60% wheat, 10, 15 and 20% rye and 10, 15 and 20% hulled oat, respectively) and multigrain (80W+10R+10O, 70W+15R+15O, 60W+20R+20O – flours with 80, 70 and 60% wheat, 10, 15 and 20% rye and 10, 15 and 20% hulled oat, respectively) flours

The increase of the rye and hulled oat levels resulted in the decrease of the parameters that defined the starch behavior during heating and cooling. In particular, the decrease of C4 value is due the amylase activity of rye which is higher with respect to the wheat (Aprodu and Banu, 2016).

According to Dubat and Boinot (2012) the Mixolab curve of rye indicated high value of C3 followed by a drop of C4 torque due to the low stability of the hot starch. The starch retrogradation also decreased with increasing the level of rye and hulled oat. This observation is in agreement with Dubat and Boinot (2012) that reported lower retrogradation for rye compared to wheat. They explained this behaviour through the different type of starch existent in the two investigated cereals.

The specific volume of bread decreased from 1.84 to 1.63 cm^3, and from 1.93 to 1.71 cm^3 for composite and multigrain flours, respectively, with increasing the level of rye and hulled oat addition to the wheat (Table 4). These results can be explained by the disruption effect caused by rye and hulled oat on the gluten network formed by wheat. In Table 4 are presented the specific volume and texture properties of the bread obtained out of composite and multigrain flours. When comparing the specific volume of the bread samples prepared out of flour samples with the same level of rye and hulled oat, one can see that this is higher in case of the multigrain flours. Significant positive correlations were registered between the specific volume and GPI (0.98, $p<0.05$), as well as between specific volume and LA-SRC (0.85, $p<0.05$).

Regarding the crumb firmness, this property was higher in case of bread samples prepared with multigrain flours than with composite flours. Moreover, a significant positive correlation ($p<0.05$) was established between crumb firmness and C5 value from the Mixolab curve.

Table 4. Specific volume and crumb firmness of breads

Flours	Specific volume, cm^3	Firmness, g force
Composite		
80W+10R+10O	1.84	1423.9
70W+15R+15O	1.77	1708.5
60W+20R+20O	1.63	1854.8
Multigrain milling		
80W+10R+10O	1.93	1502.0
70W+15R+15O	1.85	1811.6
60W+20R+20O	1.71	1936.3

CONCLUSIONS

The result of this study revealed the differences in terms of technological properties between composite and multigrain flours based wheat with different levels of rye and hulled oat.

The ash, fat, crude fiber and protein contents were higher for multigrain flours compared to the composite flours. One major difference between the two types of flours was related to the particle size estimated through module values.

Although the protein contents in the multigrain flours were low, the quality of these proteins appreciated through the SDS index and GPI were higher than in case of composite flours. Additionally, the LA-SRC values of multigrain flours were higher than for composite flours, and the specific volume of breads made with multigrain flours were higher than of breads made with composite flours.

At the same time, significant positive correlations were found between specific volume and SDS index, LA-SRC and GPI.

The increase of the level of rye and hulled oat addition resulted in the decrease of the specific volume of the bread due to gluten dilution when substituting the wheat with other cereals.

Future studies on comparative analysis of composite vs multigrain flours will be realized using a type of experimental roller mill that include break and reduction rolls.

ACKNOWLEDGMENTS

This work was supported by a grant of the Romanian National Authority for Scientific Research and Innovation, CNCS/CCCDI - UEFISCDI, project number PN-III-P2-2.1-BG-2016-0143, within PNCDI III.

REFERENCES

AACC International. Approved Methods of Analysis, 11th Ed. Methods 54-60.01 and 56-11.02. American Association of Cereal Chemists International, St. Paul, MN, U.S.A. http://dx.doi.org/10.1094/AACCIntMethod-54-60.01/56-11.02.

Aprodu I., Banu I., 2017. Milling, functional and thermo-mechanical properties of wheat, rye, triticale, barley and oat. Journal of Cereal Science, 77, 42-48.

Aprodu I., Banu I., 2016. Comparative analyses of physicochemical and technological properties of triticale, rye and wheat. The Annals of the University Dunarea de Jos of Galati. Fascicle VI – Food Technology, 40(2), 31-39.

ASRO, 2008. Romanian standards catalog for cereal and milling products analysis. SR ISO 71:2005, SR ISO 2171/2002, SR 91/2007, Bucharest.

Banu I., Stoenescu G., Ionescu V., Aprodu I., 2010. Physicochemical and Rheological Analysis of Flour Mill Streams. Cereal Chemistry, 87, 112-117.

Doehlert D.C., Moore W.R., 1997. Composition of oat bran and flour prepared by three different mechanism of dry milling. Cereal Chemistry, 74(4), 403-406.

Dubat A., Boinot N., 2012. Mixolab applications handbook. Rheological and enzymes analyses, Chopin Technology, Villenueve.

Godon B., Willm C., 1994. Primary Cereal Processing a Comprehensive Sourcebook. VCH, New York.

Kweon M., Slade L., Levine H., 2011. Solvent Retention Capacity (SRC) Testing of Wheat Flour: Principles and Value in Predicting Flour Functionality in Different Wheat-Based Food Processes and in Wheat Breeding - A Review. Cereal Chemistry, 88(6), 537-552.

Seabourn B.W., Xiao Z.S., Tilley M., Herald T.J., Park S.H., 2012. A Rapid, Small-Scale Sedimentation Method to Predict Breadmaking Quality of Hard Winter Wheat, Crop Science, 52, 1306-1315.

Shiferaw B., Smale M., Braun H.J., Duveiller E., Reynolds M., Muricho G., 2013. Crops that feed the world 10. Past successes and future challenges to the role played by wheat in global food security. Food Security, 5, 291-317.

Tulse S.B., Reshma V., Inamdar A.A., Sakhare S.D., 2014. Studies on multigrain milling and its effects on physical, chemical and rheology characteristics of milled streams. Journal of Cereal Science, 60, 361-367.

Scientific Papers. Series D. Animal Science. Vol. LXI, Number 1, 2018
ISSN 2285-5750; ISSN CD-ROM 2285-5769; ISSN Online 2393-2260; ISSN-L 2285-5750

EFFECTS OF PULSED LIGHT TREATMENT ON GERMINATION EFFICIENCY OF PULSES

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Abstract

Germination tests were performed on chickpeas, broad beans and green lentils, with the aim of identifying the processing conditions that allow improving germination efficiency and bioactivity profile of the germinated pulses. Germination experiments were carried out under different lighting conditions. The preliminary treatment of the soaked pulses through pulsed light of different fluency values before germination was also tested. Regardless of the investigated pulses, the dark or light regime had no significant influence on the total germination efficiency. Anyway, the light had a positive effect on the germination rate of chickpeas in the first 24 hours. A more pronounced increase of the antioxidant capacity was observed for samples germinated under dark conditions. The investigated pulses reacted differently to the pulsed light stimuli. Only in the case of lentils an increase of the percentage of germinated seeds was observed after the pulsed light treatment. Regarding the synthesis of both proteins and antioxidant compounds, the most promising results were registered for germinated broad beans after the pulsed light treatment at fluence of 43.2 J/cm^2.

***Key words**: lentil, chickpea, broad beans, light pulses, germination.*

INTRODUCTION

Pulses are remarkable sources of valuable nutrients such as high quality proteins, vitamins, minerals and fibers (Bassett et al., 2010). However, the nutritional value of pulses can be compromised because of the presence of trypsin inhibitors, vicilin, covicilin, and tannins, and because of the reduced digestibility of proteins and starch. Different studies showed the possibility of enhancing protein and starch digestibility, together with reducing the content of phytic acid, tannins, lectins and protease inhibitors through germination (Ghavidel and Prakash, 2007; Freitas et al., 2007; Khandelwal et al., 2010; Gan et al., 2017). Germination is recognized as an inexpensive process for increasing the nutritional value of crops (Cáceres et al., 2014; Gan et al., 2017). The germination process refers to embryo development, when partial hydrolysis of the starch, proteins, hemicellulose and cellulose occurs, leading to important transformations in the morphological structure and seeds composition. The efficiency of the germination process is influenced by seeds quality and hydration prior to germination, and environmental factors such as presence of light, temperature, humidity and oxygen (Seo et al., 2009). The importance of light in the germination is twofold, being the source of both energy and information, in terms of photoperiodicity (day/night), phototropism (light direction) and photomorphogenesis (quantity and quality of light). Seeds are provided with sophisticated equipment to monitor and determine the extent to which the environmental parameters are suitable for germination and subsequent plant growth (Seo et al. 2009). Thus the interaction between light and hormonal signals plays an essential role in the control of germination process. Many studies have investigated the effect of different regions of the electromagnetic radiation field (static magnetic field, type of illumination and pulses light) on the germination efficiency of small seed crops (Lindig-Cisneros and Zedler, 2001; Rajendra et al., 2005; Seo et al., 2009; Shine et al., 2011; Vashisth and Nagarajan, 2010). Anyway, the knowledge on the effect of

light on pulses germination efficiency and nutritional value is scarce.

The present study aimed to determine the effect of the pulsed light on the germination efficiency and antioxidant properties of different pulses under light and dark conditions. Three pulses were considered in the study, namely broad bean (*Vicia faba*), chickpea (*Cicer arienatum*) and green lentils (*Lens culinaris*).

MATERIALS AND METHODS

Materials

Commercial broad beans (*Vicia faba*), chickpeas (*Cicer arienatum*) and green lentils (*Lens culinaris*) retailed on the local market (Galati, Romania) were used in the study.

Germination process

Prior to germination, the beans were rinsed with tap water, sanitized by soaking for 15 min with aqueous ethanol solution (70%), and finally rinsed with tap water again. The beans were then subjected to swelling in tap water under dark conditions, for a period of time adequate to assure the needed humidity for germination. Preliminary tests (results not shown) were performed, for each investigated legume type, to establish the optimum swelling time, as follows: 24 hours for broad bean, 12 hours for chickpea, and 7-8 h for green lentils. Afterward, beans were subjected to germination at 22 ± 2°C using the automatic Easy Green germinator, equipped with fog generator.

In order to determine the impact on germination power and the accumulation of biologically active compounds in the investigated pulses, the following germination conditions were considered in the study: the pulsed light treatment of the soaked beans and the dark/light regime during germination. The pulsed light treatment, with fluence values of 19.2 J/cm^2 and 43.2 J/cm^2,was applied immediately after completing the swelling step. Afterwards, the pulsed light treated samples were subjected to germination under dark (germination under total (24/24h) darkness) or light (germination under daylight (12h/24h)) regime. The study was conducted in September 2017, when the daylight regime is of approximately 12 h. Light pulses were generated by an IFP xenon type lamp with the following characteristics: electromagnetic field (λ) of 200-1000 nm and impulse regime of 10^{-1} - 10^{-4} s, as indicated by Turtoi and Nicolau (2007).

The germination time varied with the type of legume; the broad beans and chickpeas were germinated for 48 h, while green lentils were allowed to germinate for 24 h. The broad beans and chickpeas sprouted after the first 24 h of germination were counted, and allowed then to germinate for additional 24 h. At the end of the germination step all sprouted beans were counted to estimate the germination power. The germinated samples were afterwards dried at 55°C for 24 - 30 h in a convection oven (LabTech LDO-030E, Daihan Lab Tech Co., LTD, Kyonggi-Do, Koreea). Germinated broad beans and chickpeas were dehulled prior to drying. The dried native and germinated beans were finally grinded into flours with particles size lower than 500 μm using a laboratory mill (WZ-2, Sadkiewicz Instruments, Bydgoszcz, Poland).

For each legume considered in the study, the control sample consisted of beans processed through swelling, dehulling, drying and grinding, under the same conditions as mentioned before. This type of processing of the control samples was considered necessary to assure the uniformity of the samples. In this respect López-Amorós et al. (2006) stated that swelling process leads to a significant decrease of total phenols content. Finally, 21 samples were obtained and coded as shown in Table 1.

Proximate composition

The proximate composition of the flours obtained from native and germinated legumes was determined as follows: the moisture content using the AACC 44-51 method (AACC International, 2010); the protein content through the semimicro-Kjeldahl method (Raypa Trade, R Espinar, SL, Barcelona, Spain) using the nitrogen conversion factor of 6.00; the fiber content through the AOAC Official Method 962.09, using Gerhardt Fibertech equipment (C. Gerhardt GmbH & Co. KG); and the ash content using SR ISO 2171: 2002 Method (ASRO, 2008).

Table 1. Codification of the lentil (L), broad bean (B) and chickpea (C) samples subjected to germination under different conditions

Sample code	Applied treatment
$L_0B_0C_0$	Control samples
L_1 B_1 C_1	Samples germinated in darkness (24/24h)
L_2 B_2 C_2	Samples germinated in daylight (12/24h)
L_3 B_3 C_3	Samples treated with light pulses of 43.2 J/cm^2 fluence and germinated in darkness (24/24h)
L_4 B_4 C_4	Samples treated with light pulses of 43.2 J/cm^2 fluence and germinated in daylight (12/24h)
L_5 B_5 C_5	Samples treated with light pulses of 19.2 J/cm^2 fluence and germinated in darkness (24/24h)
L_6 B_6 C_6	Samples treated with light pulses of 19.2 J/cm^2 fluence and germinated in daylight (12/24h)

Extraction for determination of total phenolics and antioxidant activity

The studied flours were subjected to extraction with 80% methanol solution, while stirring for 2 h at room temperature, using a magnetic stirrer. The mixture was then centrifuged at 9690×g for 10 minutes (Martinez Villaluenga et al., 2009). The supernatant was collected for further determinations.

Determination of total phenolic content

The Folin-Ciocalteu method was used to determine the concentration of total phenolic compounds. A volume of 0.2 ml extract solution was mixed with Folin-Ciocalteu reagent (1.5 mL, previously diluted with water 1:10, v/v). After 10 min of resting period at room temperature, 1.5 mL of 60 g/L sodium carbonate was added. The ad mixture was let to rest for another 90 min and then the absorbance was read at 725 nm. The total phenolic compounds were quantified and expressed as mg ferulic acid equivalents (FA)/g of sample.

Determination of antioxidant activity by DPPH method

The ability of the investigated samples to scavenge DPPH free radicals was determined by mixing 0.1 mL of extract with 3.9 mL of 6×10^{-5} M solution of DPPH in methanol. After the reaction was allowed to take place in the dark for 30 min, the absorbance at 515 nm was recorded to quantify the remaining DPPH. A control was prepared using solvent instead of sample extract and was used to measure the maximum DPPH absorbance. The DPPH radical scavenging activity was expressed as IC50 values, corresponding to the amount of antioxidant (mg of legume flours) necessary to decrease the absorbance by 50% (López-Amorós et al., 2006). Thus smaller IC50 values are related to higher antioxidant activities. All samples were prepared and measured separately in duplicate.

Statistical analysis

Two independent germination experiments were conducted and all measurements were performed in duplicate. Statistical analysis was performed using Microsoft Excel Software. Correlation analysis was performed to identify potential relationships between germination process and antioxidant activity of germinated pulses. The results are reported as mean values together with standard deviations.

RESULTS AND DISCUSSION

Influence of light and pulsed light treatment on the germination of pulses

The influence of light and pulsed light treatment on the germination process was quantified by determining the percentage of germinated seeds, and the results are shown in Figure 1. Regardless of the investigated sample, the light or dark regime during the germination operation had no significant influence on the germination power ($p>0.05$). Anyway, in the case of chickpea and broad bean samples, a significantly higher percentage ($p<0.05$) of seeds germinated under light regime was observed after the first 24 hours (samples C2 in Figure 1b and B2 in Figure 1c). Similar observation regarding the lack of influence of different light conditions on the percent of germination have been reported by Martín-Cabrejas et al. (2008) when investigating the soybean andnon-conventional legume slike cowpea, jack bean, mucuna, and dolichos.

An increase of the germination power was noticed in case of all investigated legume samples treated with pulsed light at high fluence value. Regardless of the light condition

during germination, the increase of the germination performance was significant ($p<0.05$) only in case of the lentil sample subjected to pulsed light treatment at fluence of of 43.2 J/cm^2, when the germination power reached 94% (samples L3 and L4 in Figure 1a).

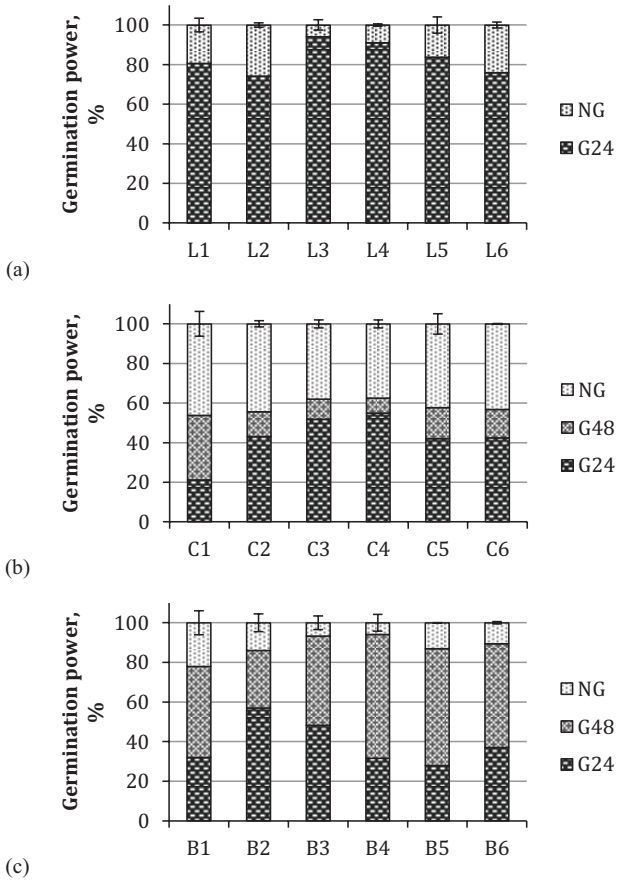

Figure 1. Germination performance of lentil (a), chickpea (b) and broad bean (c) at different light conditions.
NG - non-germinated seeds, G24 – percent of seeds germinated after 24 h, G48 – percent of seeds germinated after 48 h

The proximal chemical composition of the lentil, chickpea and broad bean samples germinated under different light conditions after eventual pulsed light treatment is presented in Table 2. The highest protein contents were determined for broad bean samples, whereas lentil and chickpea had similar protein contents. When compared to the controls, one can see that germination ensured the significant increase of the protein content in case of all studied samples ($p<0.05$). Regardless of the light conditions during lentil and chickpea germination, the most significant increase of the protein content was observed for the samples treated with light pulses of 43.2 J/cm^2 fluence. The protein content increase during germination was higher in case of lentils (from 21.25±0.09 g/100g DM corresponding to L_0, up to 26.74±0.28 g/100g DM corresponding to L_4) compared to chickpeas (from 21.16±0.35 g/100g DM corresponding to C_0, up to 24.73±0.09 g/100 g DM corresponding to C_3). On the other hand, germination without prior pulsed light treatment appeared to be more efficient in terms of increasing the protein content of the broad beans. Germination carried out under daylight conditions (12/24 h) resulted in the protein content increase from 29.94±0.41 g/100 g DM (B_0) to 36.21±0.21 g/100 g DM (B_2). Our results are in agreement with Yu-Wei

and Wang (2015) who reported significant increase of the protein content from 25.18% to 28.56% when germinating the broad beans. The increase of the protein content is the result of synthesis of cell constituents and enzymes, on the account of degrading other constituents of the cells (Yu-Wei and Wang, 2015; Lee and Karunanithy, 1990). Moreover an improvement of the protein quality was reported in case of germinating *Glycine* and *Phaseolus* beans; the total essential amino acids increased by 76% and 52%, respectively (Lee and Karunanithy, 1990).

Table 2. Effect of germination under different conditions on the proximate composition of lentils, chickpeas and broad beans

Sample	Moisture g/100g	Ash g/100g DM	Proteins, g/100g DM	Fibers, g/100g DM
L_0	13.03±0.01	3.03±0.06	21.25±0.09	4.85±0.22
L_1	7.15±0.04	2.86±0.12	22.28±0.02	3.91±0.12
L_2	8.43±0.05	3.00±0.07	24.70±0.01	4.37±0.01
L_3	7.41±0.08	2.97±0.08	25.47±0.40	4.00±0.00
L_4	6.21±0.13	2.93±0.07	26.74±0.28	4.46±0.30
L_5	6.52±0.35	2.80±0.25	25.96±0.62	3.04±0.20
L_6	6.32±0.18	3.02±0.19	24.54±0.44	3.85±0.40
C_0	8.60±0.01	2.70±0.01	21.16±0.35	1.28±0.17
C_1	7.89±0.04	2.69±0.02	22.66±0.05	0.90±0.11
C_2	7.49±0.05	2.69±0.08	22.93±0.35	0.86±0.03
C_3	7.26±0.05	2.73±0.06	24.73±0.09	0.89±0.45
C_4	5.67±0.25	2.68±0.12	24.54±0.00	0.64±0.18
C_5	6.22±0.00	2.62±0.01	23.96±0.82	1.87±0.50
C_6	6.73±0.17	2.62±0.10	24.21±0.43	1.19±0.00
B_0	8.14±0.17	4.17±0.20	29.94±0.41	1.78±0.06
B_1	6.15±0.15	4.09±0.61	35.83±0.64	2.06±0.51
B_2	6.69±0.22	4.30±0.40	36.21±0.21	2.44±0.16
B_3	5.89±0.15	4.35±0.48	33.69±0.20	1.96±0.23
B_4	8.74±0.18	4.56±0.19	34.84±0.16	1.45±0.22
B_5	6.60±0.00	4.31±0.38	32.47±0.21	1.84±0.09
B_6	6.73±0.15	4.51±0.07	33.32±0.23	1.98±0.19

Results represent mean values of two replicates ± standard deviations

Among the conventional techniques used for food processing, germination is one the few processes that ensures significant increase in nutritional value by increasing the bioavailability of nutrients, vitamins, biominerals and other biologically active substances. Studies conducted by Ghavidel and Prakash (2007) on lentils, chickpeas, black eye beans and Indian green beans highlighted the fact that germination leads to the reduction of lipid content due to the use as energy source, for supporting specific cellular processes, and the increase of protein and thiamine contents through biosynthesis.

The ash content of the investigated legumes was not affected by germination (Table 2). Regardless of the preliminary treatment and light conditions during germination, only the broad beans showed insignificant increase of the ash content. Our results are in agreement with the observations of Yu-Wei and Wang (2015) on the ash content evolution during germination of different pulses and legumes. Anyway, other studies indicated the reduction of the mineral content in the germinated seeds, mainly as a consequence of solubilization and loss of these compounds in the soaking step (Lee and Karunanithy, 1990; Ghavidel and

Prakash, 2007). A number of antinutritive factors that exist in the raw materials diminish or even disappear during germination, allowing for a more efficient biological utilization of the nutrients. The increase of the bioavailability of iron and calcium from legumes during germination was reported by Ghavidel and Prakash (2007), as well as the reversed relationship with tannins, fibers and phytic acid contents.

The dietary fibre content varied with the investigated sample, pulsed light treatment and presence of light during germination (Table 2). Lentils had the highest amount of fibers (4.85±0.22 g/100 g DM), followed by broad beans (1.78±0.06 g/100 g DM) and chickpeas (1.28±0.17 g/100 g DM). Legumes germination resulted in the overall decrease of the dietary fiber content, except for the broad beans germinated under light without prior pulsed light treatment (Table 2). Concerning the fate of the fibers during legumes germination, Ghavidel and Prakash (2007) reported the increase of the total and soluble fiber contents, and significant reduction of the insoluble fiber fraction. Previous studies report on the significant variation of the dietary fiber fractions in different legumes with the germination conditions (Martín-Cabrejas et al., 2003).

Influence of light and pulsed light treatment on the antioxidant properties of germinated pulses

Germination is one of the cheapest processes which is effective in improving the profile of the biologically active compounds, and the bioavailability of the nutrient components in the seeds (Cáceres et al., 2014; Gan et al., 2017), thus allowing to obtain products with high nutritional value. Germination is therefore an alternative to controlled food fortification by the addition of nutritive compounds obtained through chemical methods.

The effect of different environmental conditions during the legume germination process on the synthesis of antioxidant compounds has been estimated by determining the antiradical activity (DPPH RSA) and the amount of total phenols. The results obtained for lentil, chickpea and broad bean samples are shown in Figures 2, 3 and 4.

The antioxidant properties of the lentil samples varied with the pulsed light treatment and presence of light during germination (Figure 2).

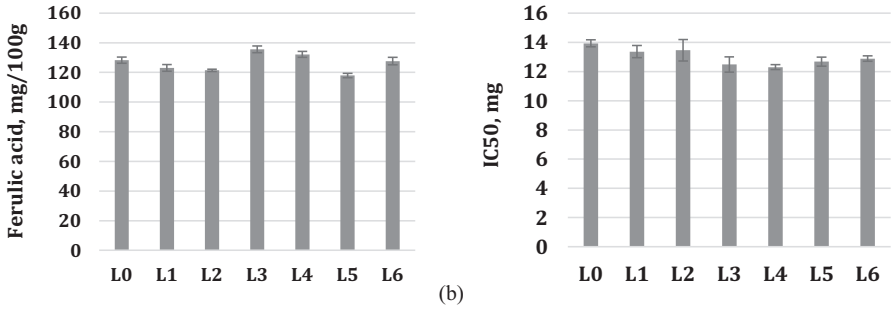

Figure 2. Effect of germination under different conditions on the total phenolic content (a) and antioxidant activity (b) of lentils

The highest total phenolic content and the lowest IC50 value were recorded for the L3 sample, treated with light pulses with fluency of 43.2 J/cm^2 and germinated in the dark. On the other hand, lentils treatment with pulsed light of lower fluence (19.2 J/cm^2) caused the most important decrease of the antioxidant properties when germination was carried out in the dark (L5 in Figure 2).

Regardless of the pulsed light treatment and light conditions, germination had a positive effect on the antioxidant capacity of chickpeas ($p<0.05$). As in case of lentil sample, the highest antioxidant properties (high content of phenolic compounds and low IC50 value) were recorded for the chickpea sample germinated in the dark, after treatment through pulsed light with fluency of 43.2 J/cm^2(C3 in Figure 3).

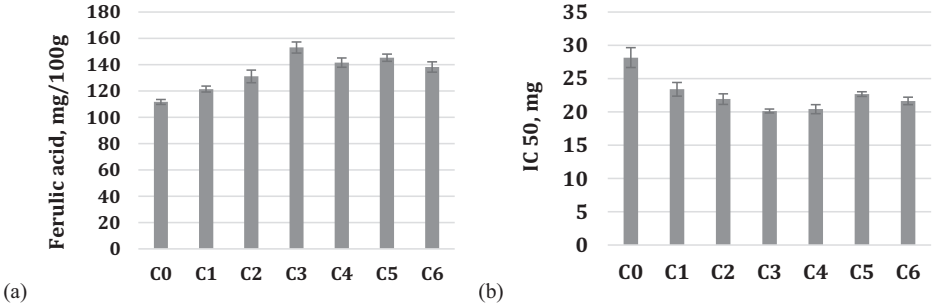

Figure 3. Effect of germination under different conditions
on the total phenolic content (a) and antioxidant activity (b) of chickpeas

Regarding the influence of the light/dark regime during germination, one can see in Figure 3b that chickpea samples germinated in the presence of natural light (C2) had slightly higher antioxidant capacity compared to the sample germinated under dark (C1). Anyway, no significant differences were observed between IC50 values of C1 and C2 ($p>0.05$). On the other hand, samples treated with pulsed light, for both fluence values considered in the experiment, developed higher contents of total phenols in the course of germination under dark (C3 and C5) compared to the daylight conditions (C4 and C6).

In case of broad beans, germination resulted in significant increase of the total phenols content for all studied variants (Figure 4a). The total phenolic content was not significantly influenced by the dark/light regime during germination ($p>0.05$), but rather by the prior treatment through pulsed light ($p<0.05$). The highest value of the total phenols content was recorded for B4 sample, treated with pulsed light with fluency of 43.2 J/cm^2.

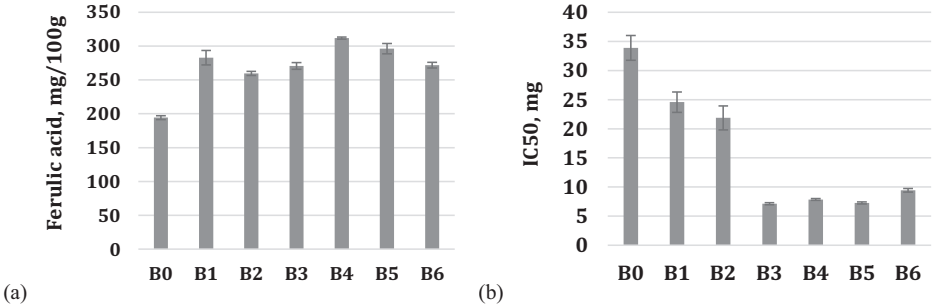

Figure 4. Effect of germination under different conditions
on the total phenolic content (a) and antioxidant activity (b) of broad beans

Among the three investigated pulses, the most significant increase of the antiradical activity, when compared to the control sample, was obtained in case of the germinated broad beans ($p<0.05$). The greatest impact on antiradical capacity, i.e. the minimum value of IC50, was recorded for the broad bean sample treated with light pulses with fluency of 43.2 J/cm^2 and germinated under dark conditions (B3). However, when considering the light/dark regime during the germination process, no significant influence on the antioxidant activity of the broad bean samples ($p>0.05$) was observed (Figure 4b). The favorable effect of germination on the antioxidant capacity of legumes was previously reported by Duenas et al. (2009) and Gharachorloo et al. (2013).

The phytochemical profile of the seeds of different origins is significantly improved by germination. Considering the involvement of free radicals in the occurrence and progress of different diseases, particular attention was paid

to studying the influence of germination on the antioxidant pool. Intensification of particular metabolic processes during germination results in the accumulation of various compounds that form redox systems including B vitamins in their structure. Various seeds may have very low or even non-detectable amounts of vitamin C, but this compound may accumulate significantly during germination by *de novo* synthesis (Gan et al., 2017). The accumulation of tocopherols, the presence of superoxide dismutase and the increased catalase activity contribute to the antioxidant potential of germinated seeds. Moreover, the increased amounts of free SH groups have also been reported to play important role in metabolic mechanisms. The most important biological functions of phytochemical compounds in the germinated seeds are antioxidant, anti-inflammatory, antidiabetic, antibacterial and antitumor effects (Hayat et al., 2014; Gan et al., 2017). Many of these effects of germinated seeds have been associated with the accumulation of biologically active compounds such as polyphenols, leading to the gradual orientation of nutritionists towards the widespread use in the diets of germinated cereals and legumes. Moreover, this trend complies with the traditional medicine recommendations on the great importance of germinated seeds for human health.

CONCLUSIONS

The results obtained in the present work indicate that the composition and the antioxidant properties of green lentils, chickpeas and broad beans can be modulated through germination under different conditions. Germination efficiency was not significantly affected by the dark or light regime. All germinated pulses accumulated higher amounts of proteins compared to the raw materials. The most significant increase of the antioxidant capacity of the germinated pulses was observed in case of the samples preliminary treated through pulsed light at high fluence value of 43.2 J/cm^2, followed by germination underdarkness in case of chickpeas and broad bens, or under daylight conditions in case of lentils.

ACKNOWLEDGMENT

This work was supported by a grant of the Romanian National Authority for Scientific Research and Innovation, CNCS/CCCDI - UEFISCDI, project number PN-III-P2-2.1-PED-2016-0155, within PNCDI III.

REFERENCES

AACC International, 2010. Approved Methods of Analysis, 11th ed. St. Paul, MN: AACC International, 44–51.

ASRO, 2008. Romanian standards catalog for cereal and milling products analysis. Bucharest: Romanian Standards Association, National Standardizations Body.

Bassett C., Boye J., Tyler R., Oomah B.D., 2010. Molecular, functional and processing characteristics of whole pulses and pulse fractions and their emerging food and nutraceutical applications. Food Research International, 43, 397-398.

Cáceres P.J., Martínez-Villaluenga C., Amigo L., Frias J., 2014. Maximising the phytochemical content and antioxidant activity of Ecuadorian brown rice sprouts through optimal germination conditions. Food chemistry, 152, 407-414.

Duenas M., Hernandez T., Estrella I., Fernandez D., 2009. Germination as a process to increase the polyphenol content and antioxidant activity of lupin seeds (*Lupinus angustifolius* L.). Food Chemistry, 117(4), 599-607.

Gan R.Y., Lui W.Y., Wu K., Chan C.L., Dai S.H., Sui Z.Q., Corke H., 2017. Bioactive compounds and bioactivities of germinated edible seeds and sprouts: An updated review. Trends in Food Science & Technology, 59, 1-14

Gharachorloo M., Tarzi B. G., Baharinia M., 2013. The effect of germination on phenolic compounds and antioxidant activity of pulses. Journal of the American Oil Chemists' Society, 90(3), 407-411.

Ghavidel R.A., Prakash J., 2007. The impact of germination and dehulling on nutrients, antinutrients, *in vitro* iron and calcium bioavailability and in vitro starch and protein digestibility of some legume seeds. LWT-Food Science and Technology, 40(7), 1292-1299.

Freitas R.L., Teixeira A.R., Ferreira R.B., 2007. Vicilin-type globulins follow distinct patterns of degradation in different species of germinating legume seeds. Food Chemistry, 102(1), 323-329.

Hayat I., Ahmad A., Masud T., Ahmed A., Bashir S., 2014. Nutritional and health perspectives of beans (*Phaseolus vulgaris* L.): An overview. Critical Reviews in Food Science and Nutrition, 54, 580-592.

Khandelwal S., Udipi S. A., Ghugre, P., 2010. Polyphenols and tannins in Indian pulses: Effect of soaking, germination and pressure cooking. Food Research International, 43(2), 526-530.

Lee C.K., Karunanithy R., 1990. Effects of germination on chemical composition of glycine and phaseolus beans. Journal of Science of Food and Agriculture 51, 437-445.

Lindig-Cisneros R., Zedler J., 2001. Effect of light on seed germination in *Phalarisa rundinacea* L. (reed canary grass). Plant Ecology, 155(1), 75-78.

López-Amorós M. L., Hernández T., Estrella I., 2006. Effect of germination on legume phenolic compounds and their antioxidant activity. Journal of Food Composition and Analysis, 19(4), 277-283.

Martín-Cabrejas M. A., Ariza N., Esteban R., Mollá E., Waldron K., López-Andréu F. J., 2003. Effect of germination on the carbohydrate composition of the dietary fiber of peas (*Pisum sativum* L.). Journal of agricultural and food chemistry, 51(5), 1254-1259.

Martín-Cabrejas M. A., Díaz M. F., Aguilera Y., Benítez V., Mollá E., Esteban R. M., 2008. Influence of germination on the soluble carbohydrates and dietary fibre fractions in non-conventional legumes. Food Chemistry, 107(3), 1045-1052.

Martinez Villaluenga C., Michalska A., Frías J., Piskula M.K., Vidal-Valverde C., Zieliński H., 2009. Effect of flour extraction rate and baking on thiamine and riboflavin content and antioxidant capacity of traditional rye bread. Journal of Food Science, 74(1), C49-C55.

Seo M., Nambara E., Choi G., Yamaguchi S., 2009. Interaction of light and hormone signals in germinating seeds. Plant molecular biology, 69(4), 463.

Shine M.B., Guruprasad K.N., Anand A., 2011. Enhancement of germination, growth, and photosynthesis in soybean by pre-treatment of seeds with magnetic field. Bioelectromagnetics, 32(6), 474-484.

Rajendra P., Sujatha Nayak H., Sashidhar R. B., Subramanyam C., Devendranath D., Gunasekaran B., Aradhya R.S.S., Bhaskaran A., 2005. Effects of power frequency electromagnetic fields on growth of germinating *Vicia faba* L., the broad bean. Electromagnetic Biology and Medicine, 24(1), 39-54.

Turtoi M., Nicolau A., 2007. Intense light pulse treatment as alternative method for mould spores destruction on paper-polyethylene packaging material. Journal of Food Engineering, 83(1), 47-53.

Vashisth A., Nagarajan S., 2010. Effect on germination and early growth characteristics in sunflower (*Helianthus annuus*) seeds exposed to static magnetic field. Journal of plant physiology, 167(2), 149-156.

Yu-Wei L., Wang Q., 2015. Effect of processing on phenolic content and antioxidant activity of four commonly consumed pulses in China. Journal of Horticulture, 1-5.

Scientific Papers. Series D. Animal Science. Vol. LXI, Number 1, 2018
ISSN 2285-5750; ISSN CD-ROM 2285-5769; ISSN Online 2393-2260; ISSN-L 2285-5750

EFFECTS OF POMEGRANATE PEEL AND PROPOLIS POWDERS AND THEIR COMBINATIONS ON PHYSICO-CHEMICAL AND MICROBIOLOGICAL PROPERTIES OF TURKISH DRY-FERMENTED SAUSAGE (SUCUK) WITH VARIOUS NITRITE LEVELS

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Abstract

The study aimed to evaluate the effects of pomegranate peel (PP) and propolis (PR) powders (2%) and their combinations (1%PP and 1%PR) on physico-chemical and microbiological properties of sucuks produced with various nitrite (N) doses (0, 50, 100 and 150 ppm) during fermentation (10 d) and storage periods (4°C; 30 d). The results showed that the pH values of all sucuks decreased during fermentation whereas the pH increased during storage (P<0.05). Water activity (aw) decreased during both the fermentation and storage periods (P<0.05). The lowest aw was obtained in samples with PR (P<0.05), whereas nitrite doses did not have a significant effect on aw. Oxidation-Reduction Potential (ORP) increased during fermentation and storage (P<0.05). PP and PR powders and different nitrite concentrations had no effect on pH and ORP. The results indicated that L values increased with PP, and decreased with PR during fermentation and storage (P<0.05). Furthermore, a increased and b* decreased during fermentation, whereas a* and b* values decreased during storage in all sucuk groups (P<0.05). The lowest a* was determined in samples with PR (P<0.05). There was a gradual increase in TBARS in all sucuks during fermentation and storage (P<0.05). The results showed that PP and PR powders were effective in reducing the TBARS levels (P<0.05). PP inhibited TBARS formation more effectively than PR in nitrite-free samples, whereas this difference between PP and PR was not determined in samples containing different nitrite levels (P<0.05). Total mesophilic aerobic bacteria counts increased during the fermentation and storage period whereas the yeast-mould counts generally decreased at the end of the fermentation (P<0.05). Furthermore, coliform bacteria counts did not change during fermentation and storage.*

Key words*: pomegranate, propolis, powder, nitrite, sucuk.*

INTRODUCTION

Chemical and microbiological changes are major cause of meat quality deterioration. For this purpose, food additives are natural or synthetics substances used to extend the shelf life by preserving the quality of meat products (Bobko et al., 2015). However, there are concerns and limitations about the use of synthetic additives because recent scientific studies have shown potential toxic effects and high costs, and consumer concerns about food additives are increasing. For these reasons, consumer demand for natural products has shifted the food industry to the use of natural additives in meat products (Şimşek and Kılıç, 2012).

Sucuk is one of the most popular and widely consumed dry-fermented meat products in Turkey (Bozkurt and Erkmen, 2007; Kilic, 2009). Lamb and/or beef, water buffalo meat, beef fat or tail fat, salt, sugar, nitrite and/or nitrate, garlic, and various spices such as black pepper, red pepper, and cumin are used in the sucuk production (Kilic, 2009). Sucuk is a meat product resistant to spoilage because of salt, nitrite, low pH and water activity. Nitrite is a synthetic food additive and is concerned about the consumption of nitrite-containing products due to its health effects. For this reason, some researchers are working on natural food additives that can be an alternative to nitrite (Li et al., 2013; Kurcubic et al., 2014).

The pomegranate (*Punica granatum* L.) is an edible fruit with composed of many pieces that is slightly sweet and sour and is widely grown in many tropical and subtropical countries (Yasoubi et al., 2007). Pomegranate peel and seeds are by products obtained during processing of pomegranate juice (Devatkal et al., 2011). Pomegranate peel are reported to possess a significant level of antioxidant and antimicrobial activity due to polyphenolic substances such as ellagic tannins, ellagic acid and gallic acid (Yasoubi et al., 2007; Qin et al., 2013). There are a number of studies investigating the antioxidant and antimicrobial effects of pomegranate peel or seeds in various meat products (Devatkal et al., 2010; Malviya et al., 2014; El-Nashi et al., 2015).

Propolis is a resinous, rubbery and balsamic substance collected from buds and exudates of flowers and trees by honey bees (Ali et al., 2010). Propolis has functional properties such as antioxidant, antibacterial, antifungal, local anesthetic and anti-inflammatory activities. These functional properties are due to components such as resins, aromatic and ethereal oils, flavonoid pigment, vanillin, isovanilin, caffeic, benzoic and ascorbic acids as well as benzyl alcohol and cinnamic acid (Ali et al., 2010; Temiz et al., 2011).There are numerous studies on the antioxidant and especially antimicrobial effects of propolis in meat products (Lu et al., 2005; Ali et al., 2010; Vargas-Sanchez et al., 2015). The purpose of this work is to evaluate the effects of PP and PR powders (2%) and their combinations (1% PP and 1% PR) on physico-chemical and microbiological properties of sucuks produced with various nitrite doses (0, 50, 100 and 150 ppm) during fermentation (10 d) and storage periods (4^{o}C; 30 d).

MATERIALS AND METHODS

Beef meat (*Longissimus thoracis et lumborum, LL*) and fat were purchased from a local slaughterhouse for each of two replications on separate production days. Spices, propolis powder, sodium nitrite and starter culture mix (Bitec Starter LS 25) were supplied by Arifoglu Spices and Food Industry (Istanbul, Turkey), Marmaris Balcısı (Muğla, Turkey), Merck (Germany) and Etol Aroma ve Baharat Gıda Ürünleri San ve Tic. A.Ş (Kocaeli, Turkey), respectively. Pomegranate peel were dried in an air circulatory drier (FN 500, Nüve, Turkey) at 40°C for 48 h, and ground in an analytical mill to a grain diameter of less than 0.5 mm.

Turkish dry-fermented sausage (Sucuk) production. Sucuk (approximately 1000 g each) was manufactured with respect to the traditional sucuk production method (Bozkurt and Erkmen, 2007). All sucuk samples contained beef and fat (5:1), salt (2%), garlic (1%), saccharose (0.4%), red pepper (0.7%), black pepper (0.5%), 9 g cumin (0.9%), allspice (0.25%) and starter culture mix (0.025%). Sucuk batter was formulated with different level of PP, PR and sodium nitrite (Table 1).

Table 1. Coding for pomegranate peel (PP), propolis (PR) powder and sodium nitrite (N) treatments evaluated

Groups	PP, PR and sodium nitrite treatments
Control	Without any powders and sodium nitrite
PPN0	2% Pomegranate peel powder
PRN0	2% Propolis powder
PP/PR N0	1% Pomegranate peel powder and 1% propolis powder
PPN50	2% Pomegranate peel powder and 50 ppm sodium nitrite
PRN50	2% Propolis powder and 50 ppm sodium nitrite
PP/PR N50	1% Pomegranate peel powder, 1% propolis powder, 50 ppm sodium nitrite
PPN100	2% Pomegranate peel powder and 100 ppm sodium nitrite
PRN100	2% Propolis powder and 100 ppm sodium nitrite
PP/PR N100	1% Pomegranate peel powder, 1% propolis powder, 100 ppm sodium nitrite
PPN150	2% Pomegranate peel powde rand 150 ppm sodium nitrite
PRN150	2% Propolis powder and 150 ppm sodium nitrite
PP/PR N150	1% Pomegranate peel powder, 1% propolis powder, 150 ppm sodium nitrite

The fermentation process was carried out under the following conditions: 24±1°C and relative humidity (RH) 95±1% for the first day, 22±1°C and RH 90±1% for the second day, 20±1°C and RH 85±1% for the half of the third day, 20±1°C and RH 80±1% for the other half of the third day, and 18±1°C and RH 70±1% for the last 7 days. Samples for physico-chemical and microbiological analyses were taken from sucuks immediately after stuffing (0 day), and after 5 and 10 days of ripening. Sucuks were kept at 4°C for 30 days, and samples analyzed at 0 (at the end of fermentation), 15 and 30 days of storage.

Physico-chemical analyses. The pH was measured using spear electrode (FC 200, Hanna Instruments, Germany) attached to a portable pH meter (HI 9024, Hanna Instruments, Germany). Color values of sucuk samples were measured according to CIE Lab Color System using a Minolta Colorimeter (Model CR-200, Minolta corp., Ramsey, Nj, USA). The water activity (a_w) values of the sucuks were determined at 20°C by using a Novasina LabTouch-a_w. Oxidation-reduction potential (ORP) was measured in sucuks using pH meter (WTW pH 3110, Germany) set to the millivolt scale and equipped with redox electrode. Thiobarbituric acid reactive substances (TBARS) were determined according to the extraction method of Lemon (1975) as described by Kilic and Richards (2003). The TBARS values were stated as μmol TBARS per kg of sucuk samples.

Microbiological analyses. A 10 g sample was aseptically taken from sucuks and transferred in a sterile Stomacher bag, and homogenized in 90 mL sterile 0.1% peptone water. Serial decimal dilutions were prepared using with 0.1% peptone water. Total mesophilic aerobic bacteria (TMAB), yeast and mould, and total coliform bacteria counts were determined according to the spread plate techniques on plate count agar at 30°C for 2 days, potato dextrose agar at 25°C for 2-5 days, and eosin methylene blue agar at 37°C for 2 days, respectively (Maturin and Peeler, 2001).

RESULTS AND DISCUSSIONS

The changes in pH levels of sucuk samples are shown in table 2. The results showed that the pH values of all sucuk samples decreased during first 5 days of fermentation whereas the pH gradually increased during last 5 days of fermentation and during storage period (P<0.05). Similar results were reported by Kunrath et al. (2017) for the Italian-type salami samples. Researchers noted that the pH dropped rapidly during the first 6 days of maturation, and gradually increased after the 6th day. Researchers pointed out that the decline in pH over the first 6 days of maturation was probably due to the presence of lactic acid bacteria in the starter culture added to the formulation (Kunrath et al., 2017). In our study, the highest pH values were detected in groups of control and PRN150, whereas the lowest pH was detected in group of PPN0 at the beginning of fermentation (P<0.05). Furthermore, the pH values of the samples with PP were also found to be lower than the other treatment groups at the beginning of fermentation (P<0.05). Similar findings were observed by Chandralekha et al. (2012) in chicken meat balls. They reported that pomegranate rind powder extracts caused lower pH values than the other formulation groups. On the other hand, El-Nashi et al. (2015) reported that the addition of PP powder in beef sausages did not cause a significant differences in pH values. At the end of fermentation, there was not found any significant difference between all sucuk groups, whereas the lowest pH value and the highest pH value were detected in the group of PP/PRN150 and PRN150 at the end of storage, respectively (P<0.05). Bernardi et al. (2013) reported that propolis containing products showed similar results when compared with control in terms of pH values. In the study conducted by us, there was not detected a significant difference between the groups containing PR and the control, however, the higher pH values were determined in PR containing groups compared with control at the end of storage (P<0.05). Additionally, the use of various nitrite doses did not have a significant effect on pH.

Table 2. The results of pH changes of sucuk samples

	Fermentationtime (Day)			Storage time (Day)	
Groups	0	5	10	15	30
Control	5.61^{aA}±0.00	4.5^{cdE}±0.01	4.72^{abD}±0.01	5.12^{aB}±0.01	5.03^{efC}±0.01
PPN0	5.31^{hA}±0.01	4.5^{cD}±0.00	4.70^{abcC}±0.01	5.06^{bB}±0.02	5.06^{bcB}±0.00
PRN0	5.56^{cA}±0.00	4.54^{bE}±0.01	4.76^{abD}±0.01	5.12^{aB}±0.01	5.06^{bcC}±0.00
PP/PR N0	5.42^{fA}±0.00	4.54^{bE}±0.01	4.69^{abcD}±0.01	4.98^{deC}±0.01	5.04^{deB}±0.01
PPN50	5.33^{gA}±0.00	4.54^{bE}±0.01	4.65^{bcD}±0.01	4.86^{hC}±0.00	5.02^{fB}±0.01
PRN50	5.59^{bA}±0.01	4.53^{bE}±0.01	4.68^{abcD}±0.01	4.99^{dC}±0.00	5.06^{cB}±0.01
PP/PR N50	5.45^{eA}±0.01	4.49^{dE}±0.01	4.78^{abD}±0.01	4.95^{fC}±0.00	5.08^{bB}±0.01
PPN100	5.33^{gA}±0.00	4.53^{bE}±0.00	4.73^{abD}±0.00	5.05^{bC}±0.01	5.06^{bcB}±0.00
PRN100	5.59^{bA}±0.01	4.58^{aE}±0.00	4.77^{abD}±0.00	5.02^{cC}±0.00	5.18^{aB}±0.00
PP/PR N100	5.46^{dA}±0.00	4.51^{cC}±0.01	4.56^{cC}±0.21	4.93^{gB}±0.01	5.05^{cdB}±0.01
PPN150	5.33^{gA}±0.01	4.40^{eE}±0.01	4.71^{abD}±0.01	4.86^{hC}±0.00	5.00^{gB}±0.01
PRN150	5.61^{aA}±0.01	4.53^{bE}±0.01	4.8^{aD}±0.00	4.96^{efC}±0.00	5.08^{bB}±0.01
PP/PR N150	5.47^{dA}±0.01	4.5^{cE}±0.00	4.79^{abD}±0.00	5.03^{cB}±0.01	4.96^{hC}±0.01

Means±standart deviation (SD). $^{a-h}$Within the column, values superscripted with different letters are significantly different (P<0.05). $^{A-E}$Within the row, values superscripted with different letters are significantly different (P<0.05).

The a_w values are given in Table 3. The a_w values decreased during both the fermentation and storage periods (P<0.05).Similarly, Kunrath et al. (2017) reported that water activity values decreased during maturation in Italian-type salami. The water activity values of the samples with PP were obtained to be lower than the control at the end of fermentation and storage (P<0.05). In general, the lower aw values were obtained in samples with PR compared to the other sucuk groups (P<0.05), whereas nitrite doses difference did not have a significant effect on aw. Kunrath et al. (2017) and Bernardi et al. (2013) indicated that the use of PR extract in Italian-type salami production did not have a significant effect on a_w values.

The color results (data is not presented) showed that L* values increased with PP addition to sucuks, and decreased with PR addition during fermentation and storage (P<0.05). Naveena et al. (2008a) and Devatkal and Naveena (2010) reported that the PP powder addition had caused lower L* values as compared to control in raw ground goat meat and cooked chicken patties, respectively. At the end of fermentation, the lowest L* values were detected in all PR containing groups (P<0.05). Additionally, L* values increased in PR containing groups with increasing nitrite level at the end of storage (P<0.05). The lowest a* values were determined in samples with PR, whereas the highest a* values were also determined in samples with PP (P<0.05). Naveena et al. (2008b) indicated an increase in a* values as a result of PP powder extract addition in cooked chicken patties. In general, a* values increased in PR containing groups with increasing nitrite level (P<0.05), whereas a similar effect did not have on PP containing groups. Furthermore, a* values increased and b* values decreased during fermentation, whereas a* and b* values decreased during storage in all sucuk groups (P<0.05).

The results of ORP are presented in table 4. The ORP values of sucuk samples were varied between -104.05 and -49.7 at the beginning of fermentation. The lowest ORP were detected in the group of PPN0 and PRN0, whereas the highest ORP value was determined in the group of PP/PR N50 at the beginning of the fermentation period (P<0.05). Results showed that the ORP values were increased during fermentation and storage period (P<0.05). The highest ORP values were determined in the group of PPN50 at the end of the both fermentation and storage period (P<0.05). The lowest ORP value was determined in the group of PPN100 at the end of fermentation, where as the lowest ORP values were determined in the groups of PPN0 and PP/PR N50 at the end of storage (P<0.05).

TBARS changes of sucuks are given in table 5. The TBARS values of sucuks were changed between 2.13-3.87 µmol/kg at the beginning of fermentation period. There was a gradual increase in TBARS levels in all sucuks during fermentation and storage period (P<0.05).

Table 3. The results of a_w values of sucuk samples

	Fermentation time (Day)			Storage time (Day)	
Groups	0	5	10	15	30
Control	$0.93^{aA}\pm0.00$	$0.90^{aB}\pm0.00$	$0.86^{aC}\pm0.00$	$0.71^{fgD}\pm0.01$	$0.70^{aD}\pm0.00$
PPN0	$0.93^{aA}\pm0.00$	$0.87^{efB}\pm0.00$	$0.84^{bcC}\pm0.00$	$0.69^{hD}\pm0.01$	$0.63^{bE}\pm0.00$
PRN0	$0.91^{fA}\pm0.00$	$0.87^{efB}\pm0.00$	$0.76^{eC}\pm0.00$	$0.69^{hD}\pm0.01$	$0.53^{eE}\pm0.00$
PP/PR N0	$0.92^{b-eA}\pm0.00$	$0.87^{fB}\pm0.00$	$0.82^{dC}\pm0.00$	$0.73^{cdD}\pm0.00$	$0.60^{cdE}\pm0.01$
PPN50	$0.92^{b-eA}\pm0.00$	$0.89^{dB}\pm0.00$	$0.83^{cC}\pm0.00$	$0.74^{cD}\pm0.00$	$0.59^{cdE}\pm0.00$
PRN50	$0.92^{fA}\pm0.01$	$0.88^{eB}\pm0.01$	$0.82^{dC}\pm0.00$	$0.73^{deD}\pm0.01$	$0.61^{bcE}\pm0.01$
PP/PR N50	$0.92^{efA}\pm0.00$	$0.89^{bcdB}\pm0.00$	$0.84^{bC}\pm0.01$	$0.74^{cD}\pm0.00$	$0.63^{bE}\pm0.01$
PPN100	$0.92^{b-eA}\pm0.00$	$0.89^{cdB}\pm0.00$	$0.85^{aC}\pm0.00$	$0.70^{gD}\pm0.00$	$0.60^{cdE}\pm0.00$
PRN100	$0.92^{efA}\pm0.00$	$0.89^{abB}\pm0.00$	$0.83^{cC}\pm0.01$	$0.69^{hD}\pm0.01$	$0.59^{cdE}\pm0.03$
PP/PR N100	$0.92^{defA}\pm0.00$	$0.89^{dB}\pm0.00$	$0.86^{aC}\pm0.00$	$0.77^{bD}\pm0.00$	$0.60^{cdE}\pm0.01$
PPN150	$0.92^{cdeA}\pm0.00$	$0.89^{bcdB}\pm0.00$	$0.84^{bC}\pm0.00$	$0.84^{aC}\pm0.00$	$0.59^{dE}\pm0.01$
PRN150	$0.92^{efA}\pm0.00$	$0.90^{aB}\pm0.00$	$0.86^{aC}\pm0.00$	$0.74^{cdD}\pm0.01$	$0.54^{eE}\pm0.00$
PP/PR N150	$0.92^{bcA}\pm0.00$	$0.89^{abcB}\pm0.00$	$0.86^{aC}\pm0.00$	$0.72^{efD}\pm0.01$	$0.58^{dE}\pm0.00$

Means±standart deviation (SD). $^{a-h}$Within the column, values superscripted with different letters are significantly different (P<0.05). $^{A-E}$Within the row, values superscripted with different letters are significantly different (P<0.05).

Table 4. The results of ORP values of sucuk samples

	Fermentation time (Day)			Storage time (Day)	
Groups	0	5	10	15	30
Control	$-92.8^{jE}\pm0.14$	$-52.85^{kD}\pm0.07$	$48.30^{dC}\pm0.00$	$64.70^{dB}\pm0.14$	$92.65^{bA}\pm0.07$
PPN0	$-103.75^{kE}\pm2.05$	$-77.35^{lD}\pm0.21$	$15.85^{lC}\pm0.07$	$39.25^{kB}\pm0.91$	$56.35^{kA}\pm0.78$
PRN0	$-104.05^{kE}\pm0.07$	$-42.60^{jD}\pm0.14$	$25.15^{iC}\pm0.21$	$82.85^{aB}\pm0.21$	$73.95^{dA}\pm0.07$
PP/PR N0	$-73.65^{fE}\pm0.07$	$-20.85^{fD}\pm0.07$	$48.65^{eB}\pm0.21$	$37.70^{lC}\pm0.14$	$57.95^{jA}\pm0.07$
PPN50	$-87.55^{hE}\pm0.07$	$-33.65^{gD}\pm0.07$	$76.55^{aB}\pm0.07$	$70.45^{cC}\pm0.07$	$95.75^{aA}\pm0.07$
PRN50	$-90.9^{iE}\pm0.14$	$-11.55^{dD}\pm0.07$	$39.05^{fC}\pm0.07$	$79.75^{bA}\pm0.07$	$64.25^{gB}\pm0.07$
PP/PR N50	$-49.7^{aE}\pm0.14$	$-7.85^{bD}\pm0.07$	$50.00^{bC}\pm0.00$	$61.95^{eA}\pm0.07$	$56.55^{kB}\pm0.07$
PPN100	$-60.95^{dE}\pm0.07$	$-35.95^{hD}\pm0.07$	$4.65^{mC}\pm0.07$	$45.85^{jB}\pm0.21$	$60.95^{iA}\pm0.07$
PRN100	$-73.6^{fE}\pm0.14$	$-10.35^{cD}\pm0.07$	$33.85^{gC}\pm0.07$	$60.45^{fB}\pm0.07$	$63.20^{hA}\pm0.14$
PP/PR N100	$-54.3^{cE}\pm0.14$	$-7.75^{bD}\pm0.07$	$22.45^{jC}\pm0.07$	$56.40^{hB}\pm0.00$	$68.10^{fA}\pm0.14$
PPN150	$-79.45^{gE}\pm0.07$	$-39.55^{iD}\pm0.07$	$18.10^{kC}\pm0.14$	$48.50^{iB}\pm0.14$	$70.60^{eA}\pm0.14$
PRN150	$-68.5^{eE}\pm0.14$	$-5.25^{aD}\pm0.07$	$32.45^{hC}\pm0.07$	$59.45^{gB}\pm0.07$	$62.75^{hA}\pm0.07$
PP/PR N150	$-51.65^{bE}\pm0.07$	$-14.90^{cD}\pm0.14$	$45.75^{eC}\pm0.07$	$60.75^{fB}\pm0.07$	$89.95^{cA}\pm0.07$

Means±standart deviation (SD). $^{a-m}$Within the column, values superscripted with different letters are significantly different (P<0.05). $^{A-E}$Within the row, values superscripted with different letters are significantly different (P<0.05).

At the end of fermentation and storage period, the lower (P<0.05) TBARS levels were detected in PP or PR containing groups compared to control group. Similarly, Han and Park (2002) indicated that the addition of PR extract to cured pork sausages resulted in lower TBARS levels than the control groups. Additionally, Ali et al. (2010) stated that the addition of PR lowered the TBA levels in fresh oriental sausages. El-Nashi et al. (2015) pointed out that the addition of PP powder reduced values of TBA in beef sausage samples as compared to control during refrigerated storage. Similarly, Borah et al. (2014) stated that the lower TBARS values as compared to control was obtained in chicken meatball with PP powder extracts during refrigerated storage. There are some other studies showing the effect of PP addition on reducing oxidation levels of meat products (Naveena et al., 2008a; Devatkal et al., 2010; El-Gharably and Ashoush, 2011). Our study results showed that PP and PR powders were effective in reducing the TBARS levels (P<0.05). PP inhibited TBARS formation more effectively than PR in nitrite-free samples, whereas this difference between PP and PR was not determined in samples containing different nitrite levels (P<0.05).

Total mesophilic aerobic bacteria (TMAB) counts were varied between 6.01 and 6.73 Log_{10} CFU/g at the beginning of fermentation (Table 6). The highest TMAB counts were detected in groups of PPN50 and PPN100 at the end of fermentation, whereas the highest TMAB counts was found in control group at the end of storage (P<0.05).

Table 5. The results of TBARS values of sucuk samples (μmol TBARS per kg sucuk)

	Fermentation time (Day)			Storage time (Day)	
Groups	0	5	10	15	30
Control	3.50^{abcE}±0.47	4.94^{aD}±0.43	6.32^{aC}±0.39	9.25^{aB}±0.04	11.25^{aA}±0.04
PPN0	3.24^{a-dC}±0.04	3.83^{bcdC}±0.40	3.68^{eC}±0.43	5.57^{bcdB}±0.24	7.69^{cdA}±0.12
PRN0	3.87^{aC}±0.09	4.41^{abC}±0.09	4.82^{bC}±0.67	6.44^{bB}±0.36	9.69^{bA}±0.28
PP/PR N0	3.29^{abcC}±0.44	3.53^{c-fC}±0.58	4.84^{bB}±0.11	5.60^{bcdB}±0.42	8.30^{cA}±0.29
PPN50	3.58^{abC}±0.35	4.19^{abcBC}±0.29	4.19^{bcBC}±0.26	4.49^{eB}±0.46	6.42^{fA}±0.27
PRN50	3.17^{a-dC}±1.12	3.47^{c-fC}±0.07	4.58^{bcBC}±0.20	5.56^{bcdAB}±0.60	7.51^{cdeA}±0.11
PP/PR N50	2.64^{bcdC}±0.41	3.22^{defC}±0.18	4.37^{bcB}±0.10	5.09^{cdeB}±0.37	7.06^{defA}±0.44
PPN100	2.38^{cdC}±0.51	3.42^{c-fBC}±0.15	4.38^{bcB}±0.13	4.40^{eB}±0.38	6.90^{defA}±0.80
PRN100	2.43^{cdE}±0.05	3.73^{b-eD}±0.08	4.61^{bcC}±0.15	5.71^{bcB}±0.24	7.42^{cdeA}±0.04
PP/PR N100	3.12^{a-dB}±0.67	3.30^{defB}±0.53	4.43^{bcAB}±0.77	4.99^{cdeA}±0.62	6.94^{defA}±0.38
PPN150	2.73^{bcdC}±0.37	2.96^{efC}±0.32	3.68^{cBC}±0.47	4.71^{deAB}±0.41	6.69^{efA}±0.49
PRN150	2.13^{dD}±0.14	2.74^{fC}±0.08	3.75^{cB}±0.44	4.35^{eB}±0.22	7.27^{defA}±0.07
PP/PR N150	2.37^{cdD}±0.06	3.29^{defCD}±0.56	4.19^{bcBC}±0.57	4.60^{eB}±0.01	7.03^{defA}±0.62

Means±standart deviation (SD). $^{a-f}$Within the column, values superscripted with different letters are significantly different (P<0.05). $^{A-E}$Within the row, values superscripted with different letters are significantly different (P<0.05).

Table 6. The results of total mesophilic aerobic bacteria counts of sucuks (Log_{10} CFU/g)

	Fermentation time (Day)			Storage time (Day)	
Groups	0	5	10	15	30
Control	6.34^{bcD}±0.05	7.75^{eC}±0.21	8.78^{bcB}±0.00	10.11^{aA}±0.00	10.15^{aA}±0.05
PPN0	6.54^{abD}±0.04	8.04^{dC}±0.27	8.13^{dBC}±0.03	8.44^{dAB}±0.04	8.57^{bcA}±0.04
PRN0	6.47^{abE}±0.06	8.54^{aA}±0.09	8.14^{dB}±0.13	7.90^{gC}±0.00	7.30^{efD}±0.00
PP/PR N0	6.44^{bcD}±0.04	8.29^{a-dB}±0.13	8.19^{dBC}±0.11	7.91^{gC}±0.18	8.73^{bA}±0.00
PPN50	6.49^{abE}±0.04	8.47^{abD}±0.01	9.83^{aA}±0.00	9.08^{bB}±0.00	8.87^{bC}±0.09
PRN50	6.46^{abC}±0.16	8.22^{a-dA}±0.06	8.69^{bcA}±0.30	8.26^{eA}±0.00	7.15^{efB}±0.21
PP/PR N50	6.73^{aC}±0.04	8.51^{aA}±0.00	8.49^{bcdA}±0.01	8.48^{dA}±0.00	7.99^{dB}±0.00
PPN100	6.31^{bcD}±0.17	8.14^{cdC}±0.19	9.53^{aA}±0.06	8.86^{cB}±0.00	8.18^{cdC}±0.00
PRN100	6.01^{dC}±0.08	8.40^{abcA}±0.20	8.43^{cdA}±0.00	8.18^{efA}±0.00	7.00^{fB}±0.00
PP/PR N100	6.41^{bcD}±0.04	8.33^{a-dC}±0.04	8.52^{bcdB}±0.09	8.84^{cA}±0.09	8.75^{bA}±0.01
PPN150	6.36^{bcC}±0.25	8.22^{a-dB}±0.06	8.62^{bcAB}±0.40	8.88^{cA}±0.04	8.79^{bA}±0.06
PRN150	6.42^{bcD}±0.13	8.49^{abAB}±0.01	8.88^{bA}±0.04	8.12^{fBC}±0.05	7.43^{eC}±0.60
PP/PR N150	6.18^{cdB}±0.14	8.17^{bcdA}±0.02	8.48^{bcdA}±0.28	8.12^{fA}±0.05	8.10^{dA}±0.02

Means±standart deviation (SD). $^{a-f}$Within the column, values superscripted with different letters are significantly different (P<0.05). $^{A-E}$Within the row, values superscripted with different letters are significantly different (P<0.05).

The lowest TMAB counts were determined in groups of PRN0, PRN50, PRN100 and PRN150 at the end of storage (P<0.05). The increase in the nitrite doses did not have a significant effect on the TMAB counts. In general, TMAB counts increased during the fermentation and storage period (P<0.05). The lower (P<0.05) TMAB counts were detected in PP or PR containing groups compared to control group at the end of fermentation and during storage. El-Nashi et al. (2015) indicated that PP powder addition reduced TMAB counts in beef sausages as compared to control during refrigerated storage. Similar results regarding the reduction of the TMAB counts of PP powder extract additions were reported by Chandralekha et al. (2012).

The yeast-mould counts generally decreased during fermentation and storage period in all groups except for control (P<0.05). Whereas the yeast-mould counts did not significantly change during the fermentation in control group, its increased during storage period (P<0.05; Table 7). At the end of fermentation, the highest yeast-mould counts were detected in groups of PPN0 and control (P<0.05). On the other hand, the highest yeast-mould counts were obtained in the control group at the end of storage (P<0.05). The lower yeast-mould counts were obtained in PP and PR containing groups with increasing nitrite levels in the 15th and 30th days of storage (P<0.05). El-Nashi et al. (2015) stated that PP powder decreased yeast and mould counts in beef sausages as compared to control during refrigerated storage, and this reducing effect of PP powder addition on yeast and mould counts also increased with increasing powder levels.

Table 7. The results of yeast and mould counts of sucuk samples (Log_{10} CFU/g)

	Fermentation time (Day)			Storage time (Day)	
Groups	0	5	10	15	30
Control	$4.64^{defC}\pm0.09$	$4.78^{fgC}\pm0.04$	$4.59^{aC}\pm0.09$	$5.00^{aB}\pm0.00$	$5.43^{aA}\pm0.04$
PPN0	$4.61^{efB}\pm0.02$	$5.31^{bcA}\pm0.04$	$4.63^{aB}\pm0.02$	$3.95^{eC}\pm0.07$	$3.47^{cdD}\pm0.18$
PRN0	$4.51^{fB}\pm0.23$	$4.99^{defA}\pm0.01$	$4.06^{bcdC}\pm0.03$	$4.60^{cB}\pm0.00$	$4.23^{bC}\pm0.02$
PP/PR N0	$5.00^{bB}\pm0.06$	$5.79^{aA}\pm0.04$	$4.39^{abC}\pm0.04$	$4.73^{bBC}\pm0.01$	$3.24^{dD}\pm0.34$
PPN50	$4.79^{cdeA}\pm0.05$	$4.30^{hA}\pm0.09$	$3.65^{eB}\pm0.50$	$3.00^{iC}\pm0.00$	$3.22^{dBC}\pm0.02$
PRN50	$4.97^{bcA}\pm0.09$	$5.06^{cdeA}\pm0.35$	$4.06^{bcdB}\pm0.03$	$3.60^{fB}\pm0.00$	$2.98^{dC}\pm0.18$
PP/PR N50	$5.27^{aA}\pm0.06$	$5.06^{cdeB}\pm0.01$	$3.00^{fD}\pm0.00$	$3.13^{hC}\pm0.07$	$3.00^{dD}\pm0.00$
PPN100	$4.55^{fB}\pm0.01$	$5.68^{aA}\pm0.06$	$3.72^{deC}\pm0.33$	$4.30^{dB}\pm0.00$	$3.14^{dD}\pm0.09$
PRN100	$4.56^{fB}\pm0.02$	$5.54^{abA}\pm0.09$	$3.00^{fE}\pm0.00$	$3.48^{gD}\pm0.00$	$3.86^{bcC}\pm0.11$
PP/PR N100	$4.54^{fA}\pm0.04$	$4.68^{gA}\pm0.01$	$3.65^{eB}\pm0.07$	$3.00^{iC}\pm0.00$	$2.30^{eD}\pm0.42$
PPN150	$4.47^{fA}\pm0.09$	$4.26^{hA}\pm0.21$	$4.13^{bcA}\pm0.03$	$3.00^{iB}\pm0.00$	$2.15^{eC}\pm0.21$
PRN150	$4.63^{defA}\pm0.10$	$4.70^{fgA}\pm0.10$	$3.88^{cdeB}\pm0.04$	$3.00^{iC}\pm0.00$	$2.39^{eD}\pm0.13$
PP/PR N150	$4.84^{bcdA}\pm0.05$	$4.87^{efgA}\pm0.14$	$3.95^{cdeB}\pm0.00$	$3.48^{gC}\pm0.00$	$2.24^{eD}\pm0.34$

Means±standart deviation (SD). $^{a-i}$Within the column, values superscripted with different letters are significantly different (P<0.05). $^{A-E}$Within the row, values superscripted with different letters are significantly different (P<0.05).

Table 8. The results of coliform bacteria counts of sucuk samples (Log_{10} CFU/g)

	Fermentation time (Day)			Storage time (Day)	
Groups	0	5	10	15	30
Control	$4.32^{aA}\pm0.16$	$4.30^{abA}\pm0.42$	$4.11^{eA}\pm0.10$	$4.18^{b-eA}\pm0.00$	$4.04^{bcdA}\pm0.00$
PPN0	$4.07^{aBC}\pm0.17$	$4.45^{abA}\pm0.21$	$4.30^{bcdAB}\pm0.00$	$4.50^{abA}\pm0.02$	$3.93^{b-eC}\pm0.04$
PRN0	$4.35^{aB}\pm0.00$	$4.59^{abA}\pm0.16$	$4.35^{bcB}\pm0.01$	$4.15^{b-eC}\pm0.05$	$4.12^{bcdC}\pm0.05$
PP/PR N0	$4.26^{aAB}\pm0.09$	$5.26^{aA}\pm0.79$	$4.30^{bcdAB}\pm0.06$	$4.60^{aAB}\pm0.57$	$3.87^{cdeB}\pm0.12$
PPN50	$4.15^{aA}\pm0.13$	$4.15^{bA}\pm0.21$	$4.19^{deA}\pm0.01$	$4.00^{deA}\pm0.00$	$4.19^{abA}\pm0.11$
PRN50	$3.95^{aA}\pm0.04$	$4.35^{abA}\pm0.50$	$3.95^{fA}\pm0.00$	$3.98^{eA}\pm0.04$	$4.04^{bcdA}\pm0.06$
PP/PR N50	$4.16^{aB}\pm0.11$	$4.65^{abA}\pm0.07$	$4.55^{aA}\pm0.03$	$4.15^{b-eB}\pm0.00$	$3.69^{eC}\pm0.13$
PPN100	$4.10^{aB}\pm0.09$	$4.15^{bAB}\pm0.21$	$4.45^{abA}\pm0.00$	$4.42^{abcA}\pm0.01$	$4.44^{aA}\pm0.11$
PRN100	$4.11^{aC}\pm0.03$	$4.60^{abA}\pm0.00$	$4.41^{bB}\pm0.04$	$4.28^{a-eB}\pm0.03$	$3.93^{b-eD}\pm0.11$
PP/PR N100	$4.17^{aB}\pm0.15$	$4.63^{abA}\pm0.21$	$4.31^{bcdAB}\pm0.01$	$4.00^{deBC}\pm0.00$	$3.69^{eC}\pm0.13$
PPN150	$4.44^{aB}\pm0.03$	$4.74^{abA}\pm0.06$	$4.34^{bcdB}\pm0.03$	$4.39^{a-dB}\pm0.09$	$4.14^{bcC}\pm0.09$
PRN150	$3.07^{bA}\pm1.09$	$4.00^{bA}\pm0.00$	$4.25^{cdeA}\pm0.19$	$4.09^{cdeA}\pm0.13$	$3.82^{deA}\pm0.31$
PP/PR N150	$4.01^{aA}\pm0.04$	$5.03^{abA}\pm1.03$	$4.25^{cdeA}\pm0.02$	$4.27^{a-eA}\pm0.01$	$4.11^{bcdA}\pm0.10$

Means±standart deviation (SD). $^{a-f}$ Within the column, values superscripted with different letters are significantly different (P<0.05). $^{A-E}$ Within the row, values superscripted with different letters are significantly different (P<0.05).

At the beginning of fermentation, whereas the lowest coliform count was determined in group of PRN150 (P<0.05), there was not found a significant difference between the other groups (Table 8). In general, there was no significant changes in all sucuk groups during fermentation, whereas the lowest number of coliform was determined in the group of PRN50 at the end of fermentation (P<0.05). There was a decrease in the groups of PPN0, PRN0, PP/PRN50, PRN100, PP/PRN100 and PPN150 during storage (P<0.05), whereas there was also no significant changes in other groups during storage. The highest numbers of coliform were determined in PPN50 and PPN100 groups at the end of storage (P<0.05). The results of coliform analysis shown that the changes in nitrite doses had no significant effect on the coliform counts.

CONCLUSIONS

Oxidative stability of sucuks was enhanced with the use of PP or PR or their combination.

Additionally, oxidative stability is also further improved by the use of PP or PR powders in combination with nitrite in sucuks. However, doses of nitrite did not created any difference.

PR powder was significantly effective in inhibiting of microbial growth.

The PP or PR powders did not have a negative effect on other physicochemical properties of sucuks.

The use of these powders are recommended as a natural antimicrobial and especially antioxidant additives in sucuk production.

REFERENCES

Ali F.H., Kassem G.M., Atta-Alla O.A., 2010. Propolis as a natural decontaminant and antioxidant in fresh oriental sausage. Veterinaria italiana, 46(2), 167-172.

Bernardi S., Favaro-Trindade C.S., Trindade M.A., Balieiro J.C.C., Cavenaghi A.D., Contreras-Castillo C.J., 2013. Italian-type salami with propolis as antioxidant. Italian Journal of Food Science, 25(4), 433.

Bobko M., Kročko M., Haščík P., Bobková A., 2015. Oxidative stability of chicken meat after propolis extract application in their diets. Potravinarstvo Slovak Journal of Food Sciences, 9(1), 48-52.

Borah A., Mahanta C.L., Devatkal S.K., Narsaiah K., 2014. Utilization of Fruit By-product for the Shelf Life Extension of Chicken Meat Ball. Journal of Food Research and Technology, 2(4), 153-157.

Bozkurt H., Erkmen O., 2007. Effects of some commercial additives on the quality of sucuk (Turkish dry-fermented sausage). Food chemistry, 101(4), 1465-1473.

Chandralekha S., Babu A.J., Moorthy P.S., Karthikeyan B., 2012. Studies on the effect of pomegranate rind powder extract as natural antioxidant in chicken meat balls during refrigerated storage. Journal of Advanced Veterinary Research, 2(2), 107-112.

Devatkal S.K., Naveena B.M., 2010. Effect of salt, kinnow and pomegranate fruit by-product powders on color and oxidative stability of raw ground goat meat during refrigerated storage. Meat Sci., 85(2), 306-311.

Devatkal S.K., Narsaiah K., Borah A., 2010. Anti-oxidant effect of extracts of kinnow rind, pomegranate rind and seed powders in cooked goat meat patties. Meat Science, 85(1), 155-159.

Devatkal S.K., Narsaiah K., Borah A., 2011. The effect of salt, extract of kinnow and pomegranate fruit by-products on colour and oxidative stability of raw chicken patties during refrigerated storage. Journal of food science and technology, 48(4), 472-477.

El-Gharably A.M., Ashoush I.S., 2011. Utilization impact of adding pomegranate rind powder and red beet powder as natural antioxidant on quality characteristics of beef sausage. World Journal of Dairy and Food Sciences, 6(1), 86-97.

El-Nashi H.B., Fattah A.F.A.K.A., Rahman N.R.A., El-Razik M.A., 2015. Quality characteristics of beef sausage containing pomegranate peels during refrigerated storage. Annals of Agricultural Sciences, 60(2), 403-412.

Han S.K., Park H.K., 2002. Accumulation of TBARS in cured pork sausages treated with propolis extracts. Journal of the Science of Food and Agriculture, 82(13), 1487-1489.

Kilic B., Richards M.P., 2003. Lipid Oxidation in Poultry Döner Kebab: Pro-oxidative and Anti-oxidative Factors. Journal of Food Science, 68(2), 686-689.

Kilic B., 2009. Current Trends in Traditional Turkish Meat Products and Cuisine. LWT Food Science and Technology, 42(10), 1581-1589.

Kunrath C.A., Savoldi D.C., Mileski J.P.F., Novello C.R., Alfaro A.D.T., Marchi J.F., Tonial I.B., 2017. Application and evaluation of propolis, the natural antioxidant in Italian-type salami. Brazilian Journal of Food Technology, 20.

Kurćubić V.S., Mašković P.Z., Vujić J.M., Vranić D.V., Vesković-Moračanin S.M., Okanović Đ.G., Lilić S.V., 2014. Antioxidant and antimicrobial activity of Kitaibeliavitifolia extract as alternative to the added nitrite in fermented dry sausage. Meat science, 97(4), 459-467.

Lemon D.W., 1975. An improved TBA test for rancidity. New Series Circular nr 51. Halifax, Nova Scotia: Halifax Laboratory.

Li L., Shao J., Zhu X., Zhou G., Xu X., 2013. Effect of plant polyphenols and ascorbic acid on lipid oxidation, residual nitrite and N nitrosamines formation in dry cured sausage. International Journal of Food Science and Technology, 48(6), 1157-1164.

Lu L.C., Chen Y.W., Chou C.C., 2005. Antibacterial activity of propolis against *Staphylococcus aureus*. International Journal of Food Microbiology, 102(2), 213-220.

Malviya S., Jha A., Hettiarachchy N., 2014. Antioxidant and antibacterial potential of pomegranate peel extracts. Journal of food science and technology, 51(12), 4132-4137.

Maturin L., Peeler J.T., 2001. Bacteriological analytical manual. Adam's Companie'sp, 92-94.

Naveena B.M., Sen A.R., Vaithiyanathan S., Babji Y., Kondaiah N., 2008a. Comparative efficacy of pomegranate juice, pomegranate rind powder extract and BHT as antioxidants in cooked chicken patties. Meat Science, 80(4), 1304-1308.

Naveena B.M., Sen A.R., Kingsly R.P., Singh D.B., Kondaiah N., 2008b. Antioxidant activity of pomegranate rind powder extract in cooked chicken patties. International journal of food science and technology, 43(10), 1807-1812.

Qin Y.Y., Zhang Z.H., Li L., Xiong W., Shi J.Y., Zhao T.R., Fan J., 2013. Antioxidant effect of pomegranate rind powder extract, pomegranate juice, and pomegranate seed powder extract as antioxidants in raw ground pork meat. Food Science and Biotechnology, 22(4), 1063-1069.

Şimşek A., Kılıç B., 2012. Recent Developments in Antioxidant Use in Meat and Meat Products. Academic Food Journal, 10(2), 75-83.

Temiz A., Şener A., Tüylü A.Ö., Sorkun K., Salih B., 2011. Antibacterial activity of bee propolis samples from different geographical regions of Turkey against two food borne pathogens, *Salmonella enteritidis* and *Listeria monocytogenes*. Turkish Journal of Biology, 35(4), 503-511.

Vargas-Sánchez R.D., Mendoza-Wilson A.M., Torres-cano-Urrutia G.R., Sánchez-Escalante A., 2015. Anti-radical potential of phenolic compounds fingerprints of propolis extracts: DFT approach. Computational and Theoretical Chemistry, 1066, 7-13.

Yasoubi P., Barzegar M., Sahari M.A., Azizi M.H., 2010. Total phenolic contents and antioxidant activity of pomegranate (*Punica granatum* L.) peel extracts. Journal of Agricultural Science and Technology, 9, 35-42.

Scientific Papers. Series D. Animal Science. Vol. LXI, Number 1, 2018
ISSN 2285-5750; ISSN CD-ROM 2285-5769; ISSN Online 2393-2260; ISSN-L 2285-5750

AVIAN PROTEOMICS: POTENTIAL TOOL FOR PRE- AND POST-SLAUGHTER POULTRY MUSCLE QUALITY EVALUATION – A REVIEW

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Abstract

A discipline within functional genomics, proteomics represents the method of analysis which allows a cell or a tissue's proteins (proteome) to be analyzed under certain given conditions. Functional proteomics allows scientists to address the interactions of different proteins in order to better understand the consequences of these interactions, and further on, to acquire knowledge on different specific areas, such as poultry breeding and quality of meat. Proteomics could help to characterize pathogen-host interactions in the diseases of livestock, assess the reproductive health and evaluate the muscle growth dynamics. Furthermore, it can be successfully applied in assessing the specific chemical reactions of rigor mortis (muscle quality), as well as evaluating the indicators of farm animal welfare, such as heat-induced stress.

Key words*: proteomics, muscle quality, poultry welfare.*

INTRODUCTION

Proteomics is the „analysis of the full complement of proteins of a cell or tissue under given conditions (it involves identifying and cataloguing proteins in a cell and identifying relative changes in populations between two or more states, physiological/diseases induced ones)" (Doherty et al., 2007; Kunec and Burgess, 2015).

It is a discipline within functional genomics, the context-defined analysis of complete complements of proteins, creating a bridge on „sequence-to-phenotype gap" (Burgess, 2004).

Proteomics allows the study of proteins present in a given tissue (the proteome). Its limitations are: cost, lack of good genomic data from many species of interest, a lack of awareness of the potential of this technology by animal scientists (de Almeida et al., 2015).

Proteomics offers a powerful new way to characterize the protein component of foods. It not only reveals which proteins are expressed in each tissue type, it also allows the investigation of differences in the protein composition of different tissues, and has the power to track the proteome of tissues before and after slaughter and evaluate the effect of downstream treatments such as cooking or curing (Clerens et al., 2012).

This serves as an important tool when looking for candidate genes that could influence the subcutaneous fat deposition in pigs (as an example), and since these traits are complex and even mutagenic in nature, the characterization at a molecular level would provide great benefits when correlating the proteome and transcriptome analysis (Bendixen et al., 2011).

MATERIALS AND METHODS

Proteomics studies are usually divided in three main areas:

(A) Qualitative proteomics - protein identification and characterization includes the analysis of the proteome relying on one or several separation steps followed by MS analysis. It involves:

- PMF (peptide-mass fingerprinting) uses two-dimensional gel electrophoresis (2-DE) to isolated an unknown protein, which is then enzymatically digested into peptides and subjected to MS.
- PFF (peptide-fragmentation fingerprinting) uses tandem MS (MS/MS) to produce fragment-ion data from one or more peptides from the protein to identify the protein unambiguously.

This approach requires the corresponding protein to be present in the database; if not, the

best match will probably be the entry with the closest homology, usually a related protein from a related species (Gallardo et al., 2013; Ortea et al., 2016).
(B) Differential proteomics shows relative abundance of a specific protein among different samples or the absolute amount of the protein, using three different methodologies:
(1) gel based – comparing the signal of an electrophoretically-isolated spot among different samples;
(2) label-based – proteins or peptides are labeled using a mass tag that is introduced metabolically, enzymatically or chemically, and relative quantification is obtained from the MS read-out (then quantification is based on the ration of heavy/light peptide pairs)
(3) label-free – avoid the labelling with stable isotopes, so protein amount is calculated based on the MS-derived ion-current signal of the peptides or proteins or on the number of identified MS/MS spectra (spectral counts) for the protein.
(C) Functional proteomics, which addresses the integrated analysis of the functional interactions among different proteins and the networks thereof, as well as the consequences of these interactions (Gallardo et al., 2013; Ortea et al., 2016).
Sentandreu et al. (2010) have recently developed a methodology for detecting the presence of chicken meat in other meat species through the identification of species-specific peptide biomarkers. This procedure combines protein enrichment strategies, identification and discriminating sequence from selected peptides by mass spectrometry. It might constitute an alternative to the current methods used for meat speciation such as immunoassays or DNA-based assays.
By implementing an OFFGEL enrichment fractionation step and MS detection by conventional LC-ion trap-MS/MS, it was possible to detect as low as 0,5 % w/v contaminating chicken in pork meat with high confidence due to acquisition of discriminating sequence information (Piras et al., 2016; Sentandreu et al., 2010). The discriminating power of this approach is based on detection of chicken-specific peptides originated from trypsin digestion of previously enriched myosin light chain 3, being comparable to methods based on DNA analysis. This proteomic approach displays more robustness in addressing some of the major limitations of DNA-based methods, such as optimization of the extraction procedures according to the different matrices and recovery of samples with high DNA quality. From this perspective, the primary amino acid sequence of the key peptide biomarkers used here will be considerably more resistant to food processing than DNA sequences (Sentandreu et al., 2010).

RESULTS AND DISCUSSIONS

Different results have emerged, considering the application of proteomics in poultry science, several of them applying in steps which precede the slaughter, while others have applied successfully in post-slaughter stages. In the latter case, the implications go through to food science, more specifically in muscle quality.

(1) Pre-slaughter application of proteomics in poultry

Among the uses of proteomics in poultry breeding, we can enumerate:
- characterization of pathogen-host interaction in the disease of production animals;
- assessment of reproductive health status;
- evaluation of the muscle growth dynamics (de Almeida et al., 2015; Doherty et al., 2004).
Pre-slaughter, proteome-based biomarkers are considered important for early diagnostics in veterinary medicine, so dedicated studies on farm animals could be performed to monitor the health and welfare as well as investigating the type and state of a disease (through analyses of serum and plasma, as well as milk – cattle) (Bendixen et al., 2011).
Agudo et al. (2005) studied the proteins from the stage 29 chicken embryos, identifying proteins implicated in the formation of various organs, CNS development and specific embryonic development – potential biomarkers for embryonic development.
In a global analysis of the chicken embryo liver proteome resulted in identification of proteins that were differentially expressed between two chicken lines that differed in hepatic lipid metabolism and fat deposition (Huang et al., 2010). Comparative analysis of the identified

liver proteins showed that proteins involved in gluconeogenesis, cholesterol metabolism and fatty acid oxidation were expressed earlier and more abundantly in the liver of lean-line of chickens.

During chicken myogenesis, in chicken thigh, hierarchical clustering analysis revealed the expression of 168 proteins. Their expression was affected by the developmental stage. Based on the expression profile, these were classified in 9 clusters, among which 2 were specific for the myogenesis: one which contained proteins showing increasing abundance (A) and another one the opposite (B). The proteins included in (A) were involved in the energy metabolism (α and β-enolase, creatine kinase, NADH dehydrogenase, cytochrome C reductase, succinyl CoA ligase), lipid transport (apolipoprotein B, fatty acid-binding protein 3) or calcium signaling (sarcalumenin). The expression of β-enolase and creatine kinase increased during postnatal muscle growth. Some of the proteins were expressed during embryogenesis, but not after hatch: alpha-fetoprotein, regucalcin, proteasome 26S subunit (early stages of myogenesis, day 7 *in ovo*).

Regucalcin regulates intracellular Ca^{2+} homeostatis by regulating Ca^{2+} transport systems in liver and renal cells (Picard et al., 2010).

HSP27 expression was at peak at 18 days *in ovo*, corresponding to sustained fibre differentiation or maturation; this may suggest that HSP27 plays a major role in the transition between the proliferation of myoblasts and the differentiation and/or maturation of myofibre in the different species, possibly through its anti-apoptotic actions or through its stabilising action on myofibrils.

There was also a high abundance of ApoA1 during the early stages of myogenesis, with a decrease in the last stages; ApoA1 is known for its involvement in cholesterol transport and lipid metabolism (Picard et al., 2010).

Proteomic characterization of the sarcoplasmic proteins in the *pectoralis major* and *supracoracoides* breast muscles was conducted for two different chicken genotypes, which included Ross 708 commercial broilers and Leghorn chicks, Hyline W-36. Results suggested that glycogen phosphorylase, enolase, elongation factor 1, creatine kinase, fructose-bisphosphatealdolase and glyceraldehyde 3-phosphate-dehydrogenase were different in the two strains during breast muscle growth (Schilling et al., 2017).

Tanaka et al. (1995) observed a dominance of alpha-enolase mRNA in the embryonic chick, while the expression of the beta-enolase mRNA is almost exclusive in mature muscle.

Han et al. (2005) analyzed the chicken gonadal primordial germ cells to probe the molecular and physiological mechanisms underlying avian germ cell development; among the identified proteins, vimentin (member of the intermediate filament protein family – valuable in the diagnosis of undifferentiated neoplasms), a specific tissue, developmentally regulated protein was highly expressed in these cells; in addition, albumin (and other proteins related to it) was also present, being attributed to a role in maintaining homeostasis of the cells.

Parada et al. (2005) studied the embryonic cerebrospinal fluid (ECSF), which is known to have a higher protein concentration (stages 18 to 30), higher than that of adult CSF. The ECSF plays the role of CNS development, expansion of cephalic cavities and in the survival, proliferation and differentiation of neuroectodermal stem cells, in collaboration with known organizing centres; at stage 24 can be registered the greatest rate of neural stem cell proliferation (time of beginning for the neurogenesis process). Proteins identified in this study (apolipoproteins, retinol, vitamin D carriers, proteins related to quiescence and cell death) may be important in the capacity of ECSF to contribute to the neurogenesis, via the exertion of a trophic role on the ectoderm.

McCarthy et al. (2006) analyzed the proteomes of the supporting stromal and B cells isolated from the chicken bursa of Fabricius (common experimental system for B-cell development). Proteins were isolated from the two major functional cell types of bursa by a sequential detergent extraction procedure that increased proteome coverage and helped to localize known and previous by unknown proteins to different cellular compartments. The analysis identified 5198 proteins in bursa, and of these, 1753 were B-cell specific, 1972 were stroma specific, and 1473 proteins were identified in both cell types. Functional modelling of the identified proteins provided insights about

signaling pathways involved in programmed cell death, proliferation and differentiation.
Korte et al. (2013) showed that enzymes of the retinoic acid metabolism play a crucial role in the early development of the primary avian B-cell organ. Similar observations were done in mammals, where vitamin A plays a similarly important role in the development of secondary lymphoid organs (van de Pavert et al., 2009)
Parada et al. (2006) characterized through proteomics the chicken embryonic cerebrospinal fluid (CSF), showing that 14 of the proteins are also present in human CSF, while 12 of them are altered in neurodegenerative diseases and/or neurological disorders (Table 1).

Table 1. Studies on potential pre-slaughter applications of proteomics in poultry science

Type of research, application and potential use	Reference
Proteins involved in the formation of various organs can be potential biomarkers for embryonic development.	Agudo et al. (2005)
Proteins involved in gluconeogenesis, cholesterol metabolism and fatty acid oxidation were expressed earlier in the chicken embryo liver proteome of the lean lines.	Huang et al. (2010)
It was observed that from the chicken proteome, β-enolase and creatine kinase increased during the postnatal muscle growth, thus being revealed their rule in myogenesis.	Picard et al. (2010)
Alpha-enolase mRNA is dominant in the embryonic chick, while β-enolase mRNA is almost exclusive in the mature muscle.	Tanaka et al. (1995)
Vimentin was highly expressed in the chicken gonadal primordial germ cells, while albumin was present too, with a potential role in maintaining the homeostasis of these cells.	Han et al. (2005)
Functional modelling of the proteins identified in chicken bursa of Fabricius may offer insights into the signaling pathways involved in programmed cell death, proliferation and differentiation.	McCarthy et al. (2006)
Out of the chicken embryonic cerebrospinal fluid proteins identified so far, 14 are also present in human CSF, while 12 of these are altered in neurodegenerative diseases and/or neurological disorders.	Parada et al. (2006)

In a study aiming to evaluate the supplementation of creatine pyruvate on the lipid and protein metabolism, by using a proteomic approach 32 liver mitochondrial proteins with differential profile changes were identified, some of these being involved in lipid and protein metabolism (Chen et al., 2012):
(1) ADRP[1] has the main established role of limiting the interaction of lipases with neutral lipids, therefore promoting the accumulation of the latter; the expression of ADRP was decreased with the creatine-pyruvate group, suggesting that this compound reduces the fatty acid accumulation and fat deposition;
(2) CETP[2] has a role in the transport of excess cholesterol from peripheral tissues to the liver, mediating the transfer of cholesteryl esters from HDL or LDL into triglyceride-rich lipoproteins, thereby stimulating reverse cholesterol transport; creatine-pyruvate determines a down-regulation of the CETP expression, its inhibition increasing the HDL-C levels and decreasing the triglyceride levels (Chen et al., 2012);
(3) FACS[3] plays a role in the fatty acid activation, catalyzing a two-step reaction, regulated by Mg^{2+}, to produce fatty acyl CoA esters (these esters being targeted by carnitinepalmitoyltransferase I for conversion to their acylcarnitine derivatives and subsequentsly being transported into mitochondria where they are subjected to β-oxidation); carnitine-pyruvate group manifested an up regulating of FACS, promoting the activation of fatty acids to fatty acyl CoA esters, which were then transported to the mitochondria;
(4) eIF2 and eIF2B[4] have a regulation role in the protein synthesis; eIF2B controls the activity of eIF2, more precisely its return to GTP bound-form, through nucleotide exchange (eIF2B promotes release of GDP from eIF2, by acting as a GDP-dissociation stimulator protein) - > a higher eIF2B expression level (creatine and creatine pyruvate groups) could lead to an increased eIF2 activation, enhancing the protein synthesis.
The conclusion is that creatine pyruvate reduces fat deposition by promoting the β-oxidation and triglyceride hydrolysis (Chen et al., 2012).
Doherty et al. (2004) focused on the changes in the most abundant proteins in the low-salt,

[1]adipose differentiation-related protein
[2]cholesteryl ester transfer protein
[3]fatty acyl-CoA synthetase
[4]eukaryotic initiation factor / and its exchange factor

water soluble component of the chicken skeletal (*pectoralis*) muscle. It seems that there are dramatic changes in protein expression from hatching to maturity. At one day post-hatch, the identified proteins are structural ones (actin, myosin light chain), proteins involved in the synthesis and modification of new proteins (elongation factor 2) and glycolytic proteins (enolase, triose-phosphate isomerase). At nine days post-hatch, the protein profile becomes simpler and more specialized, with an expansion of the glycolytic enzymes (greatest expansion being observed for GAPDH, while haemoglobin and ovotransferrin decreased to trace levels). In broiler chickens, the change in global protein expression occurs earlier than in layers, indicating that the muscle maturation occurs more rapidly in the developing broiler chicks.

After a comparison of the *pectoralis* proteomes of animals with different growth rates and different water holding capacity, within the same genotype, a total of 22 protein spots were found to show differential expression. Also, proteins such as creatine kinase, pyruvate kinase, triosephopsphateisomerase, ubiquitin, heat shock proteins, as well as several structural and contractile proteins were identified. Several of these were proposed as markers of water-holding capacity and also of growth rate, demonstrating once again the potential of proteomics in meat authentication, specifically in the selection of quality and productive traits markers (de Almeida, 2017).

On the topic of immune response studies using proteomics, the Acute Phase Response (APR) is the early and non-specific systemic reaction of the innate immune system to homeostatic disturbances. Pro-inflammatory cytokines and chemokines released from macrophages, monocytes and infected and damaged tissues affect the synthesis and secretion of hepatocytic proteins and drastically alter the plasma protein profile (O'Reilly et al., 2012).

Plasma proteins that change concentrations as a result of an APR are called termed acute phase proteins (APP). APP concentrations can change during infectious, inflammatory, stressful, traumatic or neoplastic events, often proportionally to the severity of the event.

This study characterized APP changes that occur due to gait abnormalities in broilers by measurement of the established APPs in chicken: ceruloplasmin (Cp), PIT54 (avian haemoglobin binding protein similar to mammalian haptoglobin) and ovotransferrin (OVT), which are known to increase in response to bacterial, viral and parasitic infection (O'Reilly et al., 2012).

Birds with obvious gait defect which affected the ability to move presented the highest values of all APPs. Cp was significantly associated with weight, heavier birds tending to have higher Cp concentrations. Not necessarily linking the weight to the gait score, in heavier birds there might a series of inflammatory events present, resulting in higher Cp concentrations. There were no significant associations between the APP concentrations and broiler breed (O'Reilly et al., 2012).

(2) Post-slaughter application of proteomics in poultry

After bleeding, muscle energy metabolism is modified: nutrients and oxygen are no longer supplied to the muscle, calcium ions may move from the sarcoplasmic reticulum into the cytoplasm of the muscle cell and activate many pathways including ATPases, and metabolites accumulate (Picard et al., 2010).

The regeneration of ATP depends first on the degradation of phosphocreatine, which has a half-life of 60-80 minutes in the postmortem muscle, the production of ATP from ADP catalyzed by myokinase, and subsequently on anaerobic glycogenolysis.

All these processes lead to the accumulation of protons and lactate, leading to acidification of the muscle.

In addition to energy metabolism, proteolysis, lipolysis and oxidation also play a major role in determining the meat quality; these underlie the process of "ageing" of the meat, considered to start after death and last for several days or weeks, depending on the species.

The ageing of meat allows the tenderization and the enhancement of taste and this process has proteins as a central part of it, once because they are targets and on the other hand they are also mediators of biochemical reactions (Picard et al., 2010).

The most significant change is that muscle remains functional and metabolically active for several days after slaughter although depleted

of the circulating blood that supplies oxygen and removes metabolic end products. This results in lactic acid accumulation and consequently in declining pH, in a process termed muscle acidification (Paredi et al., 2012). The acidification causes loss in water holding capacity (WHC) as well as in calcium release, and leads to cross-bridges being formed between myosin and actin filaments. As a consequence of the slaughter, glycogen level in the muscle decreases and therefore, the energy available to keep the muscle in a relaxed form also decreases.

The combination of these factors results in the onset of rigor mortis, a state essentially characterized by significant alterations at the level of the energy metabolism that result in further pH decrease and a simultaneous decrease in muscle flexibility (Paredi et al., 2012).

Post-slaughter, the food proteins are subjected to: side-chain oxidation, cross-link formation, backbone cleavage, which leads to consequences on the food properties: shelf life, nutritional value, digestibility, health effects (Clerens et al., 2012).

In a recent study, performed in 2013, Montowska and Pospiechproved that observed inter-species differences in protein expression in raw meat were retained in thermally processed meat and ready-made products after finishing the entire technological process. The proteins formed a specific pattern on 2-DE gels, thanks to which it was possible to identify the species in the products. In this study, phosphohistidine phosphatase was likely the first time identified in the chicken *pectoral* muscle. The identified proteins with species-specific electrophoretic mobility are the proteins of the largest amounts which can be found in the muscle tissue.

In livestock production is very important to understand how heat stress can prevent the muscle growth. Road transport induced-stress is known to increase the levels of corticosterone, whilst decreasing the abundance of several hexose phosphates, overall affecting the cytoskeleton structure and carbohydrate metabolism. Also, it was found that induced stress led to a repression of glycogenolysis and glycolysis and an alteration of the myofibrillar protein profile (Marco-Ramell et al., 2016). Following the heat stress, several proteins involved in glycolysis, glycogenesis and glycogenolysis were increased or modified, indicating enhanced glycolytic capacity in response to it.

For evaluating the indicators of farm animal welfare, in chickens, a study was conducted by applying a 2h restraint to chickens which were later euthanized and the tensor *fascia latae* and *biceps femoris* muscles were used to perform the proteomics analysis (Table 2). A total of 29 proteins were found to have differential expression, 37 % of which with a function in glycolysis and 14 % in cell structure. The conclusion was that the restraint period resulted in a repression of glycogenolysis and glycolysis in the thigh muscle (Paredi et al., 2013).

Table 2. Research on potential post-slaughter applications of proteomics in poultry science

Field of application	Type of research, application and potential use	Reference
Heat-induced stress	Following a period of heat stress, several proteins will go through changes (either increases or decreases), which indicates that there is an enhanced glycolytic activity associated to this stress.	Marco-Ramell et al. (2016)
	Chicken restraint may result in a repression of glycogenolysis and glycolysis in the thigh muscle.	Paredi et al. (2013)
	Heat stress may result in a proteomic response involving marked cellular changes in carbohydrate metabolism, structure and antioxidant processes in the skeletal muscle.	Cruzen et al. (2015)
Muscle quality	In comparison to commercial broiler chicken meat, the Thai chicken meat is firmer in texture and with an improved flavor, due to the expression and activity of glycolytic enzymes.	D'Alessandro and Zolla (2012b)
	Parvalbumin is overly expressed in *peroneus longus* muscle, in comparison to *pectoralis* muscle, showing that the first is more frequently used.	Jung *et al.* (2007)
	Correlations have been found between some key regulators of the cellular redox balance and the level of lipid oxidation during ageing as well as cooking.	Gobert et al. (2014)
	An overabundance of proteins involved in glycolytic pathways, muscle contraction,	Nair et al. (2017)

	proteolysis, ATP regeneration and energy metabolism in PSE breast might be related to quality differences between this type of meat and normal one.	
Adulteration	Separation of intact high and low abundance proteins from both hand-deboned and mechanically recovered meat.	Surowiec et al. (2011)
	The amount of haemoglobin can be used as a marker to differentiate mechanically recovered chicken meat from deboned chicken meat.	Schilling et al. (2017)
	Discrimination of meat species from fresh meat and meat mixtures is possible by comparing the electrophoretic mobility and by immunoblotting and LC-MS/MS analysis.	Kim et al. (2017)
Metabolic changes due to diet	Three of the 190 individual proteins identified in the *pectoralis major* muscle tissue correlated with methionine deficiency in the diet of broiler chicken.	Corzo et al. (2006)

Cruzen et al. (2015) examined the proteome response of skeletal muscle to acute (short duration and high intensity) heat stress. In the white fiber type portions, heat stress decreased abundance of tubulines and soluble actin and increased phosphorylated cofilin 2 abundance, indicating a loss of microtubule structure and a likely increase in stable actin microfilaments; an antioxidant response was observed, manifested through increasing the manganese superoxide dismutase abundance, decreasing at the same time the peroxiredoxin 2 abundance, the proteomic response to heat stress suggesting marked cellular changes in carbohydrate metabolism, structure and antioxidant processes in the skeletal muscle (Cruzen et al., 2015).
A few proteome studies have been aimed at characterizing protein markers that can assess development of tenderness during post-mortem storage of the carcass (Bendixen et al., 2011).

(a) Muscle quality

Muscle importance derives not only from its obvious physiological relevance, associated to specific diseases and metabolic conditionings, but also from its production-associated aspects such as the transformation of muscle to meat. When characterizing the muscle proteome of farm animals, two-dimensional electrophoresis separation is still the most widely used approach. Due to low sensitivity and lack of reproductibility of these systems, they have been replaced over the last years with differential proteomic assays based on fluorescent dyes, namely DIGE (Soares et al., 2012).
Proteomics may help understand biochemical mechanisms underlying meat quality in order to control or predict them. A useful approach is the comparison of proteomes between animals showing relatively high or low values for a specific trait within a population.
Other studies have been using proteomic analyses to understand better the processes underlying tenderness or to provide protein markers predicting tenderness which is one of the most important characteristic for consumer satisfaction.
Proteomics have also been used to understand better or further characterize various meat quality defects. A well-known defect is a pale, soft and exudative appearance of meat (PSE), which usually occurs in pig and poultry species, which have relatively high proportions of fast-twitch glycolytic muscle fibers (Picard et al., 2010).
Post mortem conversion of muscle into meat is a significant series of events connected with protein modification and breakdown, receiving substantial attention in the last years, which is now extended to changes occurring in cooked/cured products. Also, protein marker discovery for various meat quality attributes is another important area (Clerens et al., 2012).
Nakamura et al. (2010) and Soares et al. (2012) examined the allergenicity of meat obtained from transgenic chicken. They identified five IgE-binding protens, which through 2D-DIGE[5] proved to not be significantly changed when comparing non-GM with GM chicken.
Doherty et al. (2004) reported that the weight of *pectoralis* muscle increased approximately 44-fold from day 1 to day 27; since there is very large amount of energy derived from the glycolytic enzymes is required to maintain this mass of tissue, chickens showed a dramatic change in the relative expression levels of glycolytic enzyme, in particular to enolase isoforms, triosophosphate isoforms, creatine kinase isoforms and tubulin isoforms, along with their post-translational modifications. In a comparison between the Thai chicken meat and

[5]bidimensional (2D) difference gel eletrophoresis

the commercial broiler chicken meat, the Thai chicken type proved to be firmer in texture and had an improved flavor, largely attributed to the expression and activity of glycolytic enzymes (D'Alessandro and Zolla, 2012b).

Sentandreu et al. (2010) developed the method for extracting the myofibrillar proteins, subsequently enriching the target proteins using OFFGEL isoelectric focusing. Further on, LC-MS/MS[6] was used for the identification step.

Jung et al. (2007) aimed to investigate the differentially expressed muscle proteins between Cornish and White Leghorn breeds in order to address the possibility of improving meat quantity and quality in chicken. The skeletal muscles were removed and frozen at -80^{o}C (chicken breast – *pectoralis* muscle and drum – *peroneus longus* muscle). The steps were: (a) preparation of the sample – centrifugation – obtaining the protein pellet; (b) electrophoresis; (c) image analysis and gel digestion; (d) characterization of protein spots.

The results showed that more than 300 protein spots were detected, of which 25 common proteins were identified, both appearing in both *pectoralis* and *peroneus longus* muscles of Cornish and White Leghorn breeds. When comparing the *pectoralis* with *peroneus longus* muscles, the parvalbumin protein was over expressed in the latter, in both Cornish as well as White Leghorn. In the muscle tissue, pravalbumin is the high affinity Ca^{2+} binding protein and plays a crucial role in the muscle contraction by translocation Ca^{2+} from the myofibril to the sarcoplasmic reticulum (the conclusion being that the *peroneus longus* is more frequently used than the *pectoralis* muscle) (Jung et al., 2007).

Proteomics has been also used to identify specific markers for predicting the susceptibility of meat to oxidation. In a recent study, the scientists aimed to identify specific markers of lipid oxidation generated in meat during refrigerated storage and cooking. For this purpose, the early post-mortem sarcoplasmic proteome was analyzed and correlated to the level of lipid oxidation. Many proteins could be potential markers of lipid oxidation during meat ageing or cooking, but none of them can serve as marker throughout the complete meat process (from one day to four days of ageing and from raw to cooked). For example, among the three spots of albumin correlated to lipid oxidation during ageing or after cooking, two of them were negatively correlated while the third one was positively correlated.

Significant correlations were found between some proteins that are key regulators of the cellular redox balance (peroxiredoxin and thioredoxin) and the level of lipid oxidation either during ageing or after cooking (Gobert et al., 2014).

Considering the muscle food color, application of mass spectrometry (MS) and proteomics could be related to characterization of myoglobin's primary structure. In order to determine the exact molecular mass of myoglobin and to differentiate meat species, MALDI-TOF MS (Matrix Assisted Laser Desorption Ionization-Time of Flight Mass Spectrometry) and electrospray MS are successfully used. Mass spectrometric approach to determine the exact molecular mass of globin polypeptide chain has been applied to differentiate heme pigments from farm animals and for meat species identification. Mammalian and avian myoglobins are comprised of 153 aminoacids and the primary sequence of the myoglobin depends upon species. Avian myoglobins were established to be different then their mammalian counterparts in amino acid sequence. The molecular masses of avian myoglobins are, in general, 300-400 Da greater than those of livestock myoglobins (Poulson et al., 2015).

PSE meat tissue has poor protein functionality and is dry and tough after cooking, leading to economic loss for the poultry industry. A comparison between normal and PSE broiler breast meat tissue revealed 15 differentially abundant proteins. PSE broiler breast had abundance of: actin alpha, myosin heavy chain, phosphoglycerate kinase, creatine kinase M type, β-enolase, carbonic anhydrase 2, proteasome subunit alpha, pyruvate kinase, malate dehydrogenase. Normal broiler breast had an abundance of: phosphoglycerate mutase-1, α-enolase, ATP-dependent 6-phosphofructokinase, fructose 1, 6-bisphosphatase. The overabundance of proteins involved in glycolytic pathways, muscle contraction, proteolysis, ATP regeneration and

[6]liquid chromatography – mass spectrometry

energy metabolism in PSE breast could be related to the quality differences between normal and PSE meat (Nair et al., 2017).
It was demonstrated that 15 different proteins were differentially expressed between PSE and normal breast meat. In the study which proved this, male Hubbard x Cobb 500 broilers were raised *ad libitum* feed and water for 8 weeks. The normal breast samples where characterized by a 24h postomortem pH of 5.8-6.2, while PSE breast samples had 5.4-5.7 (Schilling *et al*., 2017). Phosphoglycerate kinase and beta-enolase where over-abundant in PSE meat, indicating an increased glycolytic activity and lower pH. Pyruvate kinase M type was over-abundant in PSE meat, being negatively correlated with the pH (24 h).
Another defect is the woody breast. This has decreased yields due to reduced water holding capacity, being pale, less red and more yellow than normal breast meat. In addition, it was characterized by a greater weight and a larger cross-sectional area. It typically has increased degenerative and atrophic fibers, mononuclear cell infiltration, lipidosis, and interstitial inflammation. In comparison to normal breast muscle, the woody breast contains an overabundance of myosin regulatory light chain 2 and 14-3-3 protein gamma. Also, increased abundance of serum albumin indicates increased oxidative stress in muscle cells from woody breast meat. Protein deglycase-DJ-1 was overabundant as well in the woody breast meat, this indicating both an increased need for deglycation of amino acids and oxidative stress. Phosphoglyceratemutase 1 was six times as abundant in woody breast meat in comparison to normal breast meat. This enzyme converts 3-phosphoglycerate into 2-phosphoglycerate within the glycolysis pathway (Schilling et al., 2017).

(b) Adulteration

The application of proteomics on contaminated meat is not only a matter of microorganisms or adulteration with meat/gelling agents from other species, but it also involves detection of performance enhancing agents, which are illegally used in different livestock, with the purpose of increasing food conversion and lean meat production (D'Alessandro and Zolla, 2012a). Besides chromatographic, electrophoretic and mass spectrometric approaches, it has been observed a differential protein pattern in meat from different animals, which allows easing the task of those agencies targeting adulteration frauds.
Meat proteins applications using proteomics can be:
(a) Detection of mechanically recovered meat;
(b) Detection of turkey meat in beef and pork;
(c) Detection of chicken meat in raw meat mixtures (burgers, sausages) (Kvasnicka, 2003).
Mechanically recovered meat is cheaper than raw meat and is been usually incorporated into many meat-derived products. EU regulations exclude mechanically recovered meat from the definition of meat, therefore analytical procedures are needed to differentiate it from hand-deboned meat.
In a study, Surowiec et al. (2011) showed that it is possible to separate intact high and low abundance proteins from both hand-deboned and mechanically recovered meat, which can be identified by LC-MS/MS. The methodology makes it possible to identify proteins that could serve as MRM biomarkers in food products. As an example of such proteins detection of potential chicken MRM markers – hemoglobin subunits and those similar to myosin-binding protein C were found. The next step will include validation of results in meat samples obtained from different producers and application of the methodology for detection of MRM in meat mixtures. The advantage of this approach compared to those previously used, based on SDS-PAGE separation or capillary electrophoresis is that OFF-GEL fractionation is fast and repeatable and fractions can be directly analyzed by quantitative LC-MS/MS techniques, resulting in higher selectivity, accuracy and ease of use. A proteomic approach was used for the identification of chicken meat that was mechanically recovered and hand deboned. Results suggested that the amount of haemoglobin can be used as a marker to differentiate mechanically recovered chicken meat from deboned chicken meat (Schilling et al., 2017).
TM1 (one of the muscle fiber type-related proteins) was observed as a commonly distributed protein in bovine, pork, chicken and duck meat. Using this as a marker, LC-MS/MS

spectra allows the identification of each species from fresh meat as well as meat mixtures. Better biomarkers yet were the proteins identified as TnI, enolase 3, LDH and TPI. These were found in all four types of meat samples and could be used as discrimination of mammals from poultry. Moreover, species-specific spectra from these proteins can be peptide markers for the identification of each species following additional analysis by LC-MS/MS. In conclusion, discrimination of meat species from fresh meat and their mixtures is possible by comparing the electrophoretic mobility and through immunoblotting and LC-MS/MS analysis, and we confirmed that commonly distributed proteins in all species can also be candidates for meat discrimination (Kim et al., 2017).

(c) Metabolic changes due to diet

On broiler chickens' meat, proteomic tools were helpful in evaluating the effects of dietary methionine on breast meat accretion and protein expression in the skeletal muscles of broiler chickens. From the 190 individual proteins which were identified in the *pectoralis major* muscle tissue, three correlated with methionine deficiency in the diet (Corzo et al., 2006).

CONCLUSIONS

Proteomics has the potential to offer many details about not only the development of the chicken embryo, but also about the undergoing processes of muscle development during the breeding cycle. Also, it can be successfully used in muscle quality evaluation, either when identifying the underlying causes of defects, as well as the evaluation of colour or tenderness. Furthermore, proteomic analysis has been successfully used in investigating metabolic modifications on muscle level due to diet changes, as well as identifying cases of adulteration, when poultry meat is found mixed with other types of meat, in ready to eat food products.

From these points on, many directions can be further approached considering the undergoing research, while also taking into consideration the existent ones, which can still be explored while applied on the other poultry species.

REFERENCES

Agudo D.F., Gomez-Esquer F., Diaz-Gil G., Martinez-Arribas F., Delcan K. et al., 2005. Proteomic analysis of the *Gallus gallus* embryo at stage-29 of development, Proteomics, 5(18), 4946-4957.

Almeida A.M., 2017. Proteomics in Food Science – From Farm to Fork, Colgrave M.L. (ed.) Academic Press Elsevier, U.S.A., 215-220.

Almeida A.M., Bassols A., Bendixen E., Bhide M., Ceciliani F., Cristobal S., Eckersall P.D., Hollung K., Lisacek F., Mazzucchelli G., McLaughlin M., Miller I., Nally J.E., Plowman J., Renaut J., Rodrigues P., Roncada P., Staric J., Turk R., 2015. Animal board invited review: advances in proteomics for animal and food sciences, Animal, 9(1), 1-17.

Bendixen E., Danielsen M., Hollung K., Gianazza E., Miller I., 2011. Farm animal proteomics – a review, Journal of Proteomics, 74, 282-293.

Burgess S.C., 2004. Proteomics in the chicken: tools to understanding immune responses to avian diseases, Poultry Science, 83(4), 552-573.

Chen J., Huang J., Deng J., Ma H., Zou S., 2012. Use of comparative proteomics to identify the effects of creatine pyruvate on lipid and protein metabolism in broiler chicken, The Veterinary Journal, 193, 514-521.

Clerens S., Plowman J.E., Dyer J.M., 2012. Food Proteomics: Mapping Modifications, in: Proteomic Applications in Biology, Heazlewood J.L., Petzold C.J. (eds.), InTech, DOI: 10.5772/31308. Available from: https://www.intechopen.com/books/proteomic-applications-in-biology/food-proteomics-mapping-modifications

Corzo A., Kidd M.T., Koter M.D., Burgess S.C., 2005. Assessment of dietary amino acid scarcity on growth and blood plasma proteome status of broiler chickens, Poultry Science, 84(3), 419-125.

Corzo A., Kidd M.T., Dozier W.A., Shack L.A., Burgess S.C., 2006. Protein expression of pectoralis major muscle in chickens in response to dietary methionine status, British Journal of Nutrition, 95(4), 703-708.

Cruzen S.M., Pearce S.C., Baumgard L.H., Gabler N.K., Huff-Lonergan E., Lonergan S.M., 2015. Proteomic changes to the sarcoplasmic fraction of predominantly red or white muscle following acute heat stress, Journal of Proteomics, 128, 141-153.

D'Alessandro A., Zolla L., 2012a. We are what we eat: Food Safety and Proteomics, Journal of Proteome Research, 11, 26-36.

D'Alessandro A., Zolla L., 2012b. Meat science: From proteomics to integrated omics towards system biology, Journal of Proteomics, 78, 558-577.

Doherty M.K., McLean L., Hayter J.R., Pratt J.M., Robertson D.H., El-Shafei A., et al., 2004. The proteome of chicken skeletal muscle: changes in soluble protein expression during growth in a layer strain. Proteomics, 4(7), 2082-93.

Doherty M.L., McLean L., Hayter J.R., Pratt J.M., Robertson D.H., et al., 2004. The proteome of chicken skeletal muscle: changes in soluble protein

expression during growth in a layer strain, Proteomics, 4(7), 2082-2093.
Doherty M.K., McLean L., Beynon R.J., 2007. Avian proteomics: advances, challenges and new technologies, Cytogenetic and Genome Research, 117, 358-369.
Gallardo J.M., Ortea I., Carrera M., 2013. Proteomics and its applications for food authentication and food-technology research, Trends in Analytical Chemistry, 52, 135-141.
Gobert M., Sayd T., Gatellier P., Santé-Lhoutellier V., 2014. Application to proteomics to understand and modify meat quality, Meat Science, 98(3), 539-543.
Han B.K., Kim J.N., Shin J.H., Kim J.K., Jo D.H. et al., 2005. Proteome analysis of chicken embryonic gonads: identification of major proteins from cultured gonadal primordial germ cells, Molecular Reproduction and Development, 72(4), 521-529.
Huang J., Tang X., Ruan J., Ma H., Zou S., 2010. Use of comparative proteomics to identify key proteins related to hepatic lipid metabolism in broiler chickens: evidence accounting for differential fat deposition between strains, Lipids, 45(1), 81-89.
Jung K.C., Jung W.Y., Lee Y.J., Yu S.L., Choi K.D., Jang B.G., Jeon J.T., Lee J.H., 2007. Comparisons of chicken muscles between layer and broiler breeds using proteomics. AJAS 20, 307-312.
Kim G.-D., Seo J.-K., Yum H.-W., Jeong J.-Y., Yang H.-S., 2017. Protein markers for discrimination of meat species in raw beef, pork and poultry and their mixtures, Food Chemistry, 217, 163-170.
Korte J., Frohlich T., Kohn M., Kaspers B., Arnold G.J., Hartle S., 2013. 2D DIGE analysis of the bursa of Fabricius reveals characteristic proteome profiles for different stages of chicken B-cell development, Proteomics, 13(1), 119-133.
Kunec D., Burgess S.C., 2015. Chapter 3 - Avian Proteomics, in: Sturkie's Avian Physiology (6th ed.), Scanes C.G. (ed.), Elsevier, USA, 25-37.
Kvasnicka F., 2003. Proteomics: general strategies and application to nutritionally relevant proteins, Journal of Chromatography, 787(1), 77-89.
Marco-Ramell A., de Almeida A.M., Cristobal S., Rodrigues P., Roncada P., Bassols A., 2016. Proteomics and the search for welfare and stress biomarkers in animal production in the one-health context, Molecular Biosystems, 12(7), 2024-2035.
McCarthy F.M., Cooksey A.M., Wang N., Bridges S.M., Pharr G.T., Burgess S.C., 2006. Modeling a whole organ using proteomics: the avian bursa of Fabricius, Proteomics, 6(9), 2759-2771.
Montowska M., Pospiech E., 2013. Species-specific expression of various proteins in meat tissue: Proteomic analysis of raw and cooked meat and meat products made of beef, pork and selected poultry species, Food Chemistry, 136(3-4), 1461-1469.
Nair. M.N., Costa-Limba B.R.C., Schikking M.W. Suman R.P., 2017. Proteomics in Food Science – From Farm to Fork, Colgrave M.L. (ed.) Academic Press Elsevier, U.S.A., p. 170.
Nakamura R., Nakamura R., Nakano M., Arisawa K., Ezaki R., Horiuchi H., Teshima R., 2010. Allergenicity study of EGFP-transgenic chicken meat by serological and 2D-DIGE analysis. Food and Chemical Toxicology, 48(5), 1302-1310.
O'Reilly E.L., Burchmore R.J., Sandilands V., Sparks N.H., Walls C., Eckersall P.D., 2012. The plasma proteome and acute phase proteins of broiler chickens with gait abnormalities, in: Farm Animal Proteomics: Proceedings of the 3rd Managing Committee Meeting and 2nd Meeting of the Working Group 1,2 & 3 of COST Action FA 1002, Rodrigues P. (ed.), Wageningen Academic Publishers, 177-180.
Ortea I., O'Connor G., Maquet A., 2016. Review on proteomics for food authentication, Journal of Proteomics, 147, 212-225.
Parada C., Gato A., Bueno D., 2005. Mammalian embryonic cerebrospinal fluid proteome has greater apolipoprotein and enzyme pattern complexity than the avian proteome, Journal of Proteome Research, 4(6), 2420-2428.
Parada C., Gato A., Bueno D., 2006. Mammalian embryonic cerebrospinal fluid proteome has greater apolipoprotein and enzyme pattern complexity than the avian proteome, Journal of Proteome Research, 4(6), 2420-2428.
Paredi G., Raboni S., Bendixen E., de Almeida A.M., Mozzarelli A., 2012. Muscle to meat molecular events and technological transformations: the proteomic insight, Journal of Proteomics, 75(14), 4275-4289.
Paredi G., Sentandreu M.-A., Mozzarelli A., Fadda S., Hollung K., de Almeida A.M., 2013. Muscle and meat: New horizons and applications for proteomics on a farm to fork perspectives, Journal of Proteomics, 88, 58-82.
Picard B., Berri C., Lefaucheur L., Molette C., Sayd T., Terlouw C., 2010. Skeletal muscle proteomics in livestock production, Briefings in Functional Genomics, 9(3), 259-278.
Piras C., Roncada P., Rodrigues P.M., Bonizzi L., Soggiu A., 2016. Proteomics in food: quality, safety, microbes and allergens, Proteomics, 16(5), 799-815.
Poulson J., Mahesh N.N., Surendranath P.S., 2015. Application of proteomics to characterize and improve color and oxidative stability of muscle foods, Food Research International, 76(4), 938-945.
Schilling M.W., Suman S.P., Zhang X., Nair M.N., Desai M.A., Cai K., Ciaramella M.A., 2017. Proteomic approach to characterize biochemistry of meat quality defects, Meat Science, 132, 131-138.
Sentandreu M.A., Fraser P.D., Halket J., Patel R., Bramley P.M., 2010. A proteomic-based approach for detection of chicken in meat mixes, Journal of Proteome Research, 9(7), 3374-3383.
Soares R., Franco C., Pires E., Ventosa M., Palhinhas R., Koci K., de Almeida A.M., Coelho A.V., 2012. Mass spectrometry and animal science: protein identification strategies and particularities of farm animal species, Journal of Proteomics, 75(14), 4190-4206.
Surowiec I., Koistinen K.M., Fraser P.D., Bramley P.M., 2011. Proteomic approach for the detection of chicken mechanically recovered meat, Meat Science, 89(2), 233-237.

Tanaka M., Maeda K., Nakashima K., 1995. Chicken alpha-enolase but not beta-enolase has a Src-dependent tyrosine-phosphorylation site: cDNA cloning and nucleotide sequence analysis, The Journal of Biochemistry (Tokyo), 117(3), 554-559.

van de Pavert S.A., Olivier B.J., Goverse G., Vondenhoff M.F., Greuter M., Beke P., Kusser K., Hopken U.E., Lipp M., Niederreither K., Blomhoff R., Sitnik K., Agace V.W.V., Randall T.D., de Jonge W.J., Mebius R.E., 2009. Chemokine CXCL 13 is essential for lymphnode initiation and is induces by retinoic acid and neuronal stimulation, Nature Immunology, 10(11), 1193-1199.

Zhai W., Araujo L.F., Burgess S.C., Cooksey A.M., Pendarvis K., Mercier Y., 2012. Protein expression in pectoral skeletal muscle of chickens as influences by dietary mentionine, Poultry Science, 91(10), 2548-2555.

Scientific Papers. Series D. Animal Science. Vol. LXI, Number 1, 2018
ISSN 2285-5750; ISSN CD-ROM 2285-5769; ISSN Online 2393-2260; ISSN-L 2285-5750

STUDY ON THE NUTRITIONAL QUALITY OF SOME ASSORTMENTS OF SMOKED SALMON FILLETS

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Abstract

The study aimed a comparative analysis of the nutritional-economic characteristics of smoked products from wild salmon (Oncorhynchus keta) and respectively Norwegian salmon (Salmo salar) from aquaculture. Twelve samples (three samples for each type of study product) were analyzed. The proteins, lipids, collagen and water content was determined using the automated analyzer Food Check (infrared spectrophotometer); mineral substances were determined by calcination and the carbohydrates content and energy value were determined by calculation, using conventional relations. The most important differences between the products analyzed have targeted the lipid content: to the smoked Norwegian salmon fillets was determined an almost double amount of lipids (45.5 g/kg product) compared to smoked wild salmon fillets (23.5 g/kg of product). It is interesting the price difference between the two products under study, the product based on smoked Norwegian salmon fillets being over 40% more expensive than wild salmon.

Key words*: lipids, proteins, salt, smoked salmon.*

INTRODUCTION

Fresh fish bring an important nutritional contribution in the diet, offering protein, fatty acids, vitamins and minerals; however, is a perishable commodity very susceptible to oxidation and alteration. Accordingly, conservation technologies are needed, such as smoking, to maintain the quality of the fish (Albertosa et al., 2017).

The conservation effect of smoching is due to a combination of factors, including salt addition, partial dehydration of tissues which takes place in the different stages of the process and the conservation action of the smoke components.

The smoking process slows down biological processes and oxidative degradation and confers of the final product sensory characteristics which consumers appreciate very much (Rizo, 2015).

Quality of raw material, also, represents an important factor for achievement a high quality smoked product (Lerfall, 2017; Usydus et al., 2011).

Smoked salmon is rich in polyunsaturated fatty acids, being used as ready-to-eat food, without cooking (Baek and Song, 2018). The study aimed a comparative analysis of the nutritional-economic characteristics of some smoked products from salmon fillets marketed in Romania.

MATERIALS AND METHODS

The biological material was represented by smoked products of wild salmon fillets (*Oncorhynchus keta*) of USA origin, fishing in the Pacific Ocean (FAO 67 zone) and respectively Norwegian salmon (*Salmo salar*) from aquaculture (Norway origin). The products studied were purchased from the supermarkets from Iasi.

Twelve samples of smoked salmon fillets were analyzed (six samples for each product type taken into study), the samples being chopped and homogenized with the help of an electric shredder.

The content of water, protein, fat, and collagen was determined using the automated analyzer Food Check (infrared spectrophotometer); mineral substances were determined by calcination, and the content of carbohydrates and energy value were determined by calculation, using conventional relations; energy conversion factors were: 4.27 for proteins, 9.02 for lipids and 3.87 for carbohydrates (according

to FAO relations, 2003). The results obtained were statistically processed using the classic method.

RESULTS AND DISCUSSIONS

The most important differences between the products analyzed (Table 1 and Table 2) have targeted the content of lipids: thus, to the smoked Norwegian salmon fillets was determined an almost double amount of lipids (4.45 g/100 g product) and an energy value (134.6 kcal/100 g of product) about 16% higher compared to smoked wild salmon fillets (2.35 g lipids and 115.59 kcal, respectively, for 100 g product); was noted and very high variability of the lipid content to samples of norway salmon fillets (33.7 % CV).

Table 1. Chemical composition and energy value of smoked wild salmon fillets

Chemical components	$\bar{x} \pm s_{\bar{x}}$	s	CV%	Min.	Max.
Lipids%	2.35 ±0.05	0.16	6.73	2.10	2.60
Proteins%	21.77 ±0.05	0.15	0.69	21.50	21.90
Collagen%	4.37 ±0.04	0.12	2.83	4.06	4.45
Water%	74.39 ±0.04	0.12	0.16	72.40	75.60
Ash%	4.86 ±0.13	0.41	8.48	4.24	5.26
Salt%	4.77 ±0.23	0.74	15.45	0.89	1.49
Dry matter%	24.49 ±0.03	0.08	0.34	24.30	24.70
Organic matter%	19.63 ±0.12	0.38	1.91	19.14	20.20
Carbohydrates %	0.21 ±0.17	0.54	14.02	0.08	0.96
GE kcal/100g	115.6 ±0.80	2.53	2.19	112.55	118.24
GE Kj/100g	483.2 ±4.30	13.59	2.81	464.32	505.95

GE = Gross Energy

Determined water content for the two types of products was relatively close (73-74 g/100 g product) as well as ash content (4.9-5.3 g/100 g product); little differences were highlighted in terms of content in protein, in favor of wild salmon fillets (21.77 vs. 21.55 g per 100 g of product). Noteworthy (Table 1 and Table 2) the different content of salt for the two types of products (4.9 vs. 3.3 g per 100 g product).
The collagen content (Table 1 and Table 2) was slightly lower for smoked Norwegian salmon compared to wild salmon (4.31 versus 4.37 g/100 g product).

Table 2. Chemical composition and energy value of smoked Norwegian salmon fillets

Chemical components	$\bar{x} \pm s_{\bar{x}}$	s	CV%	Min.	Max.
Lipids%	4.45 ±0.38	1.20	33.70	2.60	6.90
Proteins%	21.55 ±0.09	0.27	1.26	20.80	21.80
Collagen%	4.31 ±0.05	0.17	3.89	3.98	4.61
Water%	72.56 ±0.32	1.02	1.38	69.40	75.10
Ash%	5.27 ±0.08	0.25	4.79	4.90	5.61
Salt%	3.28 ±0.49	0.82	7.12	2.40	4.10
Dry matter%	25.15 ±0.05	0.17	0.67	24.90	27.60
Organic matter%	19.88 ±0.08	0.24	1.21	19.60	22.60
Carbohydrates %	0.24 ±0.09	0.28	5.19	0.11	2.41
GE kcal/100g	134.6 ±0.21	0.66	0.64	117.92	153.34
GE Kj/100g	562.46 ±19.77	62.50	11.11	492.91	640.96

GE = Gross Energy

The amount of proteins, lipids, ash, salt and water determined in this study are relatively similar to those observed in specialty literature for these product categories, with the remark that there is a very high variability of available data related to the chemical composition of wild and aquaculture salmon. Also, it can be noted the inconsistency and/or lack of complete presentation of the chemical composition of smoked salmon - some authors only mention the amount of fat and fatty acids (Espe et al., 2002), others the amount of water, salt (Lin et al., 2003), lipids, dry matter (Brillet, 2005), or the

one of proteins, fatty acids, minerals, vitamins (Usydus et al., 2009) etc.

Espe et al. 2002 have determined for smoked salmon fillets 4.4% lipids and 10.1% lipids for the Norwegian one of aquaculture.

Lin et al. (2003) have determined for smoked Pacific salmon fillets (*Oncorhynchus tshawytscha* and *Oncorhynchus keta*) a total salt content which ranged from 1.66 to 5.95% and respectively from 2.15 to 5.69%, and the moisture varied from 50.7 to 71.6% and respectively, from 55.5% to 69.7%.

Hanne, in 2007, has found for Atlantic salmon fillets (*Salmo salar*) a quantity of salt which varies between 2 and 5%, and Gallart-Jornet et al., in 2007, an average of 3.5%.

Hanne (2007) specifies the fact that cold smoked salmon appears to be a slightly preserved product, with a small amount of salt, more moisture and less smoke favour than in the past.

Brillet A. (2005) has noticed (in a larger study carried out in France on several batches of smoked Norwegian salmon (*Salmo salar*) weighing 4-5 kg) a wide variation in the chemical composition (lipids: 8.4-15.4%, salt 3.8-5.6%; dry substance: 35.7-41.7%), but states all results meet the current French standards.

Mol et al. (2008) have determined for smoked salmon the following values: water 60.7 ± 2.9%, proteins 19.9±1.4%, lipids 13.6±1.5%, ash 4.4±1.2%, carbohydrates 1.4±0.8% and an energy value of 247.1±7.1 Kcal/100g but does not specify which type of smoked salmon has been analyzed.

Usydus et al. (2009) have determined for Baltic wild smoked salmon (*Salmo salar*) 22.35% proteins and 11.51% lipids, versus 19.71% proteins and 15.46% lipids, for Norwegian aquaculture salmon.

Espe et al. (2002) mentions that the freshly used raw material affects smoke losses of the nutritional components of salmon fillets. The weaker the fish, the higher the fat loss of the fillets, and if the fish raw material is fatter, the fillets does not suffer a large changes in weight and fat through smoking.

The price of the two assortments analyzed was different, the Norwegian salmon product being over 40% more expensive (12.49 RON/100g product) than the wild salmon (8.79 RON/100g of product) at the same economic agent.

The two product assortments studied have been characterized and compared and through the prism the amount of nutrients (protein, fat) and respectively the energy and water offered to the consumer for a value unit (1 RON) (Table 3). It can be seen from the analysis of these data how the amount of protein and energy offered to consumers for the same value unit (1 RON) is higher for wild salmon (this having a lower price) compared to Norwegian salmon (Table 3).

Table 3. Nutritional - economic characteristics of analysed products (smoked salmon filets)

Nutritional - economic characteristics		Captured wild salmon (*Oncorhynchus keta*)		Norvegian culture salmon *(Salmo salar)*		
		values	%	values	%	± %
Price	RON/ kg	87.9	100	124.9	142.1	+ 42.1
Protein	g / kg	217.7		215.5	99.0	- 1.00
	g / RON	2.47		1.73	70.0	- 30.0
Fat	g / kg	23.5		44.5	189.4	+ 89.4
	g / RON	0.27		0.36	133.3	+ 33.3
Energy GE	kcal / kg	1156		1346	116.4	+ 16.4
	kcal / RON	13.15		10.77	81.9	- 19.1
Water	g / kg	743.9		725.6	97.5	- 2.5
	g / RON	8.46		5.81	68.7	-31.3

In the somewhat paradoxically mode, the price of the wild salmon product is much lower; a possible explanation could be based on the controversial situation in the world through is incriminated constipation of radioactive contamination with Caesium 137 of aquatic products coming from the following FAO areas: area 61: Pacific Northwest, area 67: Pacific Northeast, area 71: Pacific Western Central, area 77: Pacific Eastern Central, area 81: Pacific Southwest and area 87: Pacific Southeast.

The European Commission recommends its Member States to randomly monitor the levels of radioactive substances in the seafood captured in the FAO main fishing area 61, as well as those from FAO major fisheries areas 67, 71 and 77 - but of lesser importance of risk of contamination resulting from the Fukushima nuclear accident (WHO/FAO, 2011).

CONCLUSIONS

The amount of protein offered to consumers is similar for the two smoked salmon assortments marketed in Romania, but reported at the purchase price the salmon of aquaculture offers with 30% less protein than the wild one;

The most important differences between the products analyzed have targeted the lipid content: to the smoked Norwegian salmon fillets was determined a quantity almost double of lipids (+89.4%). Reported to purchase price of Norwegian salmon, the amount of lipids offered to consumers is over 33% higher.

The amount of salt contained of fillets of Norwegian salmon it is smaller than that of wild salmon with 1.6 g / 100g of product.

We recommend authorities in the field to introduce the obligation to mention on the labels of food products the area they come from fish raw material, given the potential hazards to which consumers may be expose if purchases products coming from the areas with a risk of radioactive contamination or of another nature. Thus, the consumers should make a conscious, informed choice between the price of the product and the culinary gustatory, properties, nutritional quality, but also the consumer's safety of the product it buys, carefully reading product labels.

REFERENCES

Albertosa Irene, Roberto J. Avena-Bustillosb, Ana Belén Martín-Dianaa , Wen-Xian Dub , Daniel Ricoa, Tara H. McHughb, 2017. Antimicrobial Olive Leaf Gelatin films for enhancing the quality of coldsmoked Salmon. Food Packaging and Shelf Life, 13, 49-55.

Baek S.-K., Song K.B., 2018. Development of Gracilaria vermiculophylla extract films containing zinc oxide nanoparticles and their application in smoked salmon packaging. LWT - Food Science and Technology, 89, 269-275.

Brillet Anne, 2005. Sélection et caractérisation de souches de Carnobacterium pour la biopréservation du salmon fumé These de Doctorat, Discipline: Biotechnologies Agroalimentaires, Sciences de l'Aliment, Spécialité: Microbiologie.

Espe M., Nortvedt R., Lie O., Hafsteinsson H., 2002 Atlantic salmon (*Salmo salar*, L) as raw material for the smoking industry. II: Effect of different smoking methods on losses of nutrients and on the oxidation of lipids. Food Chemistry, 77, 41-46.

Lerfall C J., Skuland A. V., Skare E.F., Rune Hasli P., Rotabakk B.T. WT, 2017. Quality characteristics and consumer acceptance of diploid and triploid cold smoked Atlantic salmon reared at 5, 10 and 15^0C. Food Science and Technology, 85, 45-51.

Gallart-Jornet L., Barat J.M., Rustad T., Erikson U., Escriche I., Fito P., 2007. Influence of brine concentration on Atlantic salmon fillet salting. Journal of Food Engineering, 80, 267-275.

Hanne Løje, 2007. The quality of cold smoked salmon - Influence of raw material and technological parameters. Ph. D. thesis, Technical University of Denmark Danish, Institute for Fisheries Research, Department of Seafood Research BioCentrum.

Lin M., Cavinato AG., Huang Y., Rasco BA., 2003. Predicting sodium chloride content in commercial king (*Oncorhynchus tshawytscha*) and chum (*O. keta*) hot smoked salmon fillet portions by short-wavelength near-infrared (SW-NIR) spectroscopy. Food Research International, 36(8), 761-766.

Mol Sühendan, Alakavuk Ü. Didem, Tosun Ş. Yasemin, 2008. Effects of Different Processing Technologies on the Chemical Composition of Seafoods. Food Sci. Technol. Res., 14 (5), 467-470.

Rizo Arantxa, Máñes Verónica, Fuentes Ana, Fernández-Segovia Isabel, Barat José M., 2015. A novel process for obtaining smoke-flavoured salmon using water vapour permeable bags. Journal of Food Engineering 149, 44-50.

Usydus Z., Szlinder-Richert Joanna, Polak-Juszczak Lucyna, Komar Katarzyna, Adamczyk Maria, Malesa-Ciecwierz Małgorzata, Ruczynska Wiesława, 2009. Fish products available in Polish market – Assessment of the nutritive value and human exposure to dioxins and other contaminants. Chemosphere, 74, 1420-1428.

Usydus Z., Szlinder-Richert Joanna, Adamczyk Maria, Szatkowska Urszula, 2011. Marine and farmed fish in the Polish market: Comparison of the nutritional value. Food Chemistry, 126, 78-84.

WHO/FAO, 2011. Impact on Seafood Safety on Nuclear Accident in Japan.
www.iaea.org/sites/default/files/seafoodsafety05110.pdf

Scientific Papers. Series D. Animal Science. Vol. LXI, Number 1, 2018
ISSN 2285-5750; ISSN CD-ROM 2285-5769; ISSN Online 2393-2260; ISSN-L 2285-5750

DETERMINATION OF RAT ADULTERATION IN MEATBALLS USING ENZYME - LINKED IMMUNOSORBENT ASSAY (ELISA) TECHNIQUES AT JATINANGOR EDUCATION AREA, SUMEDANG DISTRICT, WEST JAVA, INDONESIA

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Abstract

Currently, one of the most significant threats is the excessive hunting on wild animals. This is because the hunting results into a food product with turnover and big profit. The trigger for the demand for animal protein derived from the flesh of wild animals (bush meat) against certain species may lead to an increase in diseases. Adulteration processed meatballs into one type of processed replaces raw materials with rat meat. In addition, to causing economic losses, the food products consumed are not safe, healthy, whole, and halal, therefore the need for identification of these food products appears. One method that can be used to detect food product adulteration is the ELISA (Enzyme-Linked Immunosorbent Assay). This test method is an effort to detect the presence of antibodies or specific antigen in a sample. A total of 29 samples were collected from various meatball traders and chicken noodles around Jatinangor. The results showed negative results of 96.55% and positive results 3.45%. A positive result has a greater value than a negative result color (blue) indicating that rat antibody samples bind to streptavidin-peroxidase antigens, where antigen and antibodies occur in homologous process resulting in a change of color (yellow). This test has a high sensitivity level, so that on products that have experienced ripening can still be detected specific antigen. This is evidenced in the positive control of rat meatballs, and antibodies in rat can still be detected. The negative results indicate there is no adulteration meat meatball in the meatball and it is an evidence that Halal in the area of Jatinangor and surrounding areas become an important concern on food in the campus environment.

Key words*: adulteration, ELISA, rat meat.*

INTRODUCTION

Adulteration food processed products from meat is very frequent. Raw materials are replaced with other materials, that are cheaper. In 2013 in the United Kingdom a lot of processed beef products are substituted with horse or pork. One example is the beef burger products, where beef is replaced with horse meat. In China, rat and fox meat were sold as lamb. The meat is processed by adding gelatin, nitrate salts and stains, so it looks and feels like frozen goat meat. In 2017, in some areas in Indonesia are found several cases of counterfeiting cases on beef meatball products and chicken noodles. The products are substituted with pork or rats (Fumière et al., 2009).

Food products with processed rat meat can cause various losses for consumers. In addition to causing economic losses, the products consumed become not safe, healthy, whole, and halal. Foods faked with rat meat have the potential to cause various diseases.

This is related to the potential diseases or parasites found in rat meat. Research from the World Health Organization found that 1 in 10 people in the world suffer from diseases caused by the food they eat, 420,000 of whom die every year, many of them children.

Several methods have been developed based on electrophoresis, isoelectric focus, chromatography, DNA hybridization, polymerase chain reaction (PCR) and enzyme-linked immunesorbent assay (ELISA) (Aida et al., 2005).

Therefore, it is necessary to identify the falsification of processed foods. The falsification test on meat products is very difficult, especially heating processed products such as meatballs, because the heating process produces a denaturative protein. Antibodies to proteins

dissolve in a stable heat, which retains its antigenicity after high temperature processes (Olivier et al., 2009). One method that can be used to detect food product counterfeiting is the ELISA (Enzyme-Linked Immunosorbent Assay) method. This test method is an effort to detect the presence of antibodies or specific antigen in a sample (Ayaz et al., 2006).

The ELISA approach allows the identification of various types of meat mixtures in very low quantities or has undergone changes caused by processing. The test is expected to provide guarantee and guarantee of quality and safety of processed products (meatballs) produced in Jatinangor Sumedang, West Java-Indonesia.

MATERIALS AND METHODS

A number of antigens / antibodies are affixed to a surface (well), then added substances that can be converted by the enzyme into a detectable signal. A total of 29 samples were collected from various meatball traders and chicken noodles around Jatinangor.

The samples will be tested using the ELISA method with the Cat # RMT-48 Cat Test Kit from Alpha Diagnostic International.

Sample Preparation (extraction)

Samples are cut into small pieces, and mixed until homogeneous (in the code of the same sample). It is putted into tube test as much as 100 mg. The buffer is diluted 100x. Add the extraction buffer according to the instruction kit (incubation 4˚C ± 24 hours). Positive controls were prepared (concentration of raw meatball 10%, 20%, 30%, 40%, 50%, concentration of meatball cooked 10%, 20%, 30%, 40%, 50%).

ELISA Test

The microtiteris marked on the plate. The supernatant of sample extraction result ± 1 ml prepared. 100 µl positive and negative controls are added into a well. 100 µl of sample extraction are addedinto other wells. It is stired, by tapping well for 5-10 seconds. It is incubated at room temperature for 30 minutes. It is disposed of well contents (washed) and washed with wash buffer 3x (buffer used as much as 300µl).

It is added 100 µl antibody-enzyme conjugate into each well. It is stired, by tapping well for 5-10 seconds. It is incubated in room temperature for 20 minutes. It is discarded the contents well (spilled) and washed with the wash buffer as much as 4x (buffer used as much as 300 µl). It is added 100 µl TMB Substrate. It is stired, by tapping well for 5-10 seconds. Ii is incubated in room temperature for 10 minutes (well will be blue). It is added Stop Solution as much as 100µl (positive well will turn to yellow).

It is uppered on ELISA reader with wavelength 450 nm.

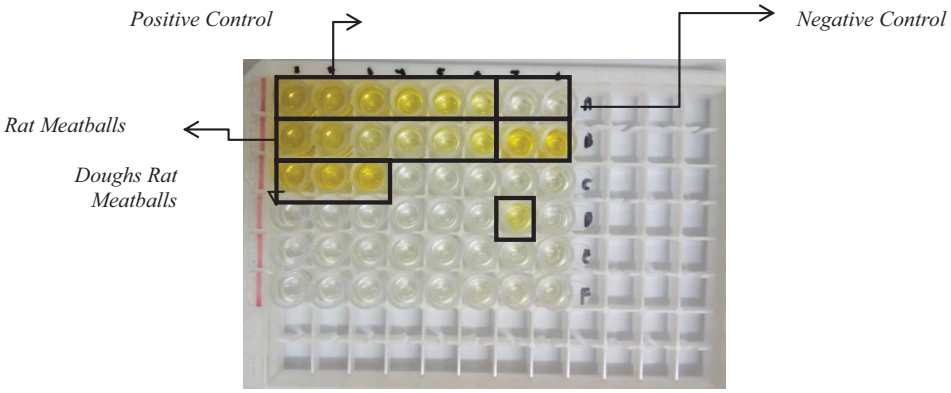

Figure 1. Result Enzyme immunoassay

RESULTS AND DISCUSSIONS

ELISA is used in order to test screening (screening test), to detect protein levels in the presence of antigens and antibodies. ELISA tests are not only sensitive, but also rapid and specific, because of complex antibodies that occur in well microplate and after substrate administration, enzymes bound to antibodies

will give color change to the fluid, giving different optical density.

Twenty-nine samples were taken from Jatinangor meatballs traders to identify adulteration on meatballs sold and tested using ELISA. The ELISA kit uses an improved biotin-avidin process. With the increase in the specific protein concentration of rat meat in the extract, more proteins bind antibodies attached to well.

After the reaction process continues, the unbound material is removed by washing. The amount of specific proteins attached to well-coated antibodies is determined by the first reaction with biotinylation and also with the conjugate streptavidin-peroxidase. After incubation, the reagents are removed by washing. Finally, the bound peroxidase activity is determined by adding a certain amount of Tetramethylbenzidine (TMB) substrate, which develops blue color (turns yellowish green with the addition of acidic reagents) in the presence of peroxidase. The development of color is proportional to the original number of certain rat proteins in the sample extract.

R/C	1	2	3	4	5	6	7	8
A	2.238	2.210	1.928	1.380	0.863	0.549	0.177	0.180
B	2.382	0.902	0.397	0.414	0.439	0.724	2.619	2.792
C	2.395	2.437	2.638	0.116	0.132	0.127	0.135	0.163
D	0.095	0.097	0.095	0.114	0.099	0.108	0.546	0.109
E	0.112	0.124	0.111	0.127	0.104	0.107	0.127	0.150
F	0.108	0.106	0.113	0.108	0.129	0.130	0.130	0.122

No. Sample B1-C3: Samples of processed rat meatballs, A1-A8: Control Samples, C4-F8: Meatballs Samples

: Negative : Positive

Figure 2. Result of ELISA Reader

At point D7 the sample has a greater value than the negative control it indicates that the rat antibody samples bind to the antigen streptavidin-peroxidase.

Where antigen and antibodies occur homologous process resulting in a change of color yellow from the process.

This test has a high sensitivity level so that on products that have experienced ripening can still be detected a specific antigen.

This is evidenced in the positive control of rat meatballs, antibodies in mice can still be detected.

This study shows that ELISA can identify the presence of rat meat in meatball dough and meatballs that have undergone a heating process. Chien et al. (2001) detected rat meat content in processed heating products by ELISA method with the lowest detection limit of 0.5% (b / w) of mice in the meat mixture. Roostita and Lengkey (2014) showed that the Enzyme-Linked Immunosorbent Assay (ELISA) check enables identification of various types of meat mixtures in very low quantities or has undergone changes caused by processing.

The test on 28 samples taken from meatballs merchants in Jatinangor showed no rat adulteration. This is because meatballs traders realize the importance of the quality of raw materials related to halal meatballs. Only 1 sample was found by traders to adulteration by mixing rat meat into meatballs, although at certain times it was profitable this can threaten the sustainability of the business. Traders already have knowledge about halal meatballs so that traders are not worried about the sale of meatballs. Halal is a sensitive issue because most of the population of Jatinangor are Muslim.

CONCLUSIONS

The test ELISA tested 29 samples from traders meatballs and showed negative results of 96.55% and positive results 3.45%. The negative results indicate no adulteration rat meat. This is evidence that halal in the area of Jatinangor and surrounding areas become an important concern for traders and consumers in the campus environment.

ACKNOWLEDGMENTS

Authors would like to thank The Center for Research and Community Services of University of Padjadjaran for funding the research and Animal Disease and Veterinary Public Health Investigation Center for helping the ELISA Test. We are grateful to Dian CaturPermatasari, Trianingtyas and Nanah, for the help during the research.

REFERENCES

Aida A.A., Che Man Y.B., Wong C.M.V.L., Raha A.R., Son R., 2005. Analysis of raw meats and fats of pigs using polymerase chain reaction for Halal authentication. Meat Sci., 69, 47–52.

Ayaz Y., Ayaz N.D., Erol I., 2006. Detection of species in meat and meat products using enzyme-linked immunosorbent assay. J. Muscle Foods, 17, 214–220

Chien C.R., Chen S.W., Hsieh C.Y., Liang J.A., Yang S.N, Huang C.Y. et al., 2001. Retrospective comparison of the AJCC 5th edition classification for nasopharyngeal carcinoma with the AJCC 4th edition: an experience in Taiwan. Jpn. J.Clin. Oncol., 31(8), 363-369.

Fumière O., Veys P., Boix Ana, von Holst C., Baeten V., Berben G., 2009. Methods of detection, species identification and quantification of processed animal proteins in feedingstuffs. Biotechnologie Agronomie Societe et Environnement, 13, 59-70, no. special.

Roostita L.B., Lengkey H.A.W., 2014. Determination of pork adulteration in meatballs using Enzyme- Linked Immunosorbent Assay (ELISA) techniques Case Study: Small medium enterprises meatballs traders at Jatinangor Education Area, Sumedang District, West Java, Indonesia. Biotechnology in Animal Husbandry, 30(2), 289-293.

Scientific Papers. Series D. Animal Science. Vol. LXI, Number 1, 2018
ISSN 2285-5750; ISSN CD-ROM 2285-5769; ISSN Online 2393-2260; ISSN-L 2285-5750

APPLICATION OF CONJOINT ANALYSIS TO DETERMINE CONSUMERS' RED MEAT PREFERENCES IN SIIRT PROVINCE

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Abstract

The aim of this paper is defining consumers' preferences for the red meat in Siirt Province. This paper illustrates the conjoint analysis application in determining consumers' preferences for the attributes of red meat according to the amount of consumption. Multiple regression analysis used for determination most valued attributes and their levels. A random sample of 160 red meat consumers was interviewed in Siirt Province. They were asked to provide demographic information and responses to several survey questions, as well as to participate in a conjoint analysis study. For the survey portion of the interview, respondents were asked to assess the importance of the following attributes: meat type, purchasing sources and price.
As a result of the study, it was found that relative importance of attributes for the regular consumers were 48.8% price, 30.7% purchasing source, 20.5% meat type, and for non-regular consumers were 37.3% meat type, 34.3% price and 28.4% purchasing source. Determination coefficients of the models for regular and non-regular consumers were found as 99.3% and 99.2%, respectively.

Key words*: conjoint analysis, red meat, preferences, attributes, consumer.*

INTRODUCTION

Meat has an important food for the people as long as human history (Aritasi, 2009). Consumer's culture level, revenue, social situation and improvement processes affect the meat consumption properties of consumers. (Arısoy and Bayramoglu, 2015). Because of importance of animal proteins such as meat, milk and egg in human nourishment, level of meat consumption is an important indicator of developed countries. Therefore, meat consumption increases as developing countries' social and economic improvements increases (Kan and Direk, 2004; Arısoy and Bayramoglu, 2015).
Income of the individuals is the most important factor determines the meat purchasing power of humans. In our country meat consumption per capita is 12 kg annually and this is very low within the other countries such as Russia (58.7 kg) and Brazil (95.1 kg). Meat prices are more expensive compared to other food items, which is reported as the reason low meat consumption in Turkey (Tomek, 1989; Onurlubaş et al., 2015). It was done some studies for determining preferences of meat consumers by means of conjoint analysis (Bernabeu and Tendero, 2005; Bernabeu et al., 2018).
The aim of this study was to determine the consumers' red meat preferences and factors affecting in Siirt Province by means of conjoint analysis.

MATERIALS AND METHODS

The questionnaire forms were personally applied to a representative sample of residents in Siirt (Figure 1) by the researchers themselves.

Figure 1. Siirt Province (Turkey)

The survey was released during the June 2017, on a random sample of residents in

the centre of Siirt. 161 questionnaires were obtained. Data analysis was performed by means of Traditional Conjoint Analysis technique (Orme, 2010). The scores given by respondents to the product characteristics (cards) was dependent variable.

The characteristics of the product (cards) or attribute levels were independent or predictor variables. The estimated regression coefficients associated with the independent variables are the part-worth utilities or preference scores for the levels. The R^2 for the regression characterizes the internal consistency of the respondent (Orme, 2010).

The attributes and their levels defining the meat preference were: price (cheap, medium, expensive), meat type (MT) (beef, sheep, goat), purchasing sources (PS) (butcher, supermarket with butcher, supermarket without butcher). A full-factorial experimental design included all possible combinations of the attributes (Orme, 2010). Cards created in this study were:

3 MT × 3 price × 3 PS = 27 cards

Depending on red meat consumption amount per capita the observations were divided to two groups. First group was regular consumers, who consume meat more than 2 kg in a month (27.3%), second group was non-regular consumers (72.7%), who consume less than 2 kg red meat in a month. The model was expressed as the following equation:

$$Y = \beta_0 + \beta_1 \times sheep + \beta_2 \times goat + \beta_3 \times medium + \beta_4 \times expensive + \beta_5 \times SB + \beta_6 \times SOB + \varepsilon$$

Where, β_i: coefficients of regression; SB: dummy variable for the supermarket with butcher; SOB: dummy variable for the supermarket without butcher; ε: term of error.

Statistical analysis was performed with the SPSS Statistical Package for Windows version 20.0 (SPSS Inc., 1999).

Descriptive statistics concerning demographic information of the respondents were given in Figure 2.

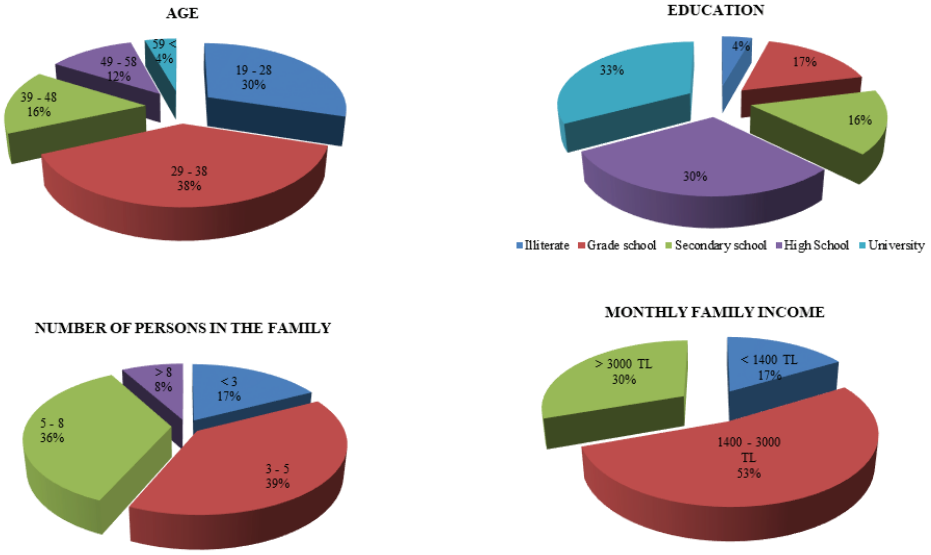

Figure 2. Sample demographic information

RESULTS AND DISCUSSIONS

Most traditional conjoint analysis problems solve a separate regression equation for each respondent (Orme, 2010). In this study regression model was applied to the average preference of respondents per cards. The regression performed for both regular and

non-regular consumers estimated determination coefficient over 99% (Table 1). According to the results obtained in Table 1, it can be said that all the parameters estimated from the model were found statistically significant. The fits obtained for both groups of consumers are similar.

Table 1. Model of estimated parameters for red meat consumers

Variables	Consumer	
	Regular	Non-regular
Sheep	-2.89**	-5.67**
Goat	-1.00**	-2.33**
Medium	-6.89**	-5.22**
Expensive	-5.67**	-2.78**
SB	1.44**	0.67**
SOB	4.33**	4.33**
Constant	17.37**	17.33**
R^2	99.3	99.2
R^2-adj.	99.1	98.9

**: p<0.01

Utilities of each level of each attribute was calculated (Table 2).

Table 2. Estimated utility of the attribute levels

Attribute	Level	Consumer	
		Regular	Non-regular
Meat type	Beef	0.00	0.00
	Sheep	-2.89	-5.67
	Goat	-1.00	-2.33
Price	Cheap	0.00	0.00
	Medium	-6.89	-5.22
	Expensive	-5.67	-2.78
Purchasing source	Butcher	0.00	0.00
	SB	1.44	0.67
	SOB	4.33	4.33

It is shown from the model that the both group of consumers prefer beef meat. This preference followed by goat and sheep meat. Goat meat is more preferable than sheep meat. From the survey study concerning red meat consumption performed in Edirne the average meat consumption differenced between 22.56 kg/year and 27.36 kg/year. Consumers preferred to beef, lamb and all of them the rate 55%, 35% and 10% respectively (Lorcu and Bolat, 2012). Average monthly meat consumption is 0.7 kg in Antalya. Lamb, goat's meat and beef meat consumption had proportion of 0.79 kg/month, 0.67 kg/month and 0.66 kg/month, respectively (Tosun and Hatırlı, 2009).

Respondents want buy meat when price is lower. Expensive meat is also preferable as shown from the Table 2. Juma et al. (2010) stated that price influenced household's chevron and mutton consumption compared to beef meat. For purchasing places SOB is most preferable place following by SB and butcher. Similar result was found in the study made in Antalya. They preferred to shopping malls exclude butcher the rate 60% (Tosun and Hatırlı, 2009). In the study of Lorcu and Bolat (2012) in Edirne, consumers' preference for place of purchase was butcher. Similar results had been got about consumer's preference and place of purchase in Elazığ and Odemis district of İzmir (Yaylak et al., 2010; Şeker et al., 2011).

The relative importance (RI) of each attribute was computed from the utilities given in Table 2. RI was defined as the percentage of the range assigned to each attribute to the variation of total ranges (Bernabeu and Tendero, 2005; Orme, 2010):

$$RI = \frac{\max U_i - \min U_i}{\sum \max U_i - \min U_i} \times \%100$$

RI of the meat attributes for regular as well as non-regular consumers was given in Figure 3.

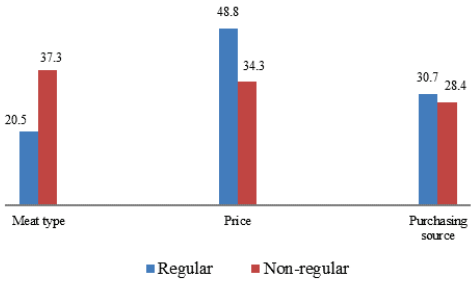

Figure 3. RI of red meat attributes

As shown in Figure 3, there are some differences. The greatest difference between groups was found in meat type. The meat type was very important for non-regular red meat consumers, rather than regular consumers. The second noticeable difference was found in price attribute. As shown in Figure 3 price was more important for regular red meat consumers, rather than non-regular consumers. Du Plessis and Du Rand (2012) determined that price was the most important factor

compared with quality and origin, however some studies reported that price is not important as other factors like origin and quality that determine consumer's decision (Bernabéu and Tendero, 2005; Mesías et al., 2005; Villalobos et al., 2010).

It can be interpreted from the Figure 3 that, non-regular red meat consumers were more selective in meat type following by price and purchasing source. But, for regular meat consumers were very important price following by purchase source and meat type.

CONCLUSIONS

Only in the smallest of problems, people would be asked to rank all possible attribute levels combinations. With this sample of three attributes and 9 total levels traditional conjoint analysis was made with 27 cards.

According to the utility of each attribute level, the red meat preferred by both consumer groups, similarly. But relative importance of attributes was found different between regular and non-regular consumer groups.

Price of the red meat was the most valued attribute by regular consumers followed by the purchasing source however, the type of the red meat was the most valued attribute for non-regular consumers followed by price.

As a result of the study, it can be noted that most of red meat consumers in Siirt prefer cheap meat from the SOB. Non-regular consumers were selective in meat type preference, when it was the last preference for regular consumers.

REFERENCES

Arısoy H., Bayramoğlu Z., 2014. Consumers' determination of red meat and meat products purchase behaviour – city of Ankara sample. Turkish Journal of Agriculture-Food Science and Technology, 31, 28-34.

Arıtasi C., 2009. Et ve balık işleme teknolojisi ders notları. Namık Kemal Üniversitesi, Tekirdağ.

Bernabéu R., Rabadán A., El Orche N.E., Díaz M., 2018. Influence of quality labels on the formation of preferences of lamb meat consumers. A Spanish case study. Meat science, 135: 129-133.

Bernabéu R., Tendero A., 2005. Preference structure for lamb meat consumers. A Spanish case study. Meat Science, 71(3), 464-470.

Du Plessis H.J., Du Rand G.E., 2012. The significance of traceability in consumer decision making towards Karoo lamb. Food Research International, 47, 210–217.

Juma G.P., Ngigi M., Baltenweck I., Drucker A.G., 2010. Consumer demand for sheep and goat meat in Kenya. Small Ruminant Research, 90(1), 135-138.

Kan A., Direk M., 2004. Course of red meat prices in the Konya province. Selcuk Universitesi Ziraat Fakultesi Dergisi, 18, 35-40.

Lorcu F., Bolat B.A., 2012. Edirne ilinde kırmızı et tüketim tercihlerinin incelenmesi. JOTAF. Tekirdağ Ziraat Fakültesi Dergisi, 9(1), 71-85.

Mesías F.J., Escribano M., De Ledesma A.R., Pulido F., 2005. Consumers' preferences for beef in the Spanish region of Extremadura: a study using conjoint analysis. Journal of the Science of Food and Agriculture, 85(14), 2487-2494.

Onurlubaş E., Yılmaz N., Doğan H.G., Kızılaslan H. 2015. A research on red meat consumption and preferences: a case study in Tekirdağ Province. Turkish Journal of Agriculture-Food Science and Technology, 3(6), 466-471.

Orme B.K., 2010. Getting started with conjoint analysis. Strategies for product design and pricing research. Second Edition. Research Publishers LLC, USA.

SPSS Inc., 1999. SPSS Categories. Version 10.0. Chicago.

Şeker İ., Özen A., Güler H., Şeker P., Özden İ., 2011. Elazığ'da kırmızı et tüketim alışkanlıkları ve tüketicilerin hayvan refahı konusundaki görüşleri. Kafkas Univ Vet Fak Derg, 17(4), 543-550.

Tömek S., 1989. Et teknolojisine giriş. Ege Üniv. Müh. Fak., Çoğaltma Yayın, 65, 38.

Tosun Ö.O., Hatirli S.A., 2009. Tüketicilerin kırmızı et satınalım yerleri tercihlerinin analizi: Antalya ili örneği. Süleyman Demirel Üniversitesi İktisadi ve İdari Bilimler Fakültesi Dergisi, 14(2).

Villalobos P., Padilla C., Ponce C., Rojas Á., 2010. Beef consumer preferences in Chile: Importance of quality attribute differentiators on the purchase decision. Chilean Journal Agricultural Research, 70(1), 85-94.

Yayla E., Taşkın T., Koyubenbe N., Konca Y., 2010. İzmir ili Ödemiş ilçesinde kırmızı et tüketim davranışlarının belirlenmesi üzerine bir araştırma. Hayvansal Üretim, 51(1), 21-30.

Scientific Papers. Series D. Animal Science. Vol. LXI, Number 1, 2018
ISSN 2285-5750; ISSN CD-ROM 2285-5769; ISSN Online 2393-2260; ISSN-L 2285-5750

A REVIEW ON THE RESULTS OBTAINED FROM THE ANALYSIS OF ANIMAL FOOD PRODUCTS FROM MEAT IN SOME EUROPEAN COUNTRIES USING GFAAS AND FAAS TECHNIQUES

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Abstract

This review paper aimed to highlight the importance of heavy metals and their presence in animal bodies and in their food products. There are specific organizations that have the role of constantly monitoring the levels of heavy metals and their consequences for human health, if the maximum admissible limits imposed by European legislation are exceeded. Their surveillance has an important role to safeguard consumers in case of a food contamination incident. They address issues such as sustainability, biological diversity, climate change, nutritional economics, population growth, water supply, and access to food. Naturally, heavy metals are not found in the animal body or in the animal food products, but they can be discovered as a result of their conscious or accidental incorporation (contamination) in food and which, by exceeding the acceptable limits, can constitute a health risk factor. As methods of analysis, Graphite Furnace and Flame Atomic Absorbtion Spectrometry were used. Therefore, it is very important to continually assess the levels of these analytes (Lead, Cadmium), to ensure that the values fall within the maximum admissible limits.

Key words: *heavy metals, human food safety, GFAAS, maximum admissible limits, European legislation.*

INTRODUCTION

Food is any substance consumed to provide nutritional support for an organism. It is usually of plant or animal origin and contains essential nutrients such as carbohydrates, fats, proteins, vitamins or minerals (Ciobanu et al., 2012).

Human nutrition is very important, as it depends, to a great extent, on ensuring the optimal development of the organism during growth, as well as the gaining and maintaining of the body's resistance to external factors (climatic, toxic or infectious).

Meat is very important in our food diet because, for most of us, it represents the main element in our nutrition (Murphy, 2002).

Animals are used as food either directly or indirectly by the products they produce. Meat is an example of a direct product taken from an animal, which comes from muscle systems or from organs.

Many food products are regularly tested for a selection of trace elements to estimate possible nutritional or toxicological associations and to warrant agreement with government regulations or food safety (Ciobanu et al., 2012; Berg et al., 2002).

The concentration of the distinct element species in food is also required to estimate the food safety and nutritional quality (Murphy, 2002).

The consumption of polluted food is the main source of Lead (Pb) and Cadmium (Cd) intake. Pb is abundant in the environment from different sources include automotive gasoline piston engines, oil burners, lead pipes, incinerators, industrial effluents and smokestack fallout (Walker, 2014).

MATERIALS AND METHODS

In order to gather the most relevant articles for the target subject, we focused our attention on literature restricted in this domain, guiding us by following keywords: lead, cadmium, meat products, organs, graphite furnace atomic absorption spectrophotometry (GFAAS), dietary intakes.

Samples collection

We selected the samples that were analyzed by GFAAS and FAAS techniques in Europe in order to create a database at least for some European country regarding heavy metals concentrations (Pb, Cd) in meat food products.

For this purpose, muscle samples from pork, beef and chicken (hearts, livers, gizzards and muscle), were randomly gathered from different farms and retail markets all over.

Samples homogenization and preparation of the analytical solutions

After the complete calcination at a maximum temperature of 450 Celsius degree, the mineralizates were processed. 5 mL of hydrochloric acid 6 Mol/L and 10 mL of 0.1 HNO_3 were added. The brushes were carefully rotated, so all the ashes came into contact with the acid. It was covered with a watch bottle and allowed to sit for 1 hour to 2 hours.

In parallel, a blank sample was prepared, with the mineralization reagents, treating the reagent blank in the same way as the samples. After this, the samples were processed by GFAAS or FAAS (Seely et al., 2009).

Estimation of Pb and Cd by graphite furnace/flame atomic absorption spectrometer (FAAS)

Standard stock solutions of Pb and Cd (1000 ppm) were prepared and diluted to the corresponding expected mass fraction recovery of trace elements in the samples. Pb and Cd were analyzed by GFAAS and FAAS. The wavelength of k = 217 nm was used for detection of Pb while the wavelength of k = 228.8 nm for Cd. Two replicate determinations were done for each sample. Sample blanks were prepared by taking 10 mL of the reagents mixture through the same procedure (Seely et al., 2009).

RESULTS AND DISCUSSIONS

Humans can be exposed to heavy metals through a variety of means, including consumption of contaminated food. Although heavy metals are usually present in foods at very low levels, long term exposure can have negative health impacts. Two of the more important toxic elements that must be monitored are cadmium (Cd) and lead (Pb), which can enter food either through environmental processes or through contamination in processing and/or packaging. As a result, it is very important to accurately measure low levels of Cd and Pb in a variety of food matrices (Dong-Gyu et al., 2015).

Lead and Cadmium are toxic metals occurring in the environment naturally and from anthropogenic activities. These heavy metals can lead to chemical contamination of products entering in the human food chain (Walker, 2014).

Determinations of these heavy metals were made in food products of animal origin in order to assess the threat to people posed by their presence. We processed and interpreted all the relevant data regarding this subject, in order to highlight their serious potential consequences.

In the past few years, a number of instrumental developments have contributed to providing more reliable results and better detection limits for trace determination of lead and cadmium by GFAAS.

These include improved electrodeless discharge (EDL) and hollow cathode (HCL) lamps for increased light output, and improved wet ashing sample preparation techniques (e.g., microwave digestion). This work will focus on the use of GFAAS for the determination of lead and cadmium (Figures 1, 2, 3) in a variety of food samples (Seely et al., 2009).

Limits of detection and quantification

An important aspect of the method performance evaluation is the calculation of the limits of detection. The limits of detection, based on the repeated analysis of blank solutions, were calculated as instrument detection limit (IDL), while the average standard deviation of repeated analysis of sample blanks (or samples containing very low concentration of the analytes) were calculated as the method detection limit (MDL).

All detection limits were obtained by analyzing more blank/samples each (Murphy, 2002).

Regarding chicken giblets, AAS analysis was employed on livers, gizzards and hearts samples sold at retail markets.

The minimum and maximum estimated concentrations of Pb were widely variable in livers, gizzards and hearts samples.

The significant Pb concentrations were found in liver samples (0.8762 ± 0.2089 ppm), whereas gizzard samples contained 0.3186 ± 0.1462 ppm and the level of Pb was estimated 0.1733 ± 0.06777 ppm in heart samples.

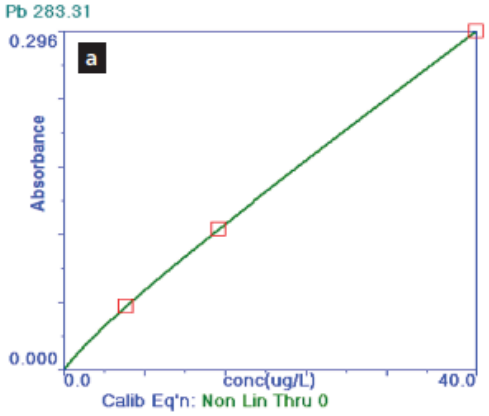

Figure 1. Three-points calibration curve for Pb (Seely et al., 2009)

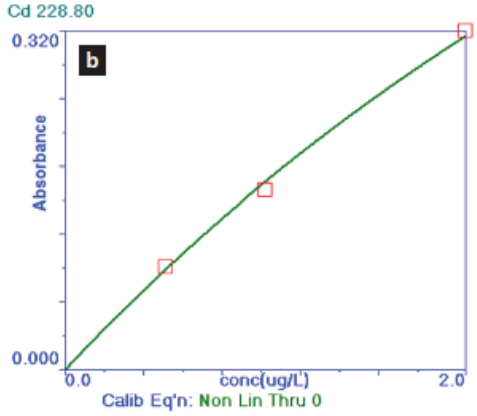

Figure 2. Three-points calibration curve for Cd (Seely et al., 2009)

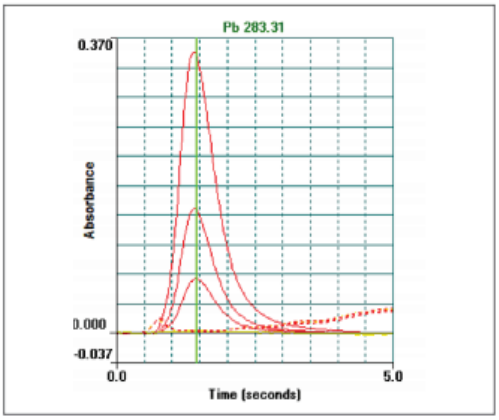

Figure 3. Overlay of spectral profiles of Pb standard solutions at 6 µg/L, 16 µg/L and 40 µg/L (Seely et al., 2009)

The results of one study indicated that broiler livers samples contain high Pb levels which exceed the maximum limit (0.5 ppm) in the Codex Alimentarius International Food Standards in some European areas.

This high level of Pb concentration could be attributed to the heavy environmental pollution with Pb which has high tendency for bioaccumulation in chicken tissue as it was deposited in kidneys (1.360 ppm), livers (0.500 ppm), ovarian tissue (0.320 ppm) and muscle (0.280 ppm) after experimental exposure of chicken-folk to chips of lead-based paint in their environment (Berg et al., 2002).

Finland

In a study made in Finland (1991), the average pb content of beef (tenderloin) was 10 µg/kg, ground beef 8 µg/kg, cow's liver 37 µg/kg, pork 9 µg/kg, pig's liver 11 µg/kg and chicken 6 µg/kg. The mean cd contents of beef, pork and chicken were lower than the limit of detection of the method employed. The mean cd content of cow's liver was 66 µg/kg (cow) and 36 µg/kg (heifer) and that of pig's liver 21 µg/kg. The contents of lead and cadmium were low in all samples studied in Finland (Tahvonen et al., 1994).

Sweden

In Sweden, the mean lead levels in pig meat, liver and kidney were < 0.005, 0.019 and 0.016 mg/kg, respectively: the mean levels in the corresponding bovine tissues were < 0.005, 0.047 and 0.097 mg/kg. The mean Cadmium levels in pig meat, liver and kidney were 0.001, 0.019 and 0.11 mg/kg, while those in the corresponding bovine tissues were 0.001, 0.070 and 0.39 mg/kg. Since toxic Pb residues were found to be in high levels in offals (the chicken livers), consistent surveillance and monitoring should be employed as its bioaccumulation could lead to serious human health problems among consumers (Jorhem et al., 1990).

Slovenia

Regarding Slovenian cattle and pigs, in a study made between 1989 and 1993, the mean concentrations of lead in bovine meat, liver and kidney were 0.05, 0.10 and 0.14 mg/kg wet weight and those in the corresponding pig tissues were < 0.05, 0.06 and 0.06 mg/kg wet weight. The mean Cadmium concentrations in

bovine meat, liver and kidney were 0.004, 0.094 and 0.373 mg/kg wet weight, respecttively, while those in the corresponding pig tissues were 0.010, 0.088 and 0.393 mg/kg wet weight. Quality assurance was carried out by analysis of certified reference materials and recovery tests.

Croatia

In rural areas from Croatia, trace element (As, Cd, Cu, Hg, Pb) concentrations were determined in the kidney of cattle, sheep, horses and pigs. The highest levels of Cd were found in horses and ranged 0.029–47.4 mg kg^{-1}, respectively. The European Union maximum levels for Cd in kidney were exceeded by 92.3% of horses, 14% of cattle and 16% of sheep. The highest levels of Pb were found in cattle (1.71 mg kg^{-1})

Toxic metals (lead, cadmium) were measured in muscle, liver and kidney samples of bovine and their relationships with heavy metal concentrations in consumed feed were studied. A total of 216 tissue samples from 72 cows and 216 feed samples from 18 farms were collected during four seasons and analyzed for heavy metals by inductively coupled plasma-optical emission spectrometry after wet digestion. The arithmetic mean concentrations (mg/kg wet weight) of lead (Pb), cadmium (Cd) were respectively, 0.221 and 0.028 in muscle, 0.273, and 0.047 in liver, 0.244, and 0.114 in kidney. All measured concentrations (with the exception of Pb in muscle) were below the European Union maximum residual limits (MRL). The Cd contents of the kidney were significantly higher than which observed in other tissues.

In addition, excessive consumption of offals originated from animals raised in Pb contaminated environment should be discouraged. Furthermore, garlic could be advised to antagonize Pb toxicity, as garlic contains chelating compounds capable of enhancing elimination of Pb. Garlic feeding can be exploited to safeguard human consumers by minimizing Pb concentrations in chicken meat which had been grown in a Pb polluted environment (Hanafy et al., 1994).

Certain meat tissues such as muscle, liver and kidney are widely consumed without any further processing, being considered a real delicacy (Doganoc, 1996).

Unfortunately, environmental pollution is increasing, creating many threats for animals and, consecutively, for humans (Murphy, 2002).

The high industrialization rate can be considered responsible for the contamination with many toxic elements such as Lead and Cadmium. They can be moved away by the air and can be deposited in soil, water, and even in the plants that the ruminants feed with (Berg et al., 2002).

According to several studies, the high accumulation of such metals in the liver and kidney was found to be directly related to their function as storage and excretory organ, respectively (Suttie, 2017).

Such unidentified sources of toxic metals could very well be attributed to high accumulation of metals from the environment onto plants, municipal wastes, lead and nickel-cadmium batteries which are non-responsibly discarded, electronic wastes, all of which are dumped and burnt in each area. Considering the intra-level distribution of Pb and Cd in the various tissues studied, liver and kidney tissues of all studied organisms showed the highest amounts of bioaccumulation as opposed to all other tissues. This strongly suggests that consumption of contaminated liver and kidney tissues may present a health risk if included regularly in the diet.

The results show a large variability of lead and cadmium concentrations in some of the meat and meat products groups. However, this variability in biological samples is considered to be normal since the sources of this metal are numerous. Furthermore the lead and cadmium concentrations, also, depend on the environmental conditions and the food production methods. Even within a certain type of food, concentration variations can be large produced by heterogeneities in the distribution of the metal (Walker, 2014). All the samples were taken in different times and seasons. Thus the exposure to different metals was not constant contributing to a larger variability.

The concentrations of the considered metals can be slightly higher in meat product samples than in meat samples. This difference may be explained by the fact that offal (mainly liver and kidney) is often used as an ingredient in meat products and is, also, an important source of lead and cadmium.

Anatomo-pathological changes

High exposure to Pb may affect neurological, renal, reproductive and haematological systems with higher vulnerability in children. Meanwhile, Cd is known to primarily affect kidneys as well as demineralisation of bones.

Therefore, repeated determinations of the levels of lead and cadmium in meat and meat products from different animal species were performed.

The main toxic effect of **Lead** is dysfunction of the nerve system of the fetus and infants. In adults, it produces: blood-side effects; reproductive dysfunction; gastrointestinal damage; nephropathy; central damage as well as the peripheral nervous system and interference in the enzymatic systems that synthesize the group. Besides, it can occur arteriosclerosis, inhibition of growth, damage or inhibits the activity of the immune system depending on the dose.

Lead is known to induce reductions in cognitive development and intellectual performance in children and to increase the number of blood pressure and cardiovascular diseases in adults (Dong-Gyu et al., 2015).

Cadmium may accumulate in the human body and may induce renal dysfunction as well, bone diseases and reproductive system deficiencies. The toxic effect of cadmium occurs in the kidneys, although it has also been associated with pulmonary lesions (including lung tumor induction) and skeletal changes in professionally exposed populations. Cadmium is relatively poorly absorbed in the body, but once absorbed, it is slowly excreted, like other metals, and accumulates in the kidney causing renal lesions. The contaminated kidneys are a major source of cadmium in the diet, although lower levels are found in many other foods (Suttie, 2017).

In addition, we discovered some of the targeted studies that indicate the fact that there have been found higher levels of heavy metals such as lead and cadmium in canned meat products, regarding imports (Walker, 2014).

This can be a real danger to consumers, since canned meat products are easily accessible (very convenient price). There is a clear need to improve quality control in the processing of these canned meat products and also in imposing stricter guidelines by the competent authorities.

CONCLUSIONS

Regarding all of these anatomo-pathological changes, both animal breeders and final consumers of animal and non-animal products should constantly monitor and assess the levels of toxic metals in the environment and food in order to highlight the degree of contamination and its sources. By doing this, people will be able to take measures in controlling these levels in the future.

REFERENCES

Berg T., Licht D., 2002. International legislation on trace elements as contaminants in food: a review. Food Additives and Contaminants, 19, 916–27.

Ciobanu C., Slencu B.G., Cuciureanu R., 2012. Estimation of dietary intake of cadmium and lead through food consumption. Rev. Med. Chir. Soc. Med. Nat., Iasi, 116, 617–23.

Doganoc D.Z., 1996. Distribution of lead, cadmium, and zinc in tissues of hens and chickens from Slovenia. Environmental Contamination Toxicology, 57, 932–7.

Dong-Gyu K., MeeKyung K., Jin Young S., Seong-Wan S., 2015. Cadmium and lead in animal tissue (muscle, liver and kidney), cow milk and dairy products in Korea, Food Additives & Contaminants, Part B, 9(1), 33-37.

Hanafy M.S., Shalaby S.M., el-Fouly M.A., Abd. el-Aziz M.I., Soliman F.A., 1994. Effect of garlic on lead contents in chicken tissues. Dtsch Tierarztl Wochenschr, 101, 157–158.

Jorhem I., Slorach S., Sundström B., Ohlin B., 1990. Lead, cadmium, arsenic and mercury in meat, liver and kidney of Swedish pigs and cattle in 1984–88. Food Additives and Contaminants, 8(2), 201-211.

Murphy S.P., 2002. Dietary Reference Intakes for the U.S. and Canada: Update on Implications for Nutrient Databases. J. Food Comp. Analysis, 15, 411-417.

Seely J.C., Hard G.C., Blankenship B., 2009. Toxicology and Applied Pharmacology, 201-208.

Suttie A., 2017. Boorman pathology of the Rat (Second Edition), Reference and Atlas, Academic Press Publishing House, Cambridge, Massachusetts, 125-166.

Tahvonen R., Kumpulainen J., 1994. Lead and cadmium contents in pork, beef and chicken, and in pig and cow liver in Finland during 1991. Food Addit. Contam., 11, 415–426.

Walker C., 2014. Ecotoxicology: Effects of Pollutants on the Natural Environment. CRC Press Publishing House, Cleveland, Ohio, 128-136.

Scientific Papers. Series D. Animal Science. Vol. LXI, Number 1, 2018
ISSN 2285-5750; ISSN CD-ROM 2285-5769; ISSN Online 2393-2260; ISSN-L 2285-5750

BEE POLLEN AS ANTIOXIDANT INGREDIENT IN READY-TO-SERVE CITRUS JUICE

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Abstract

Bee pollen is a highly nutritious food supplement, which can be easily added to any juice type and enrich its nutritional and sensory quality. The aim of the study was to develop a citrus juice recipe enriched with bee pollen, highly nutritional and with good acceptability of consumers and to compare the polyphenols content and radical scavenging activity of regular citrus juice (RJ) versus enriched citrus juice with bee pollen (JBP). Both products were evaluated for vitamin C content, antioxidant activity (DPPH method), total polyphenols (Folin Ciocalteu method) and sensory evaluation by consumers (N=125) using nine scale hedonic test. The addition of bee pollen and honey to the citrus juice did modify significantly ($p<0.05$) the total polyphenols content and antioxidant activity in JBP compared to RJ. The hedonic score of JBP was 7.29 ± 1.61, slightly lower than RJ (8.04 ± 0.73). More than 70% respondents declared they would buy this drink due to the beneficial effects of bee pollen. Bee pollen is one convenient option of many available on market used to increase the nutritional value of beverages.

Key words*: bee pollen, antioxidant activity, polyphenols, sensory evaluation, hedonic test.*

INTRODUCTION

The number of studies about consumers of bee pollen is scarce. Even more, studies about using bee pollen as ingredient in fruit juices are completely lacking as far as the author knows. Most of the studies published so far on bee pollen are focused either on bee pollen quality and composition (Bogdanov, 2004; Stangaciu, 2015; Salazar-González et al., 2016; Bobiş et al., 2017), either on the biological effects of different classes of compounds (Carpes et al., 2007; Komosinska-Vassev et al., 2015, Urcan et al., 2017). Previous literature data prove that bee pollen has the real potential to be used in nutrition and therapy (Campos et al., 2010; Bogdanov, 2014; Komosinska-Vassev et al., 2015). However, most of the studies were carried out on bee pollen extracts and not on the whole product itself (Campos et al., 2010; Bogdanov, 2014). It is debated that the outer layer of the pollen grain (exine) is heavily digestible, therefore, practitioners of Ayurveda and Traditional Chinese Medicine, recommend to add bee pollen to a slightly acidic drink or food (e.g. yoghurt) prior to ingestion in order to increase its bioavailability (Campos et al., 2010; Bogdanov, 2014; Stangaciu, 2015; Urcan et al., 2017). Recently, proposals on standardization criteria provided valuable information especially for producers and quality control services (Campos et al., 2008; Campos et al., 2010). Even in EU Regulation 1924/2006 bee pollen is viewed as functional food with beneficial effects on health. “Long term ingestion of pollen and special pollen preparations (cracked pollen, pollen extracts) can improve the physical performance and fitness of sportsmen and elderly people”, as well as "pollen intake can improve gut, gastroenterological and liver health" (EU Regulation 1924/2006). This increasing interest of the scientists in bee pollen proves its marvellous market potential (FP7-SME-2008-2). Moreover, this overlaps with the market trend for healthy “greener” food and drinks (Kasriel-Alexander et al., 2016), therefore, the consumers’ attention moved from fast-food to more conscious choices (Byers et al., 2002; Story et al., 2008).

This study aims at evaluating the influence of bee pollen in fruit juices, more specifically citrus fresh juices. Consumers have different sensorial reasons to like citrus juice during any season of the year e.g. sweet-sour or and slightly bitter taste, wonderful colour, cooling

sensation during drinking, while other consumers are attracted to its nutritional qualities - rich source of vitamins and fibers (Drewnowski et al., 2000; Fernández-Vázquez et al., 2011). Still, for consumers who are looking for a more nutritious drink, regular citrus juice is far from perfect.

In this context, the first objective of this work was to develop a citrus juice enriched with bee pollen, highly nutritional and with good acceptability of consumers. Second objective was to evaluate some quality parameters (vitamin C, polyphenols, sensory quality) of the citrus juice enriched with bee pollen.

MATERIALS AND METHODS

Preparation of citrus juices

Citrus fruits (lemons, grapefruits and oranges) were obtained from local market and kept in the refrigerator (+4°C) until further use. The fruits were washed, cut in halves, and squeezing was carried out by using a rotary hand extractor in order to obtain the juice. Fresh bee pollen samples obtained from local beekeepers were kept at -20°C in vacuumed plastic bags until further use.

Two samples of juices were freshly prepared in the days of analysis:

- *regular citrus juice* (RJ) with the following ratio between fruits orange: lemon: grapefruit, 3:1.5:1 (v/v/v);
- *citrus juice with bee pollen* (JBP) with 87% (w/v) of citrus juice (orange: lemon: grapefruit, 3:1.5:1, v/v/v), honey (8%, w/v) and bee pollen (5%, w/v). JBP was left to stand at room temperature for 15 minutes until the bee pollen was completely dissolved in the juice (the bee pollen pellets were no longer visible).

In the end, previous to any analysis, the samples were passed through a finisher with 0.5 mm holes to have a uniform appearance of the product.

The two samples were analysed for vitamin C content, antioxidant activity (DPPH method), total polyphenols (Folin Ciocalteu spectrophotometric method) and sensory evaluation by nine points hedonic test.

Evaluation of vitamin C content

Vitamin C was determined by titration with KIO_3 (0.004N) according to the method previously published by Tofana and Muresan (2011). Final results were calculated using the formula: Vitamin C (mg %) = $[(V \times V_1 \times 0.352)/M \times V_2] \times 100$, where V – the volume of KI used for titration (mL); V_1 – total volume of the extract (mL); V_2 – the volume of the extract used for titration (mL); 0.352 – the titre of KIO_3 solution; M – weight of the sample taken into analysis (g).

Determination of total polyphenols

The content of total polyphenols was estimated according to the Folin Ciocalteu method proposed by Singleton et al., 1999, using gallic acid as reference standard. Briefly, 2.5 mL Folin Ciocalteu (0.2N) reagent was added to juice sample (0.5 mL), and after 5 minutes 2 mL of Na_2CO_3 (75g/L) was added to the solution. The absorbance at 760 nm (UV-VIS 1700 Schimadzu Spectrofotometer, Japan) was then measured against a methanol blank. A standard curve of gallic acid was created using an adequately range of gallic acid solutions from 0.01 to 0.25 mg/mL (R^2=0.986). The results were expressed as Gallic Acid Equivalent (mg GAE g^{-1} dry matter sample).

Radical scavenging activity

DPPH method was used for evaluation of radical scavenging activity (RSA) (Nenadis, 2002). Methanol solution of DPPH (4 mg/100 mL) was freshly prepared in the day of the analysis. Test samples were prepared with 2.95 mL of DPPH methanol solution and 50 µl of juice extract. Methanol (50 µl) plus DPPH solution was used as negative control. Positive control was prepared using vitamin C (50 µl). After rigorous homogenisation at vortex for 10 seconds, the samples were kept in the dark for 30 minutes. Absorbance at 515 nm was read at UV-VIS 1700 Schimadzu Spectrofotometer (Japan). Radical scavenging activity of the test samples were calculated with the formula: RSA (%) = $[(A_{DPPH}-A_{sample})/A_{DPPH}] \times 100$, where A_{DPPH} was the absorbance of DPPH solution and A_{sample} was the absorbance of the sample.

Sensory analysis

Tests were conducted in Laboratory of Sensory Analysis of Foods at UASMV Cluj-Napoca, equipped with table for joint sessions and 20 computers. Samples (50 mL) were served in random order at room temperature (aprox. 20°C), in transparent glasses labelled with three-digit codes. In order to evaluate the product acceptability hedonic test was performed on consumers (N=125). Frequent consumers of citrus juices (voluntary students from 1st and 2nd year of study) were selected based on a questionnaire about their availability and willingness to participate in the test. The test design included a nine point scale hedonic test and the ranking test according to the methods previously published by Stone et al., 2010; Lawless et al., 2012. Each point of the scale had the following meaning: 1–extremely unpleasant, 2–very unpleasant, 3–unpleasant, 4–slightly unpleasant, 5–indifferent, 6–slightly pleasant, 7–pleasant, 8–very pleasant, 9–extremely pleasant (Stone et al., 2010; Lawless et al., 2012). The following sensory characteristics and attributes of the products were evaluated on the hedonic scale: colour, odour, viscosity, taste and aroma, as well as overall appreciation. Neutralisers like water and unsalted crackers were provided *ad libitum* in between samples' tastings.

Statistical analysis

Mean value and standard deviation were calculated for the three replicates of each analysis performed during the study. Hedonic score and absolute frequency of respondents were calculated for each sample on Microsoft Excel 2010.

RESULTS AND DISCUSSIONS

On the path from ancient medical tradition to nowadays trends, honey bees and their products play significant role in the quality of life. Firstly, bees play a crucial role in insect dependent plants pollination and this has a vital impact on providing food for people and feed for animals (Delaplane et al., 2000; James et al., 2008); as a consequence, undeniable economic advantages are derived from pollination. Secondly, bee products were used as medicine or as adjuvant in treatment of different diseases and health conditions since ancient times (Boukraâ, 2013). Therefore, bee pollen is one of the many options available on market to increase the nutritional value of foods, with the advantage of being easy to use, although the knowledge about their use varies greatly depending on the local culture. For example, bee products are usually highly regarded in Eastern Europe (for their therapeutic value, therefore are mainly used as food supplements in time of sickness or during cold seasons (Bogdanov, 2014; Stangaciu, 2015). In contrast, other parts of Europe consider them food or food supplements (honey is being used regularly at breakfast, while bee pollen and propolis are almost unknown or used as food supplements). Traditional Chinese Medicine and Ayurveda (Traditional Indian Medicine – Science of Life) use honey and other bee products in many applications (Stangaciu, 2015). According to World Health Organisation (WHO, 2000), about 60% of the world's population relies on traditional medicine and in some countries it is "extensively incorporated into the public health system". Even more, a close look will reveal that the main principles of traditional medicine (WHO, 2000; Boukraâ, 2013; Stangaciu, 2015) are wisely incorporated into recent consumers' trends. For example, consumers' food trends nowadays are mainly focused on eating greener and locally, avoidance of unhealthy foods, choosing seasonal foods and cutting down food waste (Kasriel-Alexander et al., 2016).

The results of the physico-chemical parameters are presented in Table 1 and discussed in further paragraphs.

Table 1. Physico-chemical parameters of citrus juice samples

Parameter	RJ (Mean ±S.D.)	JBP (Mean ±S.D.)
Vitamic C (mg%)	38.91 ± 2.07	41.53 ± 2.94
Total polyphenols (mg $GAEg^{-1}$)	13.39 ± 1.08	16.97 ± 1.57
RSA (%)	65.24 ± 3.25	81.63 ± 4.94

Vitamin C

According to previous literature data, both citrus fruits and bee pollen are rich sources of vitamins. Fruits are rich in water-soluble vitamins (Murdock, 2002). However, bee

pollen has both fat-soluble and water-soluble vitamins (Campos et al., 2008; Campos et al., 2010; Komosinska-Vassev et al., 2015). In this study, the results of vitamin C evaluation of both citrus juices RJ and JBP was high: RJ presented 38.91±2.07 mg/100 g and 41.53±2.94 mg/100 g was determined in JBP, although no significant difference ($p<0.05$) was recorded between the samples.

Total polyphenols

Polyphenols are valuable compounds with many biological activities, like antioxidant, antimicrobial, anti-inflammatory and are abundant in fruits, tea, wine, coffee (El Gharras, 2009). Regardless the botanical or geographical origin, bee pollen is a rich source of polyphenols - flavonoids and phenolic acids (Drewnowski et al., 2000; Fernández-Vázquez et al., 2011; Bogdanov, 2014; Campos et al., 2008; Komosinska-Vassev et al., 2015).

The addition of bee pollen and honey to the citrus juice did modify significantly ($p<0.05$) the total polyphenols content (16.97 mg gallic acid/g in JBP and 13.39 mg gallic acid/g in RJ). Due to the high content in polyphenols, fruits are generally highly regarded as functional foods which contribute to prevention of many disease conditions.

Radical scavenging activity

DPPH method has many advantages for laboratory work. The method is fast and easy to apply, and although DPPH is a free radical, it is stable at room temperature (Nenadis, 2002). The radical produces a violet solution in methanol or ethanol, but in the presence of an antioxidant molecule it gives rise to an uncoloured solution.

The radical scavenging activity of JBP evaluated by DPPH was significantly ($p<0.05$) higher than RJ (81.63 ± 4.94 %). Considering that there was no significant increase in the level of vitamin C or polyphenols, it means that other compounds from bee pollen may present radical scavenging activity also (other vitamins, minerals or even proteins).

Sensory analysis

Results of sensory evaluation are presented in Table 2. There is a significant statistical difference between the two samples and the sensory attributes ($p<0.01$).

The general hedonic score of JBP was 7.29 ± 1.61 on the nine point hedonic scale, which means that the juice was highly appreciated by consumers, although slightly lower than RJ which scored 8.04 ± 0.73.

Addition of bee pollen in the juice matrix changed some sensory attributes of the product. Evaluators reported in JPB sample a orange-yellow colour, “dusty feeling on the tongue” (marked as unpleasant by some of them), decreased sweetness and sourness. In spite of textural and taste changes of the juice, more than 70% respondents declared they would buy this beverage due to the beneficial effects of bee pollen. These results stress out the importance of consumers’ education about the benefits of bee pollen.

Table 2. Sensory evaluation of citrus juices samples

Sensory attributes	RJ (Mean ±S.D.)	JBP (Mean ±S.D.)
Colour	7.97 ± 1.08	7.22 ± 1.21
Odour	7.83 ± 0.52	6.94 ± 2.03
Viscosity	7.76 ± 1.47	6.85 ± 1.92
Taste and aroma	8.58 ± 1.31	8.15 ± 0.44

Even more interesting is that only 20% of them were familiar with bee pollen taste and were regular users. Sample JBP was ranked first and these results confirm that the trend for healthy and natural foods/ingredients/food supplements goes beyond employed people.

CONCLUSIONS

Consumer trends move toward more natural food products and drinks. Usually, consumers view bee products as natural and healthy products, locally and seasonally produced and although there is a slight difference between Western and Eastern European consumers’ perception of bee products, there still are some similarities: they both acknowledge the healthy potential of bee products.

Addition of honey and bee pollen to fruit beverages positively contributes not only to sensory characteristics of the final product, but also to its functional quality. To the knowledge of the author, this is the first article which uses bee pollen as ingredient in ready-to-use citrus juice. This product could be included in a

restaurant menu and provide a new, healthy option to the consumers. According to National Honey Board (U.S.A.) honey is included more and more in the restaurants' and bars' menu. While honey provides the sweet taste and specific flavour, bee pollen adds proteins, minerals and interesting texture to the new food item. It is the job of the chefs to find new creative and attractive ways to integrate these valuable products in the menu.

The findings underscore the importance of consumer education in learning to appreciate the value of bee products in daily use, not only in time of sickness. For instance, in Romania honey consumption is about 0.4-0.6 kg/year/person, among the lowest in Europe, and this is solely due to cultural heritage – honey is not seen by most of Romanian as a regular food product, but mostly as a healthy ingredient (www.romtradeinvest.ro).

As bee population is fast decreasing in the last years, it seems that consumers' understanding is increasing about bees' role and importance of bee products in maintaining a healthy life. The use of bee products in preparing various foods will help also the beekeepers to have more success in their business and will also encourage the farmers to cultivate melliferous plants, thus providing means to maintain bee populations alive. More than that, in order to protect the consumers' safety and beekeeping market, even European Parliament adopts regulations about health and nutritional claims of bee pollen (EC 1924/2006). Other vital reasons and equally important to increase the consumption of honey and bee pollen is to develop consumers awareness about improving the quality of life with bee products.

REFERENCES

Bobis O., Bonta V., Urcan A., Paşca C., Burian V., Dezmirean D., 2017. Chemical composition and microbiological parameters of commercially bee pollen. Xth Congress of Romanian Society of Apitherapy.

Bogdanov S., 2004. Quality and standards of pollen and beeswax. Apiacta, 38, 334-341.

Bogdanov S., 2014. Pollen: Production, Nutrition and Health: A Review, Bee Product Science, www.bee-hexagon.net (available online 15 feb. 2014).

Boukraâ L., 2013. Honey in traditional and modern medicine. CRC Press.

Byers T., Nestle M., McTiernan A., Doyle C., Currie-Williams A., Gansler T., Thun M., 2002. American Cancer Society guidelines on nutrition and physical activity for cancer prevention: reducing the risk of cancer with healthy food choices and physical activity. CA: A Cancer Journal for Clinicians, 52(2), 92-119.

Campos M.G., Bogdanov S., de Almeida-Muradian L.B., Szczesna T., Mancebo Y., Frigerio C., Ferreira F., 2008. Pollen composition and standardisation of analytical methods. Journal of Apicultural Research, 47(2), 154-161.

Campos M.G.R., Frigerio C., Lopes J., Bogdanov S., 2010. What is the future of Bee-Pollen. Journal of ApiProduct and ApiMedical Science, 2(4), 131-144.

Carpes S.T., Begnini R., Alencar S.M.D., Masson M.L., 2007. Study of preparations of bee pollen extracts, antioxidant and antibacterial activity. Ciência e Agrotecnologia, 31(6), 1818-1825.

Centrul Român pentru Promovarea Comerţului şi Investiţiilor Străine, 2012. Potentialul de export al României – miere. www.romtradeinvest.ro (available online 15 feb. 2014).

Delaplane K.S., Mayer D.R., Mayer D.F., 2000. Crop pollination by bees. Cabi.

Drewnowski A., Gomez-Carneros C., 2000. Bitter taste, phytonutrients, and the consumer: a review. The American Journal of Clinical Nutrition, 72(6), 1424-1435.

El Gharras H., 2009. Polyphenols: food sources, properties and applications–a review. International journal of food science & technology, 44(12), 2512-2518.

European Parliament and Council, 2007. Regulation (EC) No 1924/2006 of The European Parliament and of the Council of 20 December 2006 on Nutrition and Health Claims Made on Foods. Official Journal of the European Union L 404: L 12-3-L 12/17.

Fernández-Vázquez R.O.C.Í.O., Stinco C.M., Meléndez-Martínez A.J., Heredia F.J., Vicario I.M., 2011. Visual and instrumental evaluation of orange juice color: a consumers' preference study. Journal of Sensory Studies, 26(6), 436-444.

FP7-SME-2008-2, APIFRESH, project ID 243594, Developing European standards for bee pollen and royal jelly: quality, safety and authenticity.

James R.R., Pitts-Singer T.L. (Eds.), 2008. Bee pollination in agricultural ecosystems. Oxford University Press on Demand.

Kasriel-Alexander D., 2016. Top 10 global consumer trends for 2016. Euromonitor International, 18-21.

Komosinska-Vassev K., Olczyk P., Kaźmierczak J., Mencner L., Olczyk K., 2015. Bee pollen: chemical composition and therapeutic application. Evidence-Based Complementary and Alternative Medicine, http://dx.doi.org/10.1155/2015/297425.

Lawless H.T., Heymann H., 2010. Sensory evaluation of food: principles and practices. Springer Science and Business Media, 325-344.

Mărghitaş L.A., Stanciu O.G., Dezmirean D.S., Bobiş O., Popescu O., Bogdanov S., Campos M.G., 2009. In vitro antioxidant capacity of honeybee-collected pollen of selected floral origin harvested from Romania. Food Chemistry, 115(3), 878-883.

Murdock D.H., 2002. Encyclopedia of foods: a guide to healthy nutrition. Prepared by Medical and Nutrition

experts from Mayo Clinic, Univ. of California, LA, and Dole Food Co.
National Honey Board, www.honey.com, Trends, (available online 12 March 2018).
Nenadis N., Tsimidou M., 2002. Observations on the estimation of scavenging activity of phenolic compounds using rapid 1, 1-diphenyl-2-picrylhydrazyl (DPPH•) tests. Journal of the American Oil Chemists' Society, 79(12), 1191-1195.
Salazar-González C., Díaz-Moreno C., 2016. The nutritional and bioactive aptitude of bee pollen for a solid-state fermentation process. Journal of Apicultural Research, 55(2), 161-175.
Stangaciu S., 2015. Beekeepers, honey consumption telomers and longevity, VIIIth Congress of Romanian Society of Apitherapy.
Stone H., Bleibaum R., Thomas H. A., 2012. Sensory evaluation practices. Academic press, 291-326.
Story M., Kaphingst K.M., Robinson-O'Brien R., Glanz K., 2008. Creating healthy food and eating environments: policy and environmental approaches. Annu. Rev. Public Health, 29, 253-272.
Tofană M., Mureşan Crina, 2011. Controlul calităţii materiilor prime de origine vegetală - Caiet de lucrări practice, Ed. AcademicPres, Cluj-Napoca.
Urcan A., Bobis O., Bonta V., Moise A., Mărgăoan R., Dezmirean D., 2017. Pharmacodynamics and bee pollen digestion. Laboratory studies. Xth Congress of Romanian Society of Apitherapy.
World Health Organization, 2000. General guidelines for methodologies on research and evaluation of traditional medicine.

Scientific Papers. Series D. Animal Science. Vol. LXI, Number 1, 2018
ISSN 2285-5750; ISSN CD-ROM 2285-5769; ISSN Online 2393-2260; ISSN-L 2285-5750

THE EVOLUTION OF MINIMUM ARABLE LAND AND FOOD CONSUMPTION FROM 1961 TO 2013

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Abstract

Food security continues to be a vital subject for every country, while the main mean of supply remains agriculture. In order to evaluate the situation and to discover the tendencies, we track the main production and distribution parameters; arable land, productivity, population and food consumption from 1961 to 2013, and we explore the minimum arable land per person, a way of estimating the necessary area of productive land according to consumption levels, while also taking note of relevant dietary shifts regarding the sources of calories and proteins. Our results show a trend of significant decrease of arable land per person, but also a similar decrease of minimum arable land per person. At the dietary level, we also found increases in consumption, along with a shift towards animal based energy in developing countries, while some developed countries plateaued. In line with the field's literature, our results describe an increased consumption based on ecologically unsustainable large yield increases.

Key words*: arable land, food security, sustainability, food consumption, food footprint.*

INTRODUCTION

The 20th century has been witness to one of the best investments in international research people have made, in the form of the Green Revolution. This continuous revolution has increased food security by raising productivity and lowering prices (Evenson, 2003), making better use of arable land. Land is one of the most relevant limited resource in human history, and we can treat it as a constant to which a variable population is connected. Arable land per capita is a function that allows us to see the total amount of arable land at the disposal of a population. Minimum arable land is a more abstract function that shows us the size of arable land currently used by that population or land that will be used by a population in the future, an arable land footprint. To have food security, minimum arable land per capita must stay well below arable land per capita, and this has happened over the last half-century thanks to big increases in yields. The sustainability of productivity remains a challenge (Ray et al., 2013), but not necessarily an unexpected hurdle, as this is not the first time when a leap in efficiency in the production sector is met with Jevon's paradox – a counter-intuitive theory which predicts that gains in efficiency of use of a resource become consumed by increased demand of that resource, instead of easing the pressure on that resource (Jevons, 1865; Alcott, 2005; Sorrell, 2009). In this paper we expand on the ideas of Puia and Soran, "*Agroecosystems and human food supply*" (translation), 1981, on agricultural productivity, supply, and relative differences between dietary patterns in terms of impact on land use. These authors have observed, as others continue to do today, that the most efficient diet tends to be plant based, and adding animal components increases inefficiency and minimum arable land and decreases ecological and economical sustainability (Springmann et al., 2016; Peters et al., 2016; Shepon et al.; 2018).

MATERIALS AND METHODS

In order to explore consumption patterns, land and population we extended the methodology of Puia and Soran to cover not just a snapshots of the situation, but a timeline based on data provided by FAOSTAT ranging from 1961 to 2013. Population numbers were also provided by FAOSTAT. We used PostgresSQL and open-source software tools to manage the tables and queries. The first round of results centralized all the data into one table, including

all the parameters we explored: per capita calories, protein, plant based calories and protein, animal based calories and protein, arable land and yield. Yield is an important variable here and in the previously mentioned book the authors used a fixed value for all calculations. In our formula, we used yield relative to the actual region for increased accuracy.

$$A_m = \frac{(E_P + m * E_A) * 365}{Y * E_c}$$

A_m – minimum arable land;
Y – yield of the crop, average for cereals in this case;
E – calories: from plants, from animals, from crops; for cereals, the average value was 367.9 kcal/kg;
m – multiplier to control for inefficiency of production for animals. In this case m = 7, as suggested by Puia and Soran, to control for inefficiency of production of animals. In future studies, this multiplier could be more dynamic and fine-tuned.

These results were used for the World's situation in which the year 1961 was also added to encompass more data. For other countries and regions, further results were extracted by averaging a new set of values for each of the 5 decades inside the time line, providing a more condensed.

RESULTS AND DISCUSSIONS

The World's average situation, from 1961 to 2013, has changed consistently in a linear trend on all investigated parameters. Arable land per capita has decreased from 452 m^2 to 225 m^2, a change of -227 m^2 or -50.30%. Minimum arable land per capita has also decreased from 309 m^2 to 154 m^2, a change of -155 m^2 or -50.11%. This minimum land is dependent on the cereal yield which has grown from 1353.2 kg/ha to 3832.1 kg/ha, an increase of 2478.9 kg/ha or 183.19% (Figure 2).
In terms of energy supply, total kcal/cap/day has grown from 2196 kcal to 2884 kcal, a 688 kcal difference, 31.33% (Figure 3). Primary energy supply, measured in kcal/cap/day, has increased from 4424 kcal to 5968 kcal, an increase of 1744 kcal or 41.29%. Plant based energy supply has grown from 1858 kcal/cap/day to 2370 kcal/cap/day, an increase of 512 kcal or 27.56%. Animal based energy supply has grown from 338 kcal/cap/day to 514 kcal/cap/day, a difference of 176 kcal or 52.07%.

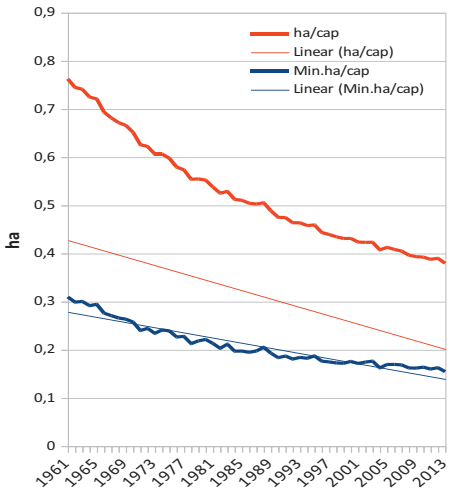

Figure 1. World average arable land per person and minimum arable land per person

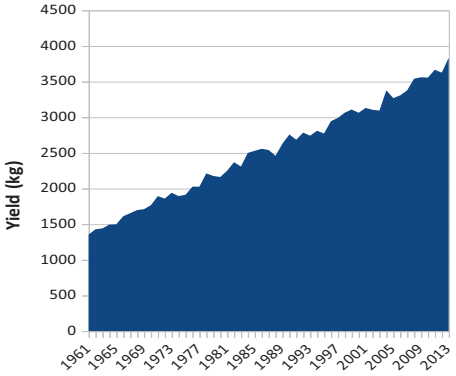

Figure 2. World average yield for cereals

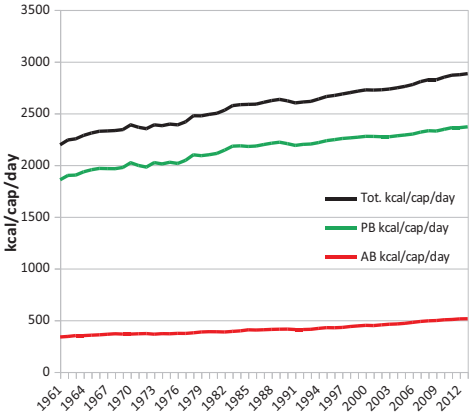

Figure 3. World average energy supply: total (black), plant based (green), animal based (red)

Protein intake has also increased, total protein in g/cap/day growing from 61.45 g to 81.23 g, an increase of 19.77 g or 32.17% (Figure 4). Plant based protein intake, in g/cap/day, has increased from 41.79 g to 49.1 g, a change of 7.3 g or 17.46%. Animal based protein intake, in g/cap/day, has grown from 19.66 g to 32.13 g, a difference of 12.47 g or 63.43%.

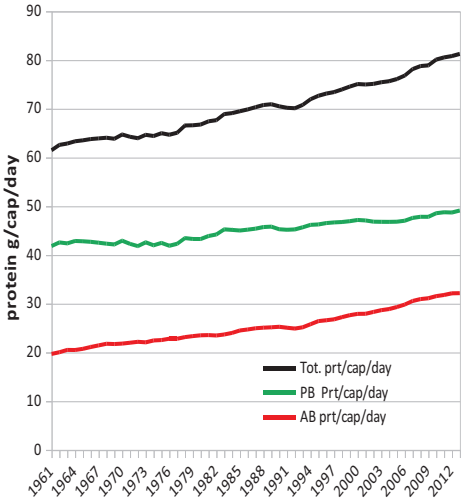

Figure 4. World average protein supply: total (black), plant based (green), animal based (red)

Linear regression (Figure 1) of the evolution of the minimum arable land per person parameter points to 2057 as the intercept with the year axis, with $R^2 = 0.90$ and Standard Error = 4.84. For the linear regression of the arable land per person parameter, the year axis intercept is also 2057, with $R^2 = 0.97$ and SE = 2.75.

The world average situation can be described as a relentless increase in overall consumption due to population growth, but also due to individual consumption increases in intensity, with rising levels across both calories and proteins. This total increase is more resource intensive due to the faster growth of consumption of animal based calories and protein, compared to the plant based increase, yet plant based nutrients still dominate the average diet. All these aspects of growth are inversely tied to arable land per person, a resource that is usually very limited and fixed. Arable land can grow, but it does so usually at the expense of grasslands, forests and wetlands, areas which are important parts of the biosphere. Arable land per person has been halved over the course of a half-century. Minimum arable land per capita also evolves, as the minimum changes to match the new total, it should increase, but, instead, it has decreased similarly to arable land per capita: by half in a half-century. This decrease has been supported by a much faster growth in yield: an almost tripling of yield for cereals, an average rate of 47 kg more per year, the negative consequences of which are not discussed in this paper, but are important to ecological sustainability and climate change (Peters, Christian J. et al., 2016; Eshel, Gidon et al., 2017). Both arable land and minimum arable land are decreasing towards 0 (ha), converging concurrently around the year 2057. While it is difficult to simulate what agricultural systems will look like then, we can notice that, as the two parameters come closer to each other and to 0, there's an increase in vulnerability to anything that might shrink the harvest, as such changes may become points of intersection in the trend lines. Yield is the most malleable factor in this equation and yield increases need to be at least 2.4% per year in order to keep up with the projected demand by 2050, but recent gains are only between 0.9% and 1.6% (Ray et al., 2013).

The following results are shown in charts. Each region shows the decade average for the period of 1962-2013. Decades are ordered new to old, while regions are ordered by the value of the most recent decade (V) descending from the biggest values to smallest values.

We can see (Figures 5, 6) how arable land per capita has decreased in most places; sharp decreases for top regions and dwindling changes for the lowest regions where the pressure was already more intense.

Concerning minimum arable land per capita (Figures 7, 8), the overall trend of decreasing values is clear, but, unlike the arable land progression, there are outliers who have had some increases along the way.

Important factors to look at in such cases would be changes in technology and related infrastructure, changes in demand for animal based foods, and more dramatic events such as conflicts that hamper, discourage, or block agricultural production.

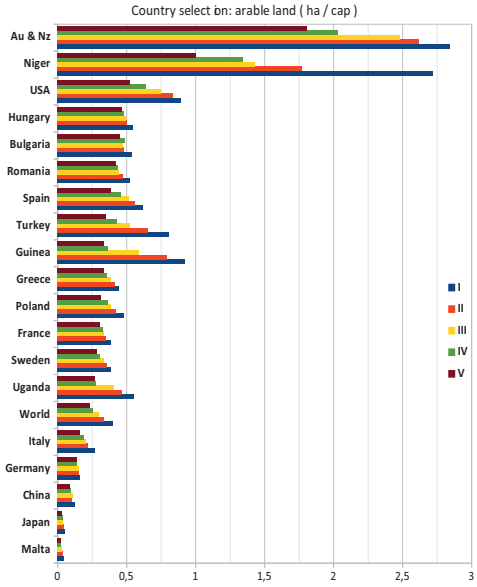

Figure 5. Change in arable land over 5 decades for a set of countries

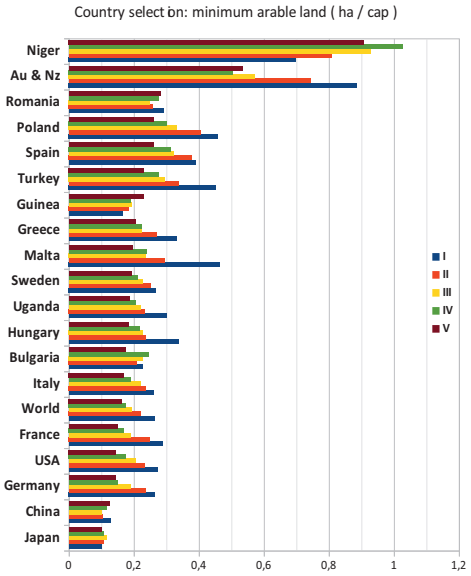

Figure 7. Change in minimum arable land over 5 decades for a set of countries

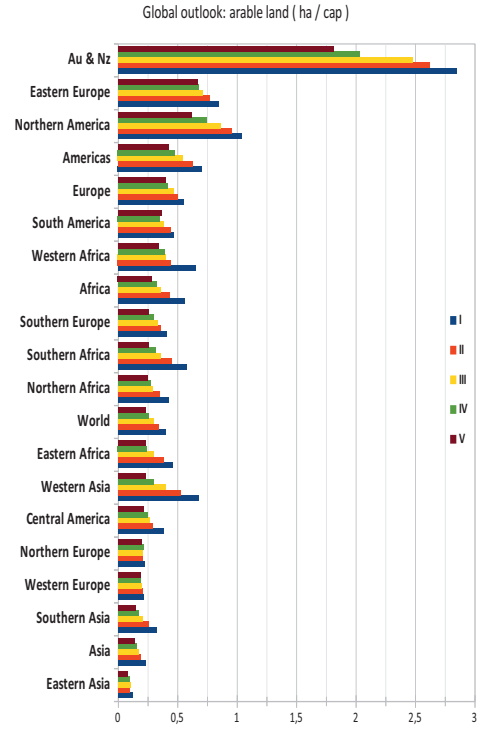

Figure 6. Change in arable land over 5 decades for global regions

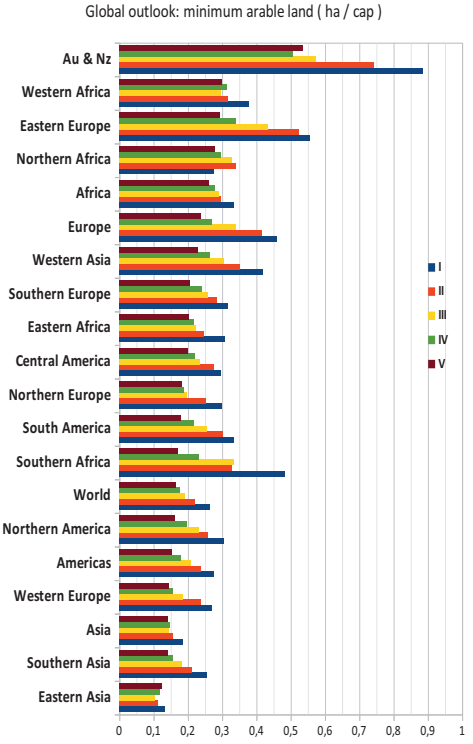

Figure 8. Change in minimum arable land over 5 decades for global regions

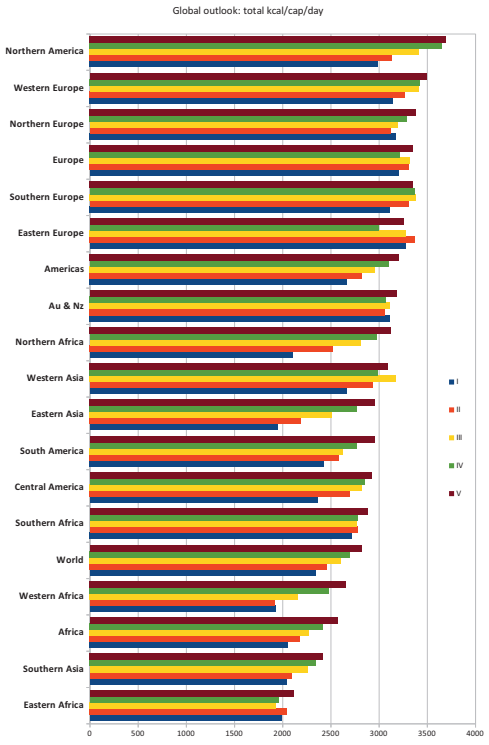

Figure 9. Change in total kcal/cap/day over 5 decades for global regions

For total calories per person, the regional changes around the world continue to support the trend of growth, even among developed regions. Not all regions have constant growth and we can see even a decrease in Southern Europe.

These averages are limited in their power to describe the level of adequate or inadequate nutrition around world, since recommended caloric intake is not a "one size fits all" parameter (FAO/WHO/UNU, 2004).

Next, we calculated the range from the starting decade to the ending decade based on their averages, illustrating the change in primary kcal/cap/day across the 5 decades. The chart (Figure 9) includes all regions and selected countries together.

The trend (Figure 10) shows an increase in the consumption of primary calories across the World, demonstrating that even if total caloric gains decelerate or plateau, there can still be increases in primary calories due to dietary shifts. However, there can be decreases too, as seen in Northern Europe, Poland, Australia and New Zealand.

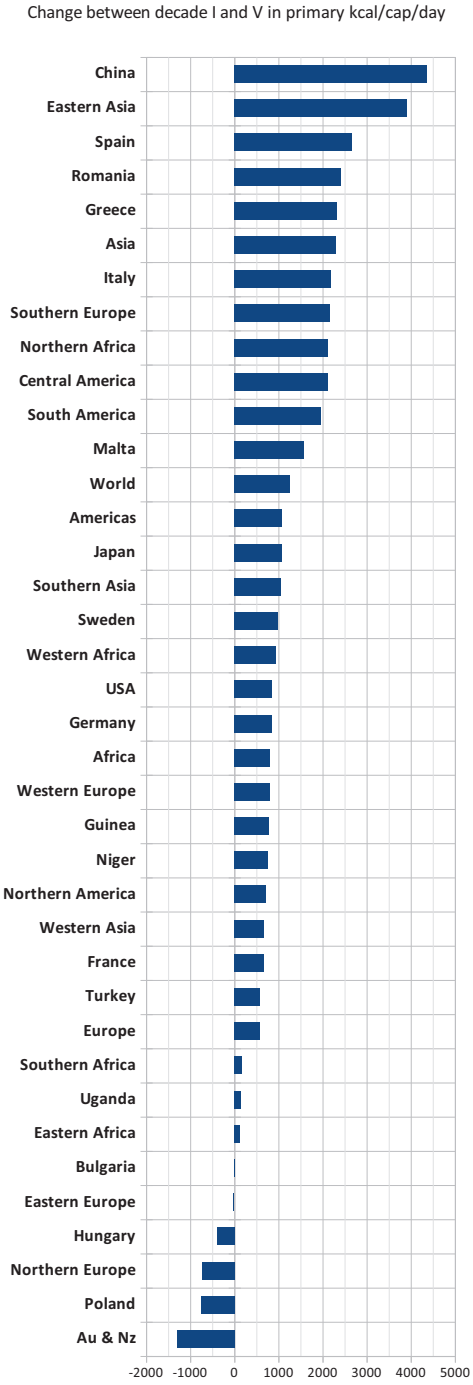

Figure 10. Change in primary kcal/cap/day from the average of decade I to the average of decade V

We also measured, from decade I to V, the shift in dietary energy supply from plant based calories to animal based calories.
The following charts (Figures 11, 12) show the change that happened with plant based calories as a percentage of the total, negative meaning a decrease in plant based and an increase animal based calories.

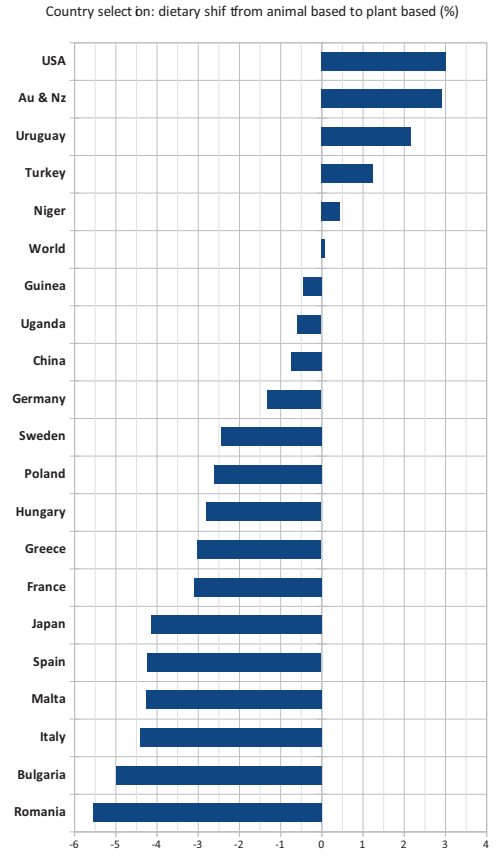

Figure 11. Changes in plant based caloric supply between the 1st decade average and the 5th decade average for a selection of countries

We can now see that a significant number of developed and developing regions (Figures 9, 10, 11, and 12) have reduced their total caloric intake and/or have replaced animal based calories with plant based calories.
We can also see that developing countries are getting closer to developed-country levels by increasing animal based calories.
The global average leans slightly towards more plant based calories and the positive trend is probably based on economic forces that promote the cheaper and more efficient plant-based ingredients, rather than on ethical forces such as environmental awareness or vegetarian movements. In some situations, this shift could be related to economic distress that makes animal products more unaffordable.

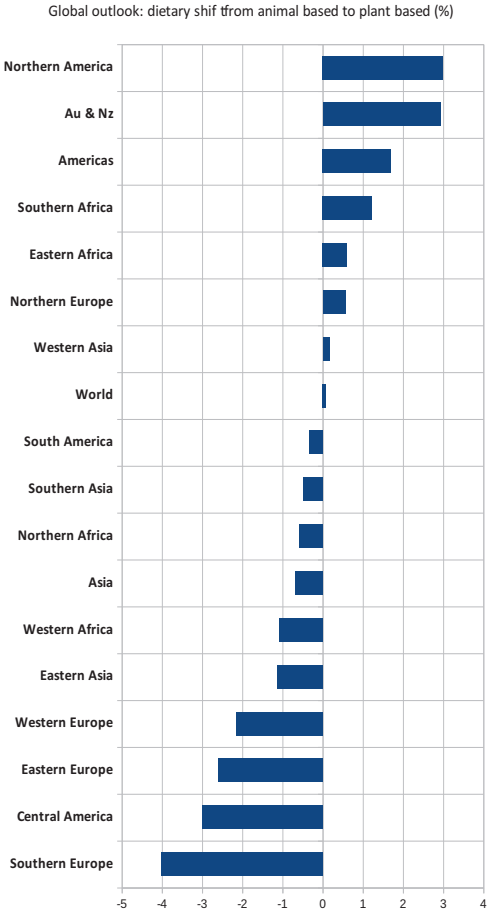

Figure 12. Changes in plant based caloric supply between the 1st decade average and the 5th decade average for global regions

CONCLUSIONS

Arable land per person has decreased continuously and consistently in the period between 1961 and 2013; someone in 1961 would have had assigned a unit of arable land twice the size of someone in 2013.
Minimum arable land has also decreased at a similar pace; a unit of arable land used by a

single person in 1961 can support two persons with greater consumption in 2013.

During this period, consumption has increased, reducing nutritional deficiencies all over World. Consumption has also become more resource intensive, with developing countries adopting a more Western diet that is rich in animal-based foods, while some developed countries have shifted slightly in the opposite direction.

The general improvement in consumption and minimum arable land that has happened is directly dependent on large gains in yield, a development that is already failing to keep up the necessary increases to match future needs 3 decades from now and is leading to a future of less food security by the middle of the 21st century.

If yield cannot keep up, other factors must change: increase available arable land, but this comes at further cost to sustainability, biodiversity, carbon sinks; decrease minimum arable land by shifting to more plant based diets.

REFERENCES

Alcott B., 2005. Jevons' paradox. Ecological Economics 54, 9–21. doi:10.1016/j.ecolecon.2005.03.020.

Erb K.-H., Lauk C., Kastner T., Mayer A., Theurl M.C., Haberl H., 2016. Exploring the biophysical option space for feeding the world without deforestation. Nature Communications 7, 11382. doi:10.1038/ncomms11382.

Eshel G., Shepon A., Shaket T., Cotler B.D., Gilutz S., Giddings D., Raymo M.E., Milo R., 2017. A model for 'sustainable' US beef production. Nature Ecology & Evolution 2, 81–85. doi:10.1038/s41559-017-0390-5.

Evenson R.E., 2003. Assessing the Impact of the Green Revolution, 1960 to 2000. Science 300, 758–762. doi:10.1126/science.1078710.

FAOSTAT. Retrieved 03/2018 from http://www.fao.org/faostat/en/.

Human energy requirements: report of a Joint FAO/WHO/UNU Expert Consultation, 2004. . Food and Agricultural Organization of the United Nations.

Jevons W.S., 1865. The coal question: an inquiry concerning the progress of the nation, and the probable exhaustion of the coal-mines. Macmillan.

Peters C.J., Picardy J., Darrouzet-Nardi A.F., Wilkins J.L., Griffin T.S., Fick G.W., 2016. Carrying capacity of U.S. agricultural land: Ten diet scenarios. Elementa: Science of the Anthropocene 4, 000116. doi:10.12952/journal.elementa.000116.

Puia I., Soran V., 1981. Agroecosistemele şi alimentaţia omenirii. Ed.Ceres, Bucuresti.

Ray D.K., Mueller N.D., West P.C., Foley J.A., 2013. Yield Trends Are Insufficient to Double Global Crop Production by 2050. PLoS ONE 8. doi:10.1371/journal.pone.0066428.

Roberts E.H., Jones J.G., Duckham A.N., 1976. Food production and consumption the efficiency of human food chains and nutrient cycles. American Elsevier.

Shepon A., Eshel G., Noor E., Milo R., 2018. The opportunity cost of animal based diets exceeds all food losses. Proceedings of the National Academy of Sciences 115, 3804–3809. doi:10.1073/pnas.1713820115.

Sorrell S., 2009. Jevons' Paradox revisited: The evidence for backfire from improved energy efficiency. Energy Policy 37, 1456–1469. doi:10.1016/j.enpol.2008.12.003.

Springmann M., Godfray H.C.J., Rayner M., Scarborough P., 2016. Analysis and valuation of the health and climate change cobenefits of dietary change. Proceedings of the National Academy of Sciences 113, 4146–4151. doi:10.1073/pnas.1523119113.

Scientific Papers. Series D. Animal Science. Vol. LXI, Number 1, 2018
ISSN 2285-5750; ISSN CD-ROM 2285-5769; ISSN Online 2393-2260; ISSN-L 2285-5750

THE ORGANOLEPTIC EXAM OF COOKED HIGH PRESSURE PROCESSED AND COOKED UNTREATED FISH FILLETS

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Abstract

Advanced food processing technologies provide us with the possibility to keep food fresh and safe for as long as possible as an alternative to refrigeration storage. One process in particular, the high pressure processing (HPP), can represent an interesting option for restaurant owners or processors of smoked fish. By using this technology, fish fillets can be kept fresh and protected from any microbial alteration that could endanger the consumer even in the case of not preparing them in the next hours after delivery. In this study, we included rainbow trout fillets as samples. The first type of samples were previously pressurized at a pressure level of 400 MPa/6 min. The second type of samples was represented by controls (untreated samples). Both of them were introduced in the sensorial exam (triangle test method) after a cooking process. The baked high pressure processed and control samples were compared with respect to texture, taste, odor and appearance. The final results revealed that the majority of the panelists found the untreated sample softer. Regarding flavour and appearance, the HPP samples and the controls were perceived as mainly similar.

Key words*: high pressure processing, organoleptic exam, trout fillets.*

INTRODUCTION

The rainbow trout (*Oncorhynchus mykiss*) is a species of salmonid which became very appreciated over the last years due to its dietary quality.

Its value as hard-fighting game fish and his remarkable benefits for human consumption make from this low-fat, sweet water fish an interesting option for fishermen to consumers. Some of the most important characteristics of rainbow trout include: excellent organoleptic properties, tender flesh, high levels of vitamins, antioxidants and omega-3 fatty acids, which convert it into an important source for a healthy food diet and respectively, a safer way of living.

The commercial value of this product has seriously grown in the late 19th century worldwide. Even though wild fish is known to have a stronger taste, being promoted as superior to farmed fish, the increased demand in the international markets, has influenced the soar of aquaculture.

See food and fish in general, beside their highly nutritional value, are very perishable (Mengden et al., 2015), having a very short shelf-life. Because fish muscles are quickly spoiled by different strains of bacteria, it can easily lead to potential microbiological risks for the consumers. For this reason, promising preservation methods that could extend shelf-life and reduce the microbial load are tested in current days (Rode et al., 2016). High pressure processing (HPP) is considered an advanced food technology which offers great advantages in this way. Even though the first attempts to use HPP date since the 19th century, the method itself became popular and beneficial in food industry in 1980 (Yagiz et al., 2007). HPP can reduce the microbial load, having a minimum effect on the flavour, nutrients, vitamins and other nutritional values of foods (Erkan et al., 2010). With this type of

processing, a better shelf-life can be obtained, but most important, the microbial risks for humans is significantly reduced (Aubourg et al., 2013). Being a non-thermal technology it is capable of inactivating spoilage and pathogenic microorganisms (Alves de Oliveira et al., 2017), modifying enzymatic activity, but preserving the freshness and the nourishing factors from foods.

The aim of this study was to evaluate the effects of an HPP pre-treatment on the sensorial quality of cooked rainbow trout fillets in comparison with untreated cooked fillets, while including the advantage of shelf-life extension. The objectives pursue comparisons regarding final product texture, flavour (odor and taste) and appearance.

MATERIALS AND METHODS

FISH SAMPLES

Fresh skinless rainbow trout fillets (weight, 60±20 g), were obtained from an aquaculture farming system (Osnabrück, Germany) and immediately transported on ice to the German Institute of Food Technologies (DIL) (Quakenbrück, Germany). The fillets were portioned in half. Each aliquot of the fillet (weight, 24,05±6,05 g) was placed in a clean, dry polyethylene bag purchased from Schulte&Co (Lohne, Germany), vacuum-sealed using Multivac Typ C200 (Wolfertschwenden, Germany) and HPP processed in 3-5 h after slaughtering and filleting.

HPP TREATMENT

Pressure treatment was carried out in a 55 L capacity high-pressure vessel HIPERBARIC (Burgos, Spain). The stainless steel vessel had 200 mm internal diameter. The instrument had a 22 m^2 surface requirement and an automatic loading/unloading system. The transmitting medium used was water. The maximum temperature of the samples during pressure treatment was 6°C. The first type of samples used in the study were placed in a cylindrical loading container and pressurized at 400 MPa at a rate of 150 MPa/min. The holding time was 6 min. The holding time refers to the time that the product was subjected to a given pressure (it does not include come up time and release time). The second type of samples used in the experiment were control samples which were not subjected to any kind of pressure treatment (untreated sample). All samples were stored in a cooling room with controlled temperature conditions of 4.0 ± 0.1°C until the sensorial exam was performed.

THE TRIANGLE TEST WITH THERMALLY PROCESSED HPP TREATED AND CONTROL SAMPLES

The triangle test is one of the commonest tests used in sensory evaluation. This type of sensory method for quality control requires trained assesors (Kilcast David, 2010). The test is useful whenever a test sample has to be compared with a control or reference sample that does not or should not change.

In accordance with the objective of our study, a number of 30 trained panelists participated in the test (ISO 4120, 2004). All samples were baked in aluminium foil at 170°C for 10 min and each panelist was presented with 3 coded samples, 2 high-pressure processed samples at a pressure level of 400 MPa/6 min and one control sample (0 MPa). The high-pressure processed sample (400 MPa/6 min) was chosen in account of the good microbiological inactivation and superior physicochemical aspect. The high-pressure processed samples were labelled as sample number 273, respectively 856 and the control sample with 138 (Table 2). The panelists are asked to identify the odd sample. Additionally, the panelists were asked to describe the difference referring to texture, taste, odor, appearance or others. If the panelists were unsure of their decision, they could write down their best guess and specify in the 'Remarks' section that their choice was based on an assumption (ISO 4120, 2004). For the assessment of results the correct answers given were counted and compared to the values of the significance level (P) (ISO 4120, 2004; Meilgaard et al., 1991) (Table 1).

Table 1. The number of assessors in a triangle test required to give correct judgments, at three different significance levels

Number of assessors	Significance level		
	5%	1%	0,1%
7	5	6	7
8	6	7	8
9	6	7	8
10	7	8	9
11	7	8	10
12	8	9	10
13	8	9	11
14	9	10	11
15	9	10	12
16	9	11	12
17	10	11	13
18	10	12	13
19	11	12	14
20	11	13	14
21	12	13	15
22	12	14	15
23	12	14	16
24	13	15	16
25	12	15	17
26	14	15	17
27	14	16	18
28	15	16	18
29	15	17	19
30	15	17	19

Sources: ISO 4120, 2004; Meilgaard et al., 1991

RESULTS AND DISCUSSIONS

The results revealed that 20 out of 30 panelists who participated at the sensorial exam identified correctly the odd sample (untreated and baked fillet) coded with the number 138 (Table 2). The answers were statistically significant ($P \geq 0.001$) expressing a level of confidence equal to 99.9%. A graphical description of the cooked untreated sample, based on the answers given by the panelists who identified the odd sample (cooked untreated sample), is shown in Figure 1. A number of 27 panelists observed differences in texture. The majority of the answers described the untreated cooked sample as softer than the high-pressure processed samples. For this quality indicator, the answers were statistically significant ($P \geq 0.05$). After HPP, the texture of fish meat is known to change towards firmness (Chouhan et al., 2015; Gómez-Estaca et al., 2007) and therefore, this property might persist even after thermal processing. The answers describing the appearance of the cooked untreated sample vs the appearance of the cooked high-pressure processed samples were not statistically significant ($P>0.05$). HPP fish meat usually becomes more opaque because the lightness increases (Chouhan et al., 2015; Gómez-Estaca et al., 2007; Gudbjornsdottir et al., 2010) (Figure 2), but in this case, it appears that after cooking there is no significant difference between untreated and high-pressure processed fish meat. The differences in flavour consisting in taste and odor were minimal. Twelve panelists noticed a difference in smell, from which 8 considered the untreated sample to have less intensive odor than the other two samples (Figure 1). The answers describing a less intensive odor andless intensive taste for the cooked untreated sample were both situated at the limit of the statistical significance ($P = 0.05$). This differences between the untreated cooked samples and the high-pressure

processed samples should not be considered at this point because the number of the answers were insufficient, but further studies should pay close attention to this parameter.

Less favour in the cooked untreated sample could automatically indicate more flavour of the cooked high-pressure processed samples and more flavour in products is always desirable. Few remarks were made concerning the shape and uniformity of the samples. This was due to the difficulty of cutting homogenously the trout fillets.

Table 2. Answers obtained after conducting the triangle test

Number of assessors	Triangle 1- sample codes			Total number of correct answers
1	273	138	856	✓
2	273	138	856	✓
3	273	138	856	✓
4	273	138	856	✓
5	273	138	856	x
6	273	138	856	✓
7	273	138	856	✓
8	273	138	856	x
9	273	138	856	x
10	273	138	856	✓
11	273	138	856	x
12	273	138	856	✓
13	273	138	856	✓
14	273	138	856	✓
15	273	138	856	x
16	273	138	856	✓
17	273	138	856	
18	273	138	856	✓
19	273	138	856	✓
20	273	138	856	x
21	273	138	856	x
22	273	138	856	x
23	273	138	856	✓
24	273	138	856	✓
25	273	138	856	x
26	273	138	856	✓
27	273	138	856	x
28	273	138	856	✓
29	273	138	856	✓
30	273	138	856	✓
				20

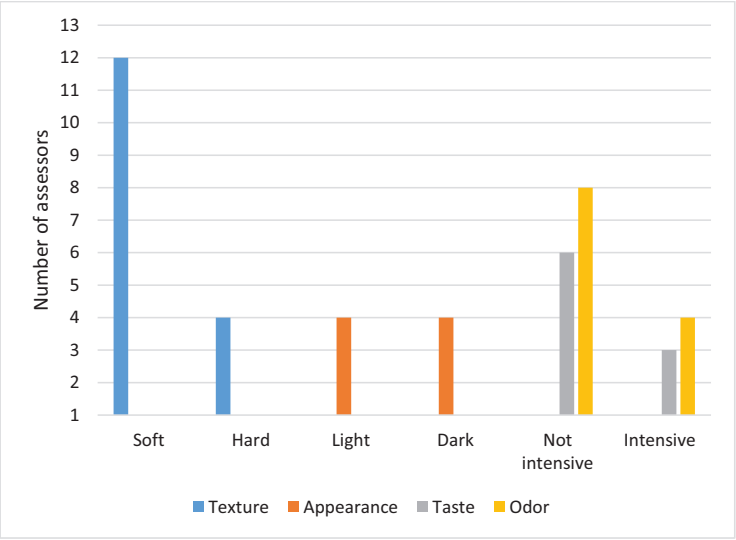
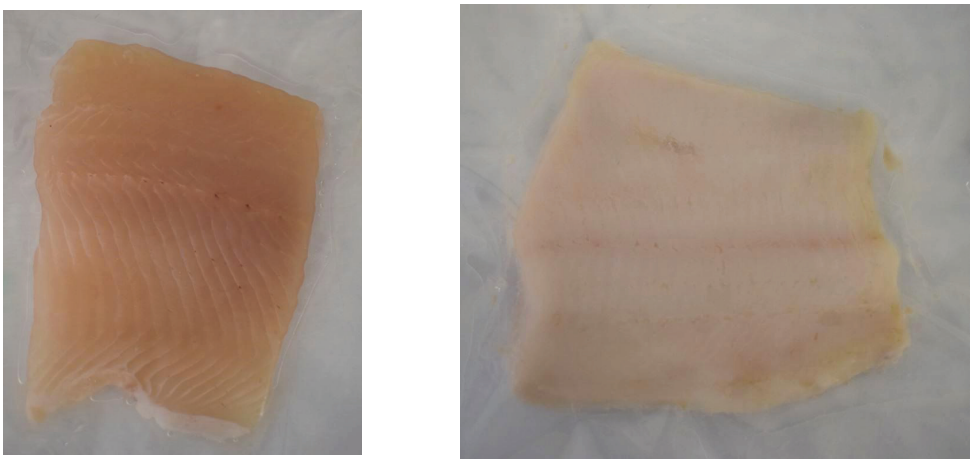


Figure 1. Description of texture, appearance, taste and odor of cooked untreated sample (code 138)



A B

Figure 2. Untreated sample before cooking (A) and sample processed at 400 MPa/6 min before cooking (B)

CONCLUSIONS

The large majority of the panelists enrolled in the organoleptic exam differentiated the untreated cooked sample from the high-pressure processed samples, mainly on texture criteria. The untreated cooked sample was softer than the cooked high-pressure processed samples. The panelists did not express their appreciation in any way for softer or firmer products. However, a more compact structure similar to the structure of the high-pressure processed samples might be considered a plus in gastronomy, for processors and consumers. No significant differences were detected concerning appearance and flavour.

ACKNOWLEDGEMENTS

This work was financially supported by the German Institute of Food Technologies (DIL), Quakenbrück, Germany. Special thanks to Dr.

rer. nat. Stefan Töpfl and Dr. rer. nat. Kemal Aganovic from the department of Advanced Research in DIL.

REFERENCES

Alves de Oliveira Fabiano N.O., 2017. Effect of high pressure on fish meat quality - A review. Trends in Food Science & Technology, 66, 1-19.

Aubourg S.P., Torres J.A., Saraiva J.A., 2013. Effect of high-pressure treatments applied before freezing and frozen storage on the functional and sensory properties of Atlantic mackerel (*Scomber scombrus*). Food Science and Technology, 53, 100-106.

Chouhan A., Kaur B.P., Rao P.S., 2015. Effect of high pressure processing and thermal treatment on quality of hilsa (*Tenualosa ilisha*) fillets during refrigerated storage. Innovative Food Science & Emerging Technologies, 29, 151-160.

Erkan Nuray, Üretener G., 2010. Effect of high pressure (HP) on the quality and shelf life of red mullet (*Mullus surmelutus*). Innovative Food Science and Emerging Technologies, 11(2), 259-264.

FAO, 2015. http://www.fao.org. Retrieved from http://www.fao.org/fishery/culturedspecies/Oncorhynchus_mykiss/en.

Gómez-Estaca J., G.-G. M. C., M. P. (2007). High pressure effects on the quality and preservation of cold-smoked dolphinfish (*Coryphaena hippurus*) fillets. *Food Chemistry*. 102: 1250–1259.

Gudbjornsdottir B., Jonsson A., Hafsteinsson H., Heinz V., 2010. Effect of high-pressure processing on *Listeria* spp. and on the textural and microstructural properties of cold smoked salmon. Food Science and Technology, 43, 366-374.

ISO 4120, 2004. Sensory analysis. Methodology. Triangle test. Geneva, Switzerland: International Organization for Standardization.

Kilcast D., 2010. Triangle Test. In Sensory analysis for food and beverage quality control- A Practical Guide (p. 91). Abington Hall, Granta Park,Great Abington, Cambridge CB21 6AH, UK: Woodhead Publishing Series in Food Science, Technology and Nutrition: Number 191.

Meilgaard M., Carr B., Civille G. (1991). Sensory Evaluation Techniques. Boca Raton: CRC Press.

Mengden Ruth R. A. (2015). High-pressure processing of mild smoked rainbow trout fillets (Oncorhynchus mykiss) and fresh European catfish fillets (Silurus glanis). *Innovative Food Science and Emerging Technologies*, 32: 9-15.

Rode Tone Mari H. M. (2016). High pressure processing extend the shelf life of fresh salmon, cod and mackerel. *Food Control*, 70: 242-248.

Yagiz Y., K. H. (2007). Effect of high pressure treatment on the quality of rainbow trout (*Oncorhynchus mykiss*) and mahi mahi (*Coryphaena hippurus*). *Journal of Food Science*, 72(9): C509-15.

WILD LIFE MANAGEMENT, FISHERY AND AQUACULTURE

Scientific Papers. Series D. Animal Science. Vol. LXI, Number 1, 2018
ISSN 2285-5750; ISSN CD-ROM 2285-5769; ISSN Online 2393-2260; ISSN-L 2285-5750

EU REGULATIONS FOR ORGANIC AQUACULTURE – A KEY FOR PRODUCING ORGANIC FOOD

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Abstract

As a result of applying conventional agriculture that involves synthesis medicines, pesticides and GMO's, the quality of food gave rise to concerns for the world population. In these circumstances organic agriculture has massively developed in the last decades. The features of this new type of agriculture were also used in aquaculture. Therefore, the European decision makers have issued the following legal acts: Regulations (EC) No 834/2007, 889/2008, 1254/2008, 710/2009 and 537/2009. As regulations they shall apply directly in the national legislation. These regulations cover the fields of pounds for aquaculture, specific organic feed and reproduction methods by organic genetic material. Another target of the regulations is the production of an important source of organic feed – seaweed - intended for the feeding of organic farm animals. In Romania the targeted species are the carp and the Asian carp varieties. There is a great potential for the seaweed organic feed. The organic aquaculture interferes in the friendly way with the environment maintaining the natural characteristics of it and also a sustainable agriculture activity.

Key words*: organic farming, feed, food, legislation, sustainability.*

INTRODUCTION

In the past two decades, the interest of consumers for foods classified as "organic" or "bio" has grown exponentially. Among the factors that determined this trend are: computer bombardment, especially via the Internet; the promotion of risks of serious diseases (cancer, diabetes, allergies) presented as foods obtained by conventional means of cultivation, growth and processing; psychosis induced by the media in connection with the various accidents in the production chain (contamination with toxic substances, increased levels of preservatives in animal or plant products); aggressive media coverage of the conflict between supporters and opponents of G.M.O.'s use. In addition to this data, a growing population segment is aware of the health risks that can arise from the use of pesticides, herbicides, antibiotics and other synthetic substances in plant cultivation and animal husbandry (http://ec.europa.eu/agriculture /organic/files/consumer-confidence/inspection-certification/EU_control_bodies_authorities_en .pdf). As a result, major retail companies have restructured their marketing strategies, offering a wider range of products under the label "organic" or "bio".

This policy has also involved the aquaculture sector. The bodies responsible for EU food safety have responded to new consumer concerns and have developed a set of normative acts to regulate: the growth, processing and marketing of animals and algae obtained under strict conditions of exploitation, according to the rules of "bio" products (http://ec.europa.eu/environment/ecolabel/documents/marketing_guide_en.pdf).

According with legislation, food means any processed, partially processed or unprocessed product, designed for human consumption.

The aquaculture products have a high nutritional value, contain good quality proteins and lipids vitamins, and minerals (Nicolae et al., 2016).

MATERIALS AND METHODS

The present study presents an overview of the literature and legal resources related to organic aquaculture production and legislation (printed and/or available online) that apply to organic food provide by aquaculture.

Organic production is regulated at EU level by: Regulation (EC) No 834/2007 on organic production and labeling of organic products and repealing Regulation (EEC) No 2092/91 (Official Journal of the European Union L 189, 2007). This is the basic normative act which in turn has undergone numerous amendments over the last ten years. They are in order: Commission Regulation (EC) No 889/2008 laying down detailed rules for the application of Council Regulation (EC) No 834/2007 on organic production and labeling of organic products as regards organic production, labeling and control (Official Journal of the European Union L 250, 2008); Regulation (EC) No 710/2009 amending Regulation (EC) No 889/2008 laying down detailed rules for the application of Council Regulation (EC) No 834/2007 as regards the establishment of detailed rules for organic production of aquaculture animals and seaweed (Official Journal of the European Union L 204, 2009); Regulation (EC) No 1254/2008 amending Regulation (EC) No 889/2008 laying down detailed rules for the application of Council Regulation (EC) No 834/2007 on organic production and labeling of organic products as regards organic production, labeling and control (Official Journal of the European Union L 337, 2008); Commission Regulation (EC) No 537/2009 amending Regulation (EC) No 1235/2008 as regards the list of third countries from which certain agricultural products produced by organic production methods must originate in order to be marketed in the Community (Official Journal of the European Union L 159, 2009).

In national legislation the definition of specific terms has been regulated by O.U.G. 34/2000, amended in 2006 (Official Gazette of Romania, Part I, no. 172, 2000). Another normative act this time a Community Directive: Directive 2006/88 on animal health conditions and applicable to aquaculture products and on the prevention of certain diseases and the measures to combat them (Official Gazette of Romania, Part I, no. 172, 2000), transposed into national legislation by Order 170/2007 of the National Sanitary-Veterinary and Food Safety Authority under the title: „Order for the approval of the sanitary veterinary norm setting animal health requirements for aquaculture animals and their products, as well as for the prevention and control of certain aquatic animal diseases" (Official Gazette of Romania, Part I, no. 679, 2007). Veterinary bodies carry out official controls on the basis of Regulation 882/2004 (Official Journal of the European Union L 165, 2004). As regards the hygiene rules applicable to organic holdings and the processing of organic products, they must comply with the provisions of Regulations 853/2004 (Official Journal of the European Union L 285, 2017) and 854/2004 (Official Journal of the European Union L 323, 2015). The procedure for registration of organic farms is regulated by Order No 1253/2013 of Ministry of Agriculture and Rural Development (M.A.R.D.) (Official Gazette of Romania, Part I, no. 687, 2013). Delegated attributions of inspection and certification competencies by M.A.R.D. to private control bodies are the basis of Order No 895/2016 (Official Gazette of Romania, Part I, no. 669, 2016).

In this work, several carp farms have been studied which received organic certifications in previous years, as well as a culture of organic spirulina in Sinoe waters.

RESULTS AND DISCUSSIONS

In this study we used the term organic or biological, respectively or ecological, whenever the term "ecological" appears in the Romanian official text. We have avoided this term for several reasons. In the linguistic nomenclature of the EU member states for agriculture, which respects the general principles and objectives of natural agriculture without artificial interference, three variants are used: biological, organic or ecological. Of the 24 official languages, six use two terms (pairs of words for the same term), eight only the term "biological", seven only use the term "organic", and the other three the term "organic". Although Romania has obtained from the European Commission the right to use the term

"ecological" it is appropriate to analyze this word from the perspective of the scientific approach. According to the opinions expressed by biologists (the term means connoisseurs of the living world as a whole), agriculture can be divided into three branches: 1. Chemistry agriculture; 2. Biological agriculture; 3. Ecological agriculture. If agriculture is defined by the phrase "conventional agriculture", organic farming refers to the "agricultural organism" (farm or household that only imports natural fertilizers - limestone, volcanic rocks, siliceous sand). Instead, the term ecological farming is coming from outside agriculture (Papacostea, 2016). It defines an agriculture that integrates into the whole of natural ecosystems, that do not pollute and as a result it is beneficial for the biotope. Ecology as a science has never developed specific technologies, but puts the label of integrated agriculture into the landscape. The final link is not man, but biotope, because the production is decreasing. For this reason, the name of ecological product is unfounded, being synonymous with the "healthy product" (Papacostea, 2009). Or all products placed on the market are considered to be healthy and good for human consumption (Simeanu, 2015).

Under specific regulations, any farm producing fish or organic algae must meet the specific requirements of any organic farm. Genetic material should come from bio-sources, feed used as a feed substrate must also be organic feed, basins must be well delimited by other sources of water, to avoid possible contamination with substances used in conventional farms. In the case of fish, compliance with animal welfare rules is mandatory for any organic farm.

To obtain certificates specific to organic holdings, the farmer must draw up the veterinary registration dossier at the County Sanitary-Veterinary Directorate, which is the competent authority at local or regional level. In the next step, the National Agency for Fisheries and Aquaculture records the dossier and grants the aquaculture license. According to the term defined by Regulation 834/2007 as "competent authority" for organic production in Romania, this is the Ministry of Agriculture and Rural Development. Certification and control bodies are a third party, independent, privately owned, carrying out inspection and certification activities in the field of organic production where the ultimate goal of production is the market. In 2017 a number of 13 control bodies were approved in Romania. Seven are native bodies, and six are branches of bodies from Italy, Germany and Austria. If the ultimate goal is to access Community funds in the specific field, the control is done by the Payment and Intervention Agency for Agriculture.

Over the last three years, Romania recorded a decrease of 23% in organic farming surface, a phenomenon reflected in the requests for organic aquaculture certificates (http://ec.europa.eu/eurostat/statistics-explained/index.php/Organic_farming_statistics).

Holdings benefiting from Community funds in previous years have not renewed their certification requests after the European Commission has suspended animal welfare subsidies. Because of the low carp price/kilo, farmers have not shown interest in this type of exploitation, the difference in price between conventional and organic products being small and therefore unattractive. Following a study started two years ago on the possibility of obtaining *Arthrospira platensis* organic spirulina crops in the Sinoe Saltwater, the results were encouraging, the alga rising in parameters similar to those in freshwater pools. At present, the physico-chemical characteristics of the Sinoe waters are within the requirements of organic aquaculture. The only biological limitation is the temperature of the environment; the algae develop only between May and October.

CONCLUSIONS

1. Aquaculture offers a great potential in Romania, especially in the indoor water sector. So far, the number of species raised has been modest compared to other countries. In order to attract an economically significant segment of buyers, the diversity of the offer must increase. In this sense, farmers have to be stimulated by means of subsidies in the first years of production. Organic spirulina produced so far only in strictly controlled artificial environments, can be cultivated in accordance with the organic production rules in Sinoe

waters, the low production cost justifies enough the method.

2. In the certification and control mechanism so far the weak link has proved the methodology for the selection of certification and control bodies by the competent authority. Also, the number of controls and their frequency create uncertainty in the certification.

ACKNOWLEDGEMENTS

This research work was carried out with the support of The National Sanitary Veterinary and Food Safety Authority.

REFERENCES

http://ec.europa.eu/agriculture/organic/files/consumer-confidence/inspection-certification/EU_control_bodies_authorities_en.pdf

http://ec.europa.eu/environment/ecolabel/documents/marketing_guide_en.pdf

http://ec.europa.eu/eurostat/statistics-explained/index.php/Organic_farming_statistics

Nicolae C.G., Bahaciu G.V., Elia E., Dumitrache F., Marin M.P., Pogurschi E., Bădulescu L. 2016. A review of the quality standards for frozen beef meat and fish. Scientific Papers. Series D. Animal Science, Vol. LIX, 302-310.

Official Gazette of Romania, Part I, no. 172, April 21, 2000.

Official Gazette of Romania, Part I, no. 679, October 5, 2007.

Official Gazette of Romania, Part I, no. 687, November 8, 2013.

Official Gazette of Romania, Part I, no. 669, August 31, 2016.

Official Journal of the European Union L 165, 30.4.2004, p. 1–141, Regulation as last amended by Commission Regulation (EU) 2017/2460 (OJ L 348, 29.12.2017, p. 6).

Official Journal of the European Union L 328, 24.11.2006, p. 14–56, Directive as last amended by Commission Implementing Directive 2014/22/EU (OJ L 44, 14.2.2014, p. 45).

Official Journal of the European Union L 189, 20.7.2007, p. 1.

Official Journal of the European Union L 250, 18.9.2008, p. 1–8.

Official Journal of the European Union L 337, 16.12.2008, p. 80–82.

Official Journal of the European Union L 159, 20.6.2009, p. 6–9.

Official Journal of the European Union L 204, 6.8.2009, p. 15–34.

Official Journal of the European Union L 323, 9.12.2015, p. 2.

Official Journal of the European Union L 285, 1.11.2017, p. 10.

Papacostea P., 2009. The truth about biodynamic organic agriculture. InfoTerapii 1/9 0:55:36.

Papacostea P., 2016. Memories and teachings at ninety years old. Revista Formula As, 1224 (http://www.formula-as.ro/2016/1224/lumea-romaneasca-24/dr-biolog-petre-papacostea-amintiri-si-invatature-la-nouazeci-de-ani-20974).

Simeanu D., 2015. Human nutrition. Ion Ionescu de la Brad Publishing House, Iasi.

Scientific Papers. Series D. Animal Science. Vol. LXI, Number 1, 2018
ISSN 2285-5750; ISSN CD-ROM 2285-5769; ISSN Online 2393-2260; ISSN-L 2285-5750

THE RATIO OF SATURATED AND UNSATURATED FAT IN FORMULATION TO PELLET STABILITY AND FISH GROWTH NILE NIRVANA

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Abstract

The purpose of this study was to determine the level and the ratio of types of vegetable fat supplements that are effective in improving the quality of pellets and growth of tilapia, with the following stages:1) Preliminary test to determine fat level, 2) The extraction of vegetable fats (hazelnuts and coconuts) was then formulated with saturated and unsaturated fatty ratios in pellets at the same fat level, and tested their physical qualities by measuring the stability of fish pellets, 3) Feeding trial of saturated fat sources (coconut extract) compared with unsaturated fats (hazelnut extract) in the formulation of growth and efficiency of tilapia seed feed. The experiment was designed completely randomly (5x3) with the treatment of saturated and unsaturated fatty ratios in low protein feed as follows: (1) basal / no oil feed, (2) coconut oil without combination with hazelnut (1: 0); (3) coconut oil mixture and hazelnut (1: 1), (4)hazelnut oil (0: 1), (5) feed control. Chemical analysis results were tested descriptively, while performance data (growth) were analyzed by variety and the difference between treatments was tested with Duncan analysis. Preliminary test results indicate that the use of 4 percent fat level can be used in the formulation of tilapia feed and in accordance with the needs of optimum fats. Chemical description of unsaturated fat sources (linoleic) in hazelnut meets the nutrient requirements of nirvana Nile seed phase. The results of test of water stability obtained that 4% vegetable fat supplement and can improve the physical quality of pellets; with stability (77.57-80.49%) after two hours. The feeding trial showed that the mixture of coconut and hazelnut fat supplements of 2% each in pellet produced physical quality, growth (daily growth rate and specific growth rate) for Nile Nirvana fish. The results of this study indicate that the ratio of saturated 1: 1 unsaturated fats from vegetable oil sources, can match the growth and efficiency achievement of fish fed with higher protein / containing ω-3.

Key words*: fat supplements, formulations, physical quality of pellets, growth, tilapia.*

INTRODUCTION

Fish cultivation can be economically valuable if it is intensively developed, one of them by paying attention to the efficiency and quality of feed. This is because feed is the biggest input in increasing fish growth in intensive. The balance of energy and protein in the formulation becomes central to feeding. Not only the adequacy of the quantity and quality of proteins that must be guaranteed, often the energy value is not as expected, so the addition of high-calorie ingredients becomes important. In addition to increasing caloric value, dietary fat reduces the dust, texture effect and increases palatability, thereby reducing the lost feed in both the pelletizing process and its delivery, but may have a decrease in the quality of fat as well as processing and duration of storage (Galli, 2000).

Energy sources can be obtained from fats and carbohydrates, so consider their use in formulations. According to Tacon (1986), omnivores can utilize fats and carbohydrates as "spare" proteins. Some carbohydrate source ingredients, have a high content of crude fiber that is less digestible by fish. An important energy source because it has a high calorie value is fat, and can be obtained from some of the raw material feed (invisible fat) or can be from fat and visible fat supplements. The main feed ingredients commonly used in fish feed are fish meal, but high fat content is usually avoided when the choice of the ingredients is easily rancid and affects the duration of drying. Other feed ingredients are generally derived

from by-products of oil-making agro-industries such as soybean meal or derived from the rest of the manufacture of other foodstuffs such as corn bran, wheat bran (polar) which most of the oil content is also lost in pressing and drying. The addition of supplements in the form of oil to be an alternative source of energy that can be pursued since preparation of pellet making.

The addition of oil as a neutral fat which is liquid at room temperature can be adjusted at the time before or after pelleting. Fats in the diet need to be considered the quality and quantity. According to Aderolu and Akinremi (2009), as well as peanut oil 5%, the use of coconut oil as much as 5% can produce better (hemoglobin, hematocrit and protein) characteristics compared to control (without oil) although his blood cholesterol increased in catfish. In addition, the use of coconut oil improves feed conversion (0.57-0.61) compared to control (0.87), with feed cost cheaper than peanut oil.

Problems that can be identified are:

(1) To what extent is the level of fat supplement use in low protein feed formulations on the physical quality of the pellets (durability and palatability).

(2) To what extent is the source of saturated fat (coconut fat) and fat sourcenot saturated (hazelnut fat) affect the growth of tilapia seeds stadiafingerling.

MATERIALS AND METHODS

Research stages

Stages of research includes preliminary scale up preparation and oil characterization of coconut pairing and hazelnut, continued pellet making and oil addition (4%) and physical pellet test (water stability or pellet dispersion). The second stage is a phase aimed at testing fatty acid supplements from alternative materials of coconut and hazelnut as a source of saturated fatty acid and unsaturated fatty acid biological tests to see the value of benefits and efficiency to the growth of tilapia which is maintained on an aquarium container.

Research materials

The research materials used are hazelnut and coconut, tilapia fish stadium fingerling (weight 10 ± 0.20 g), feed ingredients, and research container (fiber and aquarium). The chemicals used include: chemicals for fat extraction (n-hexane), protein, fatty acids, energy, ash, crude fiber.

Research procedure

The fish is acclimatized in a fiber tub. Feed is given on an ad satiation frequency twice a day (at 9:00 and 14:00 hours). Weighing the fish weight done each period and adjusted the amount of feed given. The feeding trial ended on day 60 and in the final period of the study carried out sampling of fish meat.

Variable observed

1) Water stability (%) $= \frac{\text{(Dry weight (g) after dipping)}}{\text{(Dry weight (g) before immersion in water)}}$ x 100%
(Khalil, 1999a)

2) Daily Growth Rate (g/day) $= \frac{\text{Final weight (g)} - \text{Initial weight (g)}}{\text{times of obserbed (days)}}$

3) Specific Growth Rate (%/day)$= \frac{(\text{Ln}W_2 - \text{Ln}\,W_1)}{T_2 - T_1} \times 100\%$

4) Feed Conversion Ratio $= \frac{\text{Feed intake}}{\text{Weight gain}}$

5) Protein Efficiency Ratio (PER)$= \frac{\text{Weight gain}}{\text{Protein consume}}$

Research was conducted experimentally using a Completely Randomized Design of 5 treatments and three replications.

Treatment based on the addition of fatty acid source (from vegetable oil and saturated and unsaturated), namely:

Feed A: Basal/no oil feed (protein 28%);
Feed B: Coconut oil without combination of hazelnut oil (1:0).
Feed C: Coconut oil mixture with hazelnut oil (1:1)
Feed D: Hazelnut oil, without combination of coconut oil (0:1)
Feed E: Control Feed (higher protein 32%).

RESULTS AND DISCUSSIONS

The ratio of saturated fatty acids and unsaturated supplementation results

Table 1 shows that the addition of a 4% fat dietary supplement of coconut oil and coconut oils yields different saturated and unsaturated fatty acids.

The extraction of hazelnut and coconut in this study was done steep extracted, without heating to avoid excessive polymer oxidation.

The extracted ingredients are substances such as oil, fat, or fatty acids and others more quickly and perfectly, while materials such as wax, pigment, and albumin compounds are slightly dissolved.

The results of extraction on coconut and candlenut are affected by water content.

According to Hertrampf and Pascual (2000), the reduced moisture content in the seeds causes the seeds to become hard so that the oil is difficult to remove.

Table 1. Energy Protein and Saturated/Unsaturated Fatty Acid Ratio

Nutrient	Treatments				
	A	B	C	D	E
Protein (%)	28.07	28.14	28.05	28.05	32.05
DE (kcal/kg)	2237	2248	2250	2250	2246
DE/P	7.97	7.99	8.02	8.02	7.17
Saturated Fatty Acid*) (%)	2.18	4,64	6.24	3.04	2.4
Unsaturated Fatty Acid*) (%)	3.15	4.56	2.97	6.16	3.16
SFA : FA Ratio	2 : 3	1 : 1	2 : 1	1 : 2	2 : 3
Linoleic acid (%)	0.49	1.04	0.36	1.73	0.68
Total Fat in Feed (%)	5.33	9.21	9.21	9.21	5.27

Note: DE = digestible energy

From Table 1 it can be seen that the fatty acid content contained in the hazelnut and coconut is sufficient to meet the requirements and is expected to know the balance of unsaturated fats and saturated fats needed for the nutritional benefits of tilapia.

Based on the composition of the feed formulation as well as the results of the analysis and calculation of fatty acid composition, descriptively the ratio of saturated and unsaturated fat components of the oil type exhibits different saturated and unsaturated fatty acids. Mixing the ratio of feed oil supplement ingredients of hazelnut and coconut materials representing the ratio of unsaturated fatty acids and saturated fatty acids to tilapia from the calculation and analysis. The treatment provided is: without supplements (pellet A); SFA: saturated fatty acid ratio of 1:0 (pellet B), 1:1 (pellet C), 0:1 (pellet D), containing 28% crude protein, maximum fish meal 15%, compared to control (commercial) (pellet E). The presence of fat is also required in the diet, fat is a protein sparing effect as a provider of non-protein energy so that proteins are used for growth. In each 2% fat addition derived from the seed fats of hazelnut can increase the calorific value (gross energy), which is about 150 kcal/kg of feed. The use of excess oil can affect the excess energy that quickly leads to satiety, so that protein intake can be reduced.

The content of corn linoleic is quite large, thus increasing the level of unsaturated fatty acid ω-6 although not supplemented fat. As for commercial pellets there are sources of unsaturated fatty linolenic acid other than derived from the source of fish meal, also added fish oil.

Dispersion of pellets from fat added fish

Dispersion of pellets is one of the feed factors that can affect the performance of fish. The results of the variance analysis showed no differences between treatments on the stability of the pellets in water after 30 minutes and 60

minutes. The Duncan Test results (Table 2) showed that fatty supplements in the formulation may affect the physical quality of the pellets, in this case with the stability of the pellets in water after two hours of immersion.

Table 2. Physical quality of pellets (dispersion of pellets)

Treatments	Water Pellet Dispersion (%)		
	30 minutes	60 minutes	120 minutes
A	87.22	84.01	77.57 [b]
B	87.27	85.15	78.79 [b]
C	87.36	85.90	80.33 [a]
D	86.33	84.79	79.49 [ab]
E	88.82	85.96	80.49 [a]

Table 2 shows that control pellets (E), and pellets with coconut oil supplements (C) have higher stability values than basal pellets (A). Pellet control is better than the basal pellet caused by the nature of protein flexibility and the presence of oil. Pellet control is better than the basal pellet caused by the nature of protein spasticity and the presence of fish oil. According Rasyaf (1994), the use of oil as part of the formulation usually only ranges from 1-3%, because if excessive can cause the pellets back into the form of flour. Meanwhile, according to Behnke (2001), the addition of fat in high quantities, i.e. more than 10% can inhibit gelatinization of starch, but can be overcome with the use of higher temperatures.

The addition of fat supplements of the type of coconut oil (feed C) which produces the highest durability compared to other types of oil although not significantly different. Coconut oil supplementation in basal feed formulation (low protein) can increase stability as well as high protein pellets. The use of coconut oil is more resistant to rotation of the tumbling device, due to its more saturated and non-volatile structure, will soon close the pores of the cell wall matrix and act as a lubricant between the feed particles (mash), and the heat conductor. This will have a positive effect on the hardness of the pellets. Coconut oil is one of the vegetable products that contain a lot of saturated fatty acid that is equal to 92%, whereas hazelnut contains many unsaturated fatty acid and tend to be volatile. Volatile fatty acids are fatty acids such as linoleic, linolenic, stearate, oleic, and others that are not bound by glyceride molecules.

The addition of a hazelnut oil supplement (feed D) as well as a mixture of hazelnuts (feed B) produced similar effects with other treatments, including with basal pellets (A). Fat supplements are thought to enhance the surface active properties of pelletizing agents through the formation of emulsions.

Although it can improve texture, fat as a hydrophobic compound can disrupt the binding properties of water-soluble components in feed (starch, protein and fiber) so that it can be detrimental to hardness and endurance of feed.

The presence of a carboxyl-methyl-cellulose binder (CMC) in the feedstock at all treatments may affect durability. Gelatinization of starch that occurs on the surface of the feed material is a critical point of the formation of intra-particle bonds that play an important role in the formation of strength, durability of pellets.

Stability figures of pellets in water or Pellet Dispersion after dripping 30 minutes, 60 minutes, and 120 minutes ranged from 77.57 to 8.82%. Fat treatment tends to increase the stability of the pellets in water.

Galli (2000) states that feed additive materials can improve the quality of pellets by adding moisture and surfactant either in the process before pelleting (during mixing mixing) or after pelleting (during cooling or conditioning). Surfactant and moisturizing incorporation permits many permeations of feed particles and at any given moment (the "glass transition point") causes the fat to trap into the feed particles, so this does not cause oily-looking pellets.

Growth performance and efficiency.

Based on the result of analysis of variance, it is found significance of influence to absolute weight, daily growth rate (DGR and SGR) (Table 3).

Table 3. Growth of Absolute Weight, DGR, and SGR tilapia

Treatments	Weight Gained (g)	Daily Growth Rate (g/day)	Specific Growth Rate (%)
A	21.27 [a]	0.34±0.03 [a]	1.79±0.02 [a]
B	27.33 [cd]	0.43±0.05 [cd]	2.08±004 [b]
C	25.93 [b]	0.41±0.10 [b]	2.02±0.02 [b]
D	26.71 [c]	0.42±0.03 [c]	2.04±0.02 [b]
E	27.56 [d]	0.44±0.04 [d]	2.09±0.03 [b]

The results of the variance analysis show that there is a significant difference in the types of fats (saturated, unsaturated and mixed) resulting in different effects on absolute growth, daily growth rate and specific growth rate indicating that fat addition is effective against growth of tilapia seed stadia fingerling.

The ratio of saturated and unsaturated fatty acids to 1:1 (feed C) resulted in the ratio of feed conversion and feed efficiency (%), which was better than those containing fish oil and high protein (Table 4). The results of this study indicate that feed with the dominance of unsaturated fatty acids (feed D), saturated fatty acids (feed C) and mixed saturated and unsaturated (feed B) of clear coconut oil and hazelnut oil yield the same feed efficiency with high protein pellets (E).

Table 4. Number of Feed, Feed Conversion Ratio and Feed Efficiency of tilapia fish

Treatments	Number of Feed (g)	Feed Conversion index	Feed Efficiency (%)
A	42.41	1.99±0.02 [c]	50.14±0.48 [a]
B	48.62	1.78±0.04 [ab]	56.23±1.37 [bc]
C	46.30	1.73±0.04 [a]	57.93±1.37 [c]
D	46.31	1.73±0.02 [a]	57.68±0.53 [c]
E	50.10	1.82±0.03 [b]	55.01±0.75 [b]

Note: A: basal feed/without supplements; B: a mixture of coconut and hazelnut; C: coconut oil 4%; D: hazelnut 4%; E: commercial/feed (pellet control).

Table 4 shows the best efficiency, conversion, and protein efficiency ratio of feed produced by feed C with 4% coconut oil. Aderolu and Akinremi (2009) showed that a mixture of coconut oil, fish oil, and cow fat respectively produced 1.7% of the best growth in trout. According to Ng and Chong (2004), the fat content of 5% feed can meet the minimum requirement of fat for cultivation.

Feed with 4% fat supplements from both saturated and unsaturated oils, coconut (saturated), or walnut (unsaturated) oils each showed no different conversion of feeds, ranging from 1.73 to 1.78. Although the growth of saturated and unsaturated ratio 1:1 on feed (B) and 1:2 (D) contain coconut oil supplements was higher than that of hazelnut oil on feed C (Table 5), the three treatments of fat supplements resulted in the conversion, efficiency and efficiency of feed proteins no different. The content of unsaturated fatty acids (linoleic) in feed B and D as needed (0.5-1%), compared with coconut fat (C) (Table 3), so the growth is better. The results of Hsieh et al. (2007), enzymatic activity of SCD (sterol-CoA desaturase) was highest seen in mixed-supplemented fish (SFA + PUFA) as well as SFA supplements (coconut oil) (Craigh and Helfreich, 2009), vegetable oil (palm oil, and soybean oil) can be used as a substitute for fish oil without affecting the growth and efficiency of feed in freshwater fish (Hertrampf and Pascual, 2000).

The results of this study indicate that the ratio of saturated 1:1 unsaturated fats from vegetable oil sources, can match the growth achievement and efficiency of fish fed with higher protein and contain ω-3 (fish oil).

CONCLUSIONS

The results showed that the use of level 4% fat can be used in the feed formulation of Nile fish and in accordance with the needs of optimum fats, as well as physical and chemical descriptions that meet the

requirements of Nile fish feed stadia fingerling. A 4% fat supplementation consisting of a 2% coconut oil mixture and 2% hazelnut oil in a 28% protein diet yields the best performance (physical quality of pellets, and growth).

ACKNOWLEDGEMENTS

The researchers would like to thank the Minister of Department, Aquaculture for his approval of this research can take place through the Research funded by the Disertasi of Doctoral 2017. Rector and Director of Research and Community Service and Innovation Padjadjaran University, and Dean of the Faculty of Fisheries and Marine Science of Padjadjaran University, who has given the trust to conduct this research. Head Laboratory of Fish Nutrition, and Laboratory of Hatchery, Faculty of Fisheries and Marine Science of Padjadjaran University, which have given permission to use the laboratory.

REFERENCES

Aderolu A.Z., Akinremi O.A., 2009. Dietary effects of coconut oil and peanut oil in improving biochemical characteristics of *Clarias gariepinus* juvenile. Turkish Journal of Fisheries and Aquatic Sciences, 9, 105-110.

Behnke K.C., 2001. Factors affecting pellet quality. Maryland Nutrition Conference, Department of Poultry Science and Animal Science, University of Maryland. Feed Tech.cbna.com.br.

Craigh S., Helfreich L.A., 2009. Understanding fish nutrition feeds and feeding. http:/www.ex.vt. Edu/Pubs/Fisheries

Galli J.A., 2000. Technology of the use of fats in feeds. NRA print. Singapore.

Hertramph J.W., Pascual F.P., 2000. Handbook on ingredients for aquaculture feeds. Kluwer Academic Publishers, London, pp 506.

Hsieh S.L., Hu C.Y., Hsu Y.T., Hsieh T.J., 2007. Influence of dietarylipids on the fatty acid composition and stearoyl-CoA desaturase expression in hybrid tilapia (*Oreochromis niloticus* × *O. aureus*) under cold shock. Comparative Biochemistry & Physiology, Part B, 147(3), 438-444

Khalil, 1999a. Pengaruh kandungan air dan ukuran partikel terhadap sifat fisik pakan lokal. Kerapatan Pemadatan Tumpukan dan Berat Jenis. Media Peternakan, 22 (1): 1-11.Ng, W.K and C.W. Chong, 2000. An Overview of Lipid Nutrition with Emphasis on Alternative Lipid Sources in Tilapia Feeds. Fish Nutrition Laboratory. School of Biological Sciences, University Sains Malaysia.

Rasyaf M., 1994. Makanan Ayam Broiler. Kanisius, Yogyakarta.

Tacon A.G.J., 1986. The Nutritional of feeding of farmed fish and Shrimp. FAO of the United Nations, Brazil.

Scientific Papers. Series D. Animal Science. Vol. LXI, Number 1, 2018
ISSN 2285-5750; ISSN CD-ROM 2285-5769; ISSN Online 2393-2260; ISSN-L 2285-5750

EFFECTS OF TRICAINE ON BLUE TILAPIA AT DIFFERENT SALINITIES AND CONCENTRATIONS

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Abstract

This experiment was devised to evaluate the effects of tricaine methanesulfonate (MS-222) on blue tilapia, Oreochromis aureus at five different salinities (0, 8, 16, 20, 24 ppt) and four different tricaine concentrations (200, 300, 400, 500 mg l^{-1}). Even though, a body of literature exist about the tricaine usage on fish, not much information is present on tricaine with salt. The requirement time to anaesthetize fish depends on intensity of tricaine concentration and salinity. Induction time of fish decreased as tricaine concentrations increased. When exposed to any of tricaine concentrations, fish entered a deep state of anaesthesia (induction time ranged between 0.19 and 2.54 min). Recovery time was highest at 400-500 mg l^{-1} of tricaine as salinity increased. Tricaine + salt combination is strongly recommended to use in blue tilapia culture. Ideal tricaine concentration was 200 mg l^{-1} of tricaine at 8 ppt of salinity to reduce stress in blue tilapia.

Key words: *anaesthetic, blue tilapia, salinity, tricaine.*

INTRODUCTION

Anaesthetics are generally used for sedating or immoving fish in aquacultural practices such as scientific researches and fish farming. They are crucial for decreasing stress caused by handling procesures (Hseu et al., 1998). Several chemicals have established to anaesthetize fish such as tricaine methanesulfonate, quinaldine, phenoxyethanol, clove oil, benzocaine, metomidate, sedanol (Mylonas et al., 2005; Küçük et al., 2016). Each one has its own benefits and balks (Anderson et al., 1997; Munday and Wilson, 1997; Hseu et al., 1998; Wagner et al., 2003; King et al., 2005; Mylonas et al., 2005; Weber et al., 2009; Küçük, 2010; Pramod et al., 2010; Mercy et al., 2013; Mazik and Simco, 2014).

Tricaine methanesulfonate is one of the most widely used anaesthetics in aquaculture (Ross and Ross, 1999). It is structured a white crystalline powder and is solved easily in water. It is purchased as Tricine-S or Finquel and registered only chemical by the Food and Drug Administration (FDA) to use for market fish in the USA and United Kingdom (Hseu et al., 1998; Coyle et al., 2004).

The aim of the study was to expose tilapia to four different MS-222 concentrations (200, 300, 400 and 500 mg l^{-1}) at 0, 8, 16, 20, 24 ppt of salinities and to evaluate induction time, recovery time and survival for each concentration.

MATERIALS AND METHODS

The study was done on blue tilapia, *Oreochromis aureus*, which was commercially attained from Adana, Turkey. Some of water quality parameters of water source were given in Table 1.

Fish average length (147.03±8.53 mm) and weight (47.53±6.54 g) were measured at the beginning of experiment. Fish had been starved for 24 h prior to the trial.

Tricaine methansulfonate (Sigma Aldrich catalog no E10521) stock solution (0.4%, 100 ml) buffered with 1 M Tris-Cl (pH 9.0) and working solution (0.2%, 100 ml) were prepared. Anesthetization practise was done in an aerated 500 ml beaker. Fish was exposed 200, 300, 400, 500 mg l^{-1} concentrations of MS-222 at 0, 8, 16, 20, 24 ppt of salinities at 23.7°C and pH 8.13 untill anaesthesia stage of 3 for induction and recovery times were reported for each concentration. After recovery, fish were taken care in the maintenance aquariums for 48 h in order to see any adverse event for fish situation. Experiment was undertaken on ten fish for each concentration.

Table 1. Some water quality parameters of water source

Parameter	Value
EC (µs cm^{-1})	856.2
Total hardness (mg l^{-1} $CaCO_3$)	712.6
Alkalinity (mg l^{-1} $CaCO_3$)	588.0
Bicarbonate (mg l^{-1})	360.4
Calcium (mg l^{-1})	90.2
Magnesium (mg l^{-1})	12.8
Ammonia (mg l^{-1})	0.33
Nitrite (mg l^{-1})	0.0016

The induction time was put down for each fish when fish suffered total equilibrium, its operculum rate stopped and fish did not reponse to presure on its body (SIII) (Table 2). Anaesthetized each fish was weighed and measured its length. After that, recovery time was recorded when fish began swimming as usual (RIII) (Table 2).

Table 2. Stages of induction and recovery in fish (Küçük and Çoban, 2016)

Stages of Induction	Description	Behavior/Response
	Sedation	Slight loss of reactivity to external stimuli; operculum rate slightly decreased; equilibrium normal
I	Anesthesia	Partial loss of muscle tone; swimming erratic; increased operculum rate; reactivity only to strong tactile and vibration stimuli
II		
III	Deep anesthesia	Total loss of muscle tone and equilibrium; slow but regular operculum rate; loss of spinal reflexes
IV	Death	Breathing and heart beat stop; eventual death
Stages of Recovery	**Desciption**	**Behavior/Response**
I	Deep anesthesia	No body movements but opercular movements start
II	Anesthesia	Regular opercular movements and body movements start
III	Sedation	Equilibrium regained with preanesthetic appearance

Differences between tricaine and salinity concentrations were analyzied by SSPS. Induction time, recovery times and survival value were set up by comparing each salinity and tricaine concentration. The data are given as mean±SD. Analysis of variance and Duncan's multipe range tests were followed out for signicant differences ($P \leq 0.05$).

RESULTS AND DISCUSSIONS

In the present study, induction and recovery times at each salinity and concentration were demonstrated in the Table 3. The induction time of *Oreochromis aureus* decreased as tricaine concentration increased ($P<0.05$). It ranged between 0.19 and 2.54 min at 200-500 mg l^{-1} of tricaine.

Increasing salinity did not affect induction time of blue tilapia. But, at > 8 ppt, recovery time was signifantly different.

Recovery time became shorter than that of higher salinities and tricaine concentrations. Survival was not significantly affected during trial. Even no fish died within 24 h after trial.

Table 3. Induction time, recovery time, induction rage and survival of blue tilapia in six salinities and four tricaine concentrations

Salinity (ppt)	Tricaine conc. (mg l^{-1})	Induction time (min)	Recovery time (min)	Induction rage (min)	Survival (%)
0	200	$1.34\pm0.34^{A, a}$	$0.64\pm0.35^{A, a}$	1.05-2.21	100
	300	$0.77\pm0.25^{A, b}$	$0.67\pm0.37^{A, b}$	0.43-1.01	100
	400	$0.59\pm0.23^{A, c}$	$1.39\pm0.30^{A, c}$	0.36-1.03	100
	500	$0.47\pm0.22^{A, d}$	$1.82\pm0.42^{A, c}$	0.27-1.04	100
8	200	$1.23\pm0.29^{A, a}$	$0.49\pm0.19^{C, a}$	0.51-1.54	100
	300	$0.72\pm0.34^{A, b}$	$0.83\pm0.46^{C, b}$	0.35-1.29	100
	400	$0.41\pm0.06^{A, c}$	$1.13\pm0.46^{C, c}$	0.35-0.51	100
	500	$0.34\pm0.06^{A, d}$	$1.21\pm0.11^{C, c}$	0.22-0.45	100
16	200	$1.31\pm0.44^{A, a}$	$0.52\pm0.26^{BC, a}$	0.42-2.19	100
	300	$0.90\pm0.32^{A, b}$	$0.83\pm0.26^{BC, b}$	0.51-1.25	100
	400	$0.58\pm0.23^{A,c}$	$1.24\pm0.51^{BC, c}$	0.39-1.03	100
	500	$0.39\pm0.05^{A, d}$	$1.31\pm0.31^{BC, c}$	0.28-0.45	100
20	200	$1.39\pm0.29^{A, a}$	$0.59\pm0.17^{ABC, a}$	1.00-2.01	100
	300	$0.73\pm0.32^{A, b}$	$0.90\pm0.21^{ABC, b}$	0.40-1.19	100
	400	$0.51\pm0.21^{A, c}$	$1.22\pm0.10^{ABC, c}$	0.31-1.07	100
	500	$0.33\pm0.08^{A, d}$	$1.25\pm0.32^{ABC, c}$	0.21-0.45	100
24	200	$1.40\pm0.48^{A, a}$	$0.86\pm0.25^{AB, a}$	0.90-2.54	100
	300	$0.69\pm0.29^{A, b}$	$1.08\pm0.30^{AB, b}$	0.40-1.06	100
	400	$0.41\pm0.11^{A, c}$	$1.20\pm0.17^{AB, c}$	0.29-0.58	100
	500	$0.31\pm0.09^{A, d}$	$1.18\pm0.11^{AB, c}$	0.19-0.48	100

$^{a-d, A-C}$ Mean values within a row having different superscripts are significantly different by least significant difference test; upper case for salinity; lower case for tricane concentration.

Induction time did not change significantly when salinity increased. But it decreased as tricaine concentration increased (Figure 1).

Recovery time was highest at 0 ppt and 400-500 mg l^{-1} of tricaine. It was particially suppressed by salinity at 8 ppt of salinity.

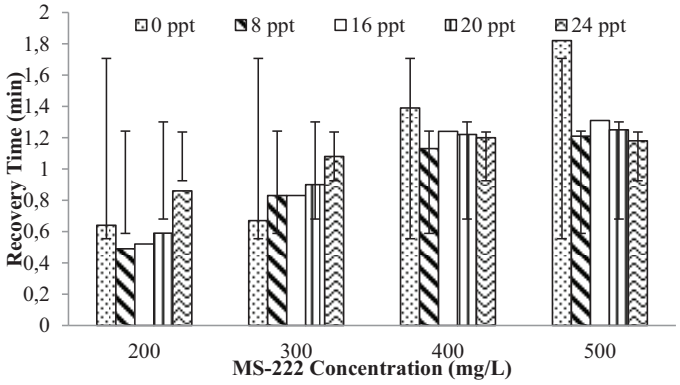

Figure 1. Mean±SD of induction time of blue tilapia immersed to anesthesia

There was negative relation between induction time and tricaine concentration. Induction times significantly decreased with increasing tricaine concentrations. It is agree with previous studies (Mylonas et al., 2005; Küçük, 2010; Pramod et al., 2010; Pawar et al., 2011 and Mercy et al., 2013). But, there was a positive relation between recovery time and tricaine concentration. Recovery time increased as tricaine concentration arised. The same relation was reported by Küçük (2010). Recovery time and mortality risk inceased when anaesthetic concentration rised up.

Ideal tricaine concentration was 200 mg l^{-1} of tricaine at 8 ppt of salinity. Induction and recovery times were 1.23 min and 0.49 min respectively. Hseu et al., (1998) verified our results. They indicated that those were 1.31 min and 0.63 min at 100 mg l^{-1} of tricaine, respectively for goldlined sea bream.

Tilapia were tolerant fish to high tricaine concentrations (200-500 mg l^{-1}) and high salinities (0-24 ppt) concentrations comparing with goldfish *Carassius auratus* exposed to 150-500 mg l^{-1} of tricaine concentration and 0-16 ppt of salinity (Küçük and Çoban, 2016). Induction time and recovery time of tilapia were 0.31 and 1.18 min at highest degrees of salinity and tricaine (24 ppt and 500 mg l^{-1} of MS-222, respectively). Those of goldfish were 0.27 and 1.27 min at 16 ppt and 500 mg l^{-1} of tricaine. The values are almost close each other. The results showed that tilapia were more tolerant than goldfish when exposed to high salinity and tricaine concentration.

When an anaesthetic is chosen, a lot of considerations are thought such as efficiency, price, handiness to use, security to fish, user and environment, structure of experiment and fish species (Mylonas et al., 2005). Tricaine provided all these considerations, except for price and withdrawal time. Cost of tricaine was the higher than other anaesthetic (Hseu et al., 1998) and before marketing fish 21 days are needed to draw the residue from fish body (Coyle et al., 2004).

Tricaine + salt combination alleviated fish from handling stress and increased survival in stripped bass (Mazik et al., 1991; Mazik and Simco, 2014). Survival percentage were also excellent (100%) in this trial. On the other hand, in our study, tilapia tolerated to high tricaine concentration. Because alkalinity and total hardness of the water source were high (Table 1). Coyle et al. (2004) mentioned that tricaine has high potency to warm water fish with low hardness. High hardness of water protected tilapia from advers effect of high tricaine concentrations.

In this study, use of anaesthetics + salt combination was tested in tilapia to explaine why tricaine was used with salt to anaesthetize fish. This combination alleviated blue tilapia. Because salt is used to reduce stress in fish. Adding 8 g l^{-1} of salt take off the difference between fish blood and environment (Wurst, 1995). For blue tilapia, 200 mg l^{-1} of tricaine + 8 g l^{-1} of salt combination is suggested to use. Even, identical combination (200 mg l^{-1} of tricaine + 12 g l^{-1} of salt) was used for goldfish by Küçük and Çoban (2016).

CONCLUSIONS

Ideal concentration of tricaine was 200 mg l^{-1} at 8 ppt of salinity for blue tilapia (1.23 min). As tricaine concentration increased, induction time decreased. Tilapia quite tolerant to tricaine and salt. Survival was perfect for all concentrations and salinities.

At high salinity tilapia recovered quickly, eventhough concentration and salinity were high. High salinity alleviated tricaine effect on recovery time of tilapia (1.18 min at 500 mg l^{-1} and 24 ppt). Recovery time was . 1.82 min at 500 mg l^{-1} and 0 ppt. As a result of that, tricaine + salt combination made induction and recovery times faster.

REFERENCES

Anderson W.G., McKinley R.S., Colavecchia M., 1997. The use of clove oil as an anesthetic for rainbow trout and its effects on swimming performance. North American Journal of Fish Management, 17, 301-307.

Coyle S.D., Durborow R.M., Tidwell J.H., 2004. Anesthetics in aquaculture. Southern Regional Aquaculture Center, SRAC Publicaton No: 3900.

Hseu J., Yeh S., Chu Y., Ting Y., 1998. Comparison of efficacy of five anesthetics in Goldlined sea bream, *Sparus sarba*. Acta Zoologica Taiwanica, 9, 35-41.

Holloway A.C., Keene J. D., Noakes G., Moccia R.D., 2004. Effects of clove oil and MS-222 on blood hormones profiles in rainbow trout *Onchorhynchus*

mykiss, Walbaum. Aquaculture Research, 35, 1025-1030.

King W., Hooper V.B, Hillsgrove S., Benton C., Berlinsky D.I., 2005. The use of clove oil, metomidate, tricaine methanesulphonate and 2-phenoxyethanol for inducing anaesthesia and their effect on the cortisol stress response in black sea bass (*Centropristis striata* L.). Aquaculture Research, 36, 1442-1449.

Küçük S., 2010. Efficacy of tricaine on *Poecilia latipinna* at different temperatures and concentrations. African Journal of Biotechnology, 9, 755-759.

Küçük S., Çoban D., 2016. Effects of tricaine as an anaesthetics on goldfish, *Carassius auratus* (Linnaeus, 1758) at different salinities and concentrations. Turkish Journal of Fisheries and Aquatic Sciences, 16, 605-610.

Küçük S., Öztürk S., Çoban D., 2016. Anaesthetics in Aquaculture. Adnan Menderes Üniversitesi Ziraat Fakültesi Dergisi, 13(2), 79-85 (in Turkish with English abstract).

Mazik P.M., Simco B.A., Parker N.C., 1991. Influence of water hardness and salts on survival and physiological characteristics of stripped bass during and after transport. Transactios of the American Fisheries Soceity 120, 121-126.

Mazik P.M., Simco B.A., 2014. The effects of size, water hardness, salt levels and MS-222 on the survival and stress response in striped bass (*Morone saxatilis*). http://fishphysiology.org/wp-content/ uploads/2014/

Mercy T.V.A., Malika V., Sajan S., 2013. Use of tricaine methanesulfonate (MS-222) to induce anaesthesia in *Puntius denisonıı* (day, 1865) (Teleostei: Cypriniformes:Cyprinidae), a threatened barb of the Western Ghats. Indian Journal of Threatened Taxa, 5, 4414-4419.

Munday P.L., Wilson S.K., 1997. Comparative efficacy of clove oil and other chemicals in anaesthetization of *Pomacentrus amboinensis*, a coral reeffish. Journal of Fish Biology, 51, 931-938.

Mylonas C.C., Cardinaletti G., Sigelaki I., Polzonetti-Magni A., 2005. Comperative efficacy of clove oil and 2-phenoxyethanol as anesthetics in the aquaculture of European sea bass (*Dicentrarchus labrax*) and gillhead sea bream (*Sparus auratus*) at different temperature. Aquaculture, 246, 467-481.

Pawar H.B., Sanaye S.V., Sreepada R.A., Harish V., Suryavanshi U., Ansari T., Ansari Z.A., 2011. Comparison of efficacy of four anaesthetic agents in the Yellow Seahorse, *Hippocampus kuda* (Bleeker, 1852). Aquaculture, 311, 155-161.

Pramod P.K., Ramachandran A., Sajeevan T.P., Thampy S., Pai S.S., 2010. Comparative efficacy of MS-222 and benzocaine as anaesthetics under simulated transport conditions of a tropical ornamental fish *Punctius filamentosus* (Valenciennes). Aquaculture Research, 41, 309-314.

Ross L.G., Ross B., 1999. Anaesthetic and sedative techniques for aquatic animals. Second edition, Blackwell publising, Oxford, 31.

Small B., 2003. Anaesthetic efficacy of metomidate and comparison of plasma cortisol responses to tricaine methanesulfonate, quinaldine and clove oil anaesthetized channel catfish *Ictalurus punctatus*. Aquaculture, 218, 177-185.

Wagner G.N., Singer T.D., McKinley R.S., 2003. The ability of clove oil and MS-222 to minimize handling stress in rainbow trout (*Oncorhynchus mykiss* Walbaum). Aquaculture Research, 34, 1139-1146.

Weber R.A., Peleteiro J.B., Gracia-Martin L.O., Aldegunde M., 2009. The efficacy of 2-phenoxyethanol, metomidate, clove oil and MS-222 as anaesthetic agent in the Senegalese Sole (*Solea senegalensis* Kaup, 1858). Aquaculture, 288, 147-150.

Wurst W.A., 1995. Using salt to reduce handling stress in channel catfish. World Aquaculture, 26(3), 80-81.

Scientific Papers. Series D. Animal Science. Vol. LXI, Number 1, 2018
ISSN 2285-5750; ISSN CD-ROM 2285-5769; ISSN Online 2393-2260; ISSN-L 2285-5750

PREY PREFERENCE OF THE LONG-SNOUTED SEAHORSE (*Hippocampus guttulatus* Cuvier, 1829) AT THE ROMANIAN BLACK SEA COAST

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Abstract

The long-snouted seahorse (Hippocampus guttulatus Cuvier, 1829) is a representative species of the Romanian coast, due to its charismatic appearance and extraordinary biology. Although it is not a commercial fish in Romania, it is subjected to harvesting to be sold as curio or for the aquarium business, and many times is by-caught in trawl or pound net fishery. The current research aimed at the examination of the gut content of wild seahorse specimens, in order to determine the prey preferences of the species along the Romanian Black Sea coast. In the wild, large prey items (Amphipoda, Balanus larvae) were identified as the preferred prey of adult specimens, indicating that size and availability are important factors in prey selection. Adult seahorses appear to prefer larger prey both in wild and controlled environments, as previous research has indicated.

Key words*: feeding behavior, gut contents, long-snouted seahorse, prey availability, zooplankton.*

INTRODUCTION

The long-snouted seahorse (*Hippocampus guttulatus* Cuvier, 1829) is a representative species of the Romanian coast, due to its charismatic appearance and extraordinary biology (Figure 1). Although it is not a commercial fish in Romania, it is subjected to harvesting to be sold as curio or for the aquarium business (Vincent et al., 2011).

Figure 1. *H. guttulatus* individual in its natural habitat (southern Romanian Black Sea coast) (Photo: Nenciu)

Moreover, everywhere in the world, seahorses are often fished with non-selective gear (trawls) (Caldwell and Vincent, 2012) and are vulnerable to the degradation of habitats they inhabit (Woodall, 2012). There is an urgent need for concrete guidelines and initiatives to ensure the conservation of seahorse populations, with emphasis on their biology and ecology. It is also extremely important to understand also the socio-economic aspects of the fishery, which can significantly affect the populations of these species, as well as the way in which anthropogenic activities and their consequences on the marine environment can affect seahorse populations (Nenciu et al., 2013).

The genus *Hippocampus* is included in Annex II of CITES (Convention on International Trade in Endangered Species of Wild Flora and Fauna) since November 2002. The presence of 3 species, namely *Hippocampus guttulatus* (Cuvier, 1829), *Hippocampus hippocampus* (Linnaeus, 1758) and *Hippocampus fuscus* (Rüppel, 1838) is reported in the Romanian Black Sea waters by bibliographic sources (Lourie et al., 2004; Foster and Vincent, 2004). In more recent works, only one species (Radu and Radu, 2008) is reported at the Romanian coast, although several species are described by previous sources (Banarescu, 1964). However, only a genetic study can confirm the presence of these three different species, which have not been done so far in the Black Sea and is currently ongoing (Nenciu et al., 2015b; Taflan et al., 2017).

Seahorses are carnivorous fish that hide in strategic places along the edges of reefs or seagrass beds waiting for prey to come within striking reach (Van Wassenbergh et al., 2011). Seahorses capture highly evasive prey such as small shrimp or larval fishes (Kendrick and Hyndes, 2005). To do so, they make use of a two-phase prey-capture mechanism that is commonly referred to as pivot feeding: a rapid upward rotation of the head is followed by suction to draw the prey into the seahorse's snout (Roos et al., 2009).

Although voracious, seahorses choose to be opportunistic instead of dynamic predators. Seahorses have no teeth and so they stalk their prey, waiting for them to get close enough before sucking them in through their tubular snout. Moreover, seahorses have no stomach, thus digestion occurs very rapidly, consequently they need to constantly hunt and consume prey.

In Romania, the first experiments conducted on the breeding and rearing in captivity of seahorses were carried out by the National Institute for Marine Research and Development (NIMRD) „Grigore Antipa" Constanta, in 2008. The results of the experiments conducted have shown that the breeding and subsequent rearing of these fish in captivity is feasible (Figure 2).

Figure 2. *H. guttulatus* male in controlled environment (captive breeding experiment) (Photo: Nenciu)

However, the major drawback in rearing *H. guttulatus* was supplying the most appropriate diet for the fry, as many individuals died of starvation before reaching maturity due to the lack of a small-sized food alternative (Nenciu et al., 2015a).
An experiment previously conducted by NIMRD experts indicated that a combined diet of the rotifer *Brachionus plicatilis* and the brine shrimp *Artemia salina* is the most recommended for rearing seahorses in captivity, due to the advantages that the two invertebrates have separately. On the one hand, rotifers develop greater densities and have a higher protein content (reflected in the protein content of the batches analyzed), while brine shrimps have a higher lipid content and are easier to hunt, being larger and more visible in the tank and always selected as preferred prey (Nenciu et al., 2015a).
Under these circumstances, the current research aimed at the examination of the gut content of wild seahorse specimens, in order to determine the prey preferences of the species along the Romanian Black Sea coast.

MATERIALS AND METHODS

For the gut content analysis of wild specimens, 30 individuals were collected from the Romanian coast, from by-catches in commercial trawls and pound nets (3 sampling Stations, Edighiol, Agigea and 2 Mai, 10 individuals each, August 2017) (Figure 3).

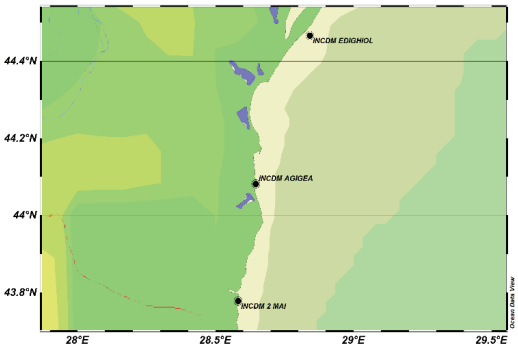

Figure 3. Sampling stations for wild *H. guttulatus* specimens

Specimens were dissected and guts were removed (Figure 4).

Figure 4. Dissection and gut content analysis (Photo: Nenciu M.I.)

The number of empty and full guts of the specimens was recorded. Gut contents were recovered and homogenized in Petri dishes and examined using a binocular stereo microscope.
The prey items were identified to the lowest possible taxonomic level and assigned to different prey categories such as Amphipoda, Isopoda, Copepoda etc.
Two types of methods were used, namely qualitative and quantitative methods (Totoiu et al., 2014). The qualitative analysis consisted in the identification of the food components found in the fish's gut. The quantitative method consisted in numerical analysis (frequency of occurrence - FO% - and numerical abundance - N - of the analyzed stomachs where a specific prey group was identified) (Hyslop, 1980; Hansson, 1998).
The frequencies of occurrence (FO%), as numerical percentages of prey items, were calculated to characterize the gut contents (Hyslop, 1980; Hansson, 1998). The frequency of occurrence calculates the percentage of the total number of guts in which the specific prey species occurs:

$$FO\% = FOi/FOt \times 100,$$

where: FOi is the number of guts in which the species "I" occurs, and FOt is the total number

of full guts. The Index of Relative Importance (IRI) was impossible to calculate due to the very small size of the prey and guts, which prevented weighing.

RESULTS AND DISCUSSIONS

The three sampling stations were not selected randomly (Figure 3), but so as to cover the northern, central and southern parts of the Romanian coast, with different habitat types. The 30 individuals investigated, all adults, 10 per each station, were equally divided between males and females (5 males and 5 females per each station). No significant differences between males and females were identified in any of the sampling station.

In the northern station, Edighiol, the main food group identified were amphipods (FO% 66.66), two thirds of the prey items in the gut contents being represented by this group. The next group as frequency was meroplankton, namely *Balanus* cypris larvae (FO% 44.44). The other three items, Isopoda, Copepoda and other (non-identified semi-digested items) held equal shares (FO% 22.22) (Table 1, Figure 5).

Table 1. Prey groups identified in *H. guttulatus* gut contents (Station Edighiol)

Group	N	FO%
Crustacea: Amphipoda	6	66.66
Crustacea: Isopoda	2	22.22
Crustacea: Copepoda	2	22.22
Meroplankton: *Balanus* cypris	4	44.44
Other (non-identified)	2	22.22

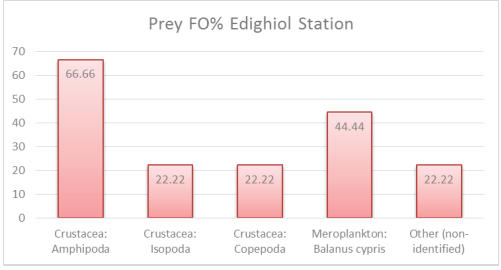

Figure 5. Frequency of occurrence (FO%) of prey groups identified in *H. guttulatus* gut contents (Station Edighiol)

In the central station (Agigea), however, the largest share was held by meroplankton - Balanus cypris larvae (FO% 77.77), followed by Amphipoda (FO% 55.55), isopod crustaceans (FO% 33.33), with the smallest share held by copepods and other non-identified items (FO% 22.22) (Table 2, Figure 6).

Table 2. Prey groups identified in *H. guttulatus* gut contents (Station Agigea)

Group	N	FO%
Crustacea: Amphipoda	5	55.55
Crustacea: Isopoda	3	33.33
Crustacea: Copepoda	2	22.22
Meroplankton: *Balanus* cypris	7	77.77
Other (non-identified)	2	22.22

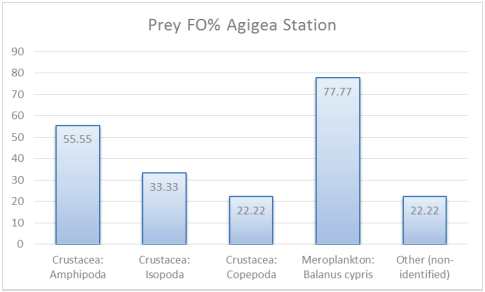

Figure 6. Frequency of occurrence (FO%) of prey groups identified in *H. guttulatus* gut contents (Station Agigea)

The southern most station (2 Mai) also recorded a clear dominance of *Balanus* cypris larvae (meroplankton), which were identified in all seahorse guts analyzed (FO% 100), followed by amphipods (FO% 50), copepods (FO% 40), other non-identified items (FO% 30), the last group as frequency being isopods (FO% 20) (Table 3, Figure 7).

Table 3. Prey groups identified in *H. guttulatus* gut contents (Station 2 Mai)

Group	N	FO%
Crustacea: Amphipoda	5	50
Crustacea: Isopoda	2	20
Crustacea: Copepoda	4	40
Meroplankton: *Balanus* cypris	10	100
Other (non-identified)	3	30

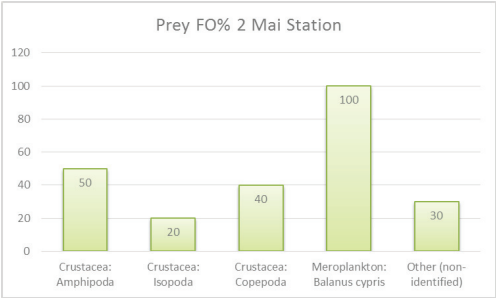

Figure 7. Frequency of occurrence (FO%) of prey groups identified in *H. guttulatus* gut contents (Station 2 Mai)

Overall, for all stations investigated, the dominant prey group was represented by *Balanus* cypris larvae (meroplankton), with FO% 70, followed by amphipods (FO% 57.14) Isopoda and Copepoda recorded close values, FO% 25 and FO% 21.42, respectively (Table 4, Figure 8).
Out of the total guts investigated, only two were lacking any prey, while all other contained, in different shares, all the major groups identified.

Table 4. Prey groups identified in *H. guttulatus* gut contents (TOTAL)

Group	N	FO%
Crustacea: Amphipoda	11	57.14
Crustacea: Isopoda	7	25
Crustacea: Copepoda	8	21.42
Meroplankton: *Balanus* **cypris**	21	75
Other (non-identified)	7	25

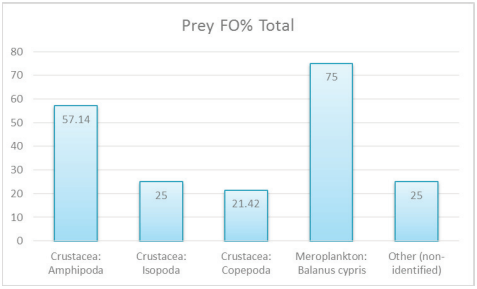

Figure 8. Frequency of occurrence (FO%) of prey groups identified in *H. guttulatus* gut contents (TOTAL)

Amphibalanus improvisus (Darwin, 1854) (*Balanus*) is a small sessile crustacean, typical for the shallow fringe of sea (less than 10 m deep), occurring in marine and brackish environments. *A. improvisus* has been dispersed by shipment outside its natural distribution area, which is considered to be the western Atlantic. It was first recorded as invasive in the Black Sea in 1844 (Skolka and Gomoiu, 2004). Since then, it has rapidly developed on rocky substrate, on man-made structures, buoys, ships' hulls, the shells of crabs and mollusks (mussels), and certain seaweeds. Its larval stages have become a significant dietary item in the food chain of coastal ecosystems, especially for small fish such as Syngnathids (seahorses and pipefish). The analyses performed on the gut contents of seahorses at the Romanian Black Sea coast revealed that *Balanus* larvae were identified in 21 of the 28 full guts (FO% 75), probably due to its high availability at the time sampling was performed (August) (Figure 9). The distribution map points out its highest abundance in the southern part of the Romanian coast, not surprisingly given the rocky habitat type and the existence of numerous hydro-technical constructions (Figure 10).

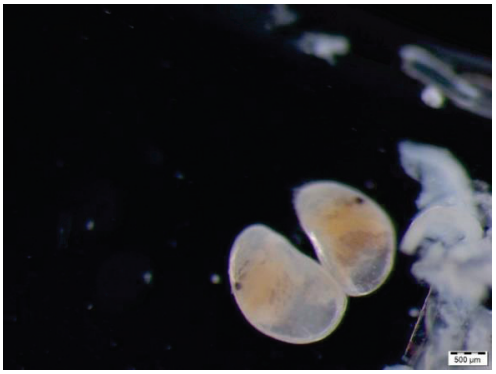

Figure 9. *Balanus* cypris larvae in seahorse gut (Photo: Harcota G.E.)

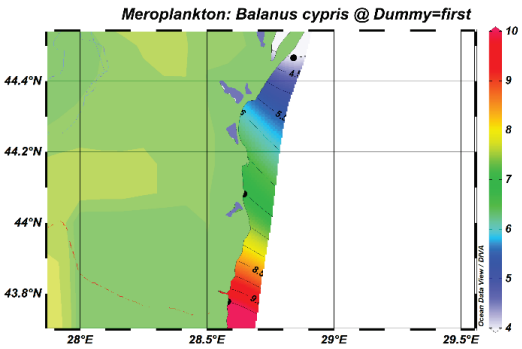

Figure 10. Distribution map of *Balanus* larvae identified in the gut content of *H. guttulatus*

The second dietary group identified were amphipod crustaceans (FO% 57.14). Amphipods are utilizers of primary and partly secondary production in marine ecosystems. Moreover, they transform sediment composition by enriching it with organic matter. These small crustaceans are dietary items for many of coastal fishes, seahorses included (Figure 11). Relatively large in size, amphipods are easily visible and provide a high nutritional value for the voracious seahorses.
The distribution map shows a higher abundance of amphipods in the northern part of the coast (Figure 12).

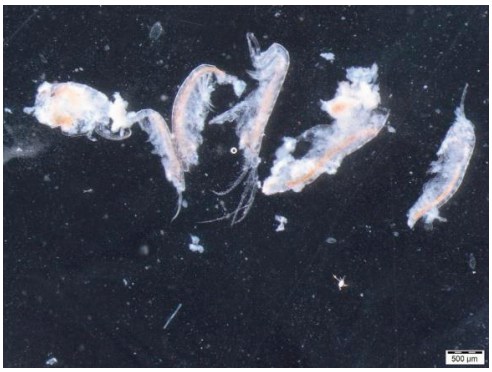

Figure 11. Amphipods identified in seahorse gut content (Photo: Harcota G.E.)

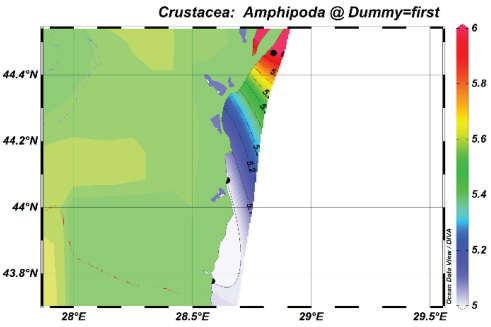

Figure 12. Distribution map of Amphipoda identified in the gut content of *H. guttulatus*

Copepods (FO% 21.42) were not so frequent in the gut contents of the investigated specimens, most likely due to the fact that they were all adults, thus preferably selecting larger prey items (Figure 13). Nevertheless, copepods are the biggest source of protein in the marine environment and are an important prey, especially for small forage fish or juveniles. The distribution map (Figure 14) also shows a dominance of copepods in the southern part of the coast.

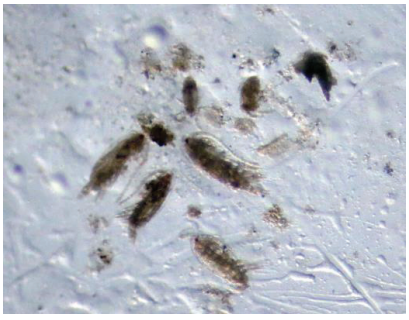

Figure 13. Copepods identified in seahorse gut content (Photo: Bisinicu E.)

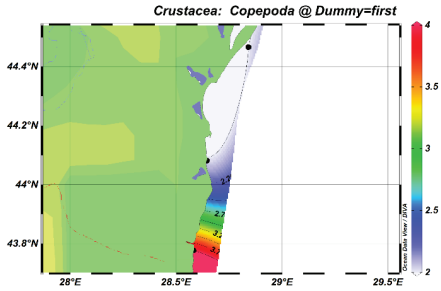

Figure 14. Distribution map of Copepoda identified in the gut content of *H. guttulatus*

Similarly to copepods, isopod crustaceans did not occur in extremely high frequencies (FO% 25). However, they are often dietary components of coastal fish, due to their large size and availability.

The distribution map (Figure 15) revealed a concentration of isopod crustaceans in the central part of the coast.

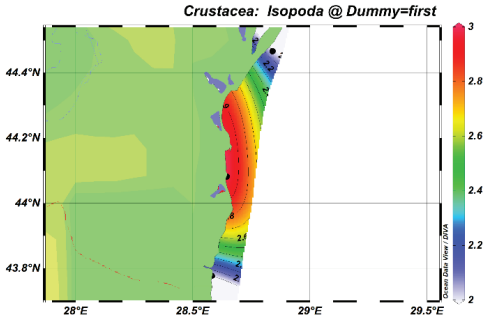

Figure 15. Distribution map of Isopoda identified in the gut content of *H. guttulatus*

The group generically called „other non-identified items" includes semi-digested prey fragments, impossible to identify (Figure 16).

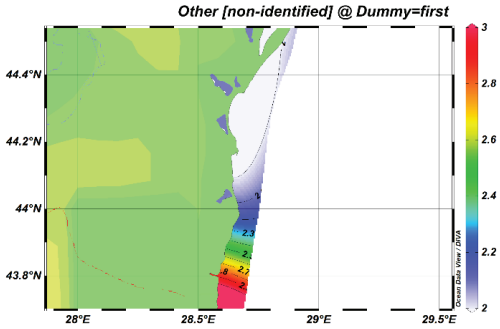

Figure 16. Distribution map of other preys (non-identified) in the gut content of *H. guttulatus*

To date, there are no other studies on the gut contents of *H. guttulatus* in the Black Sea, which makes it difficult to infer which other prey items are likely to be found in seahorses' diet.

Yet, similar investigations performed on the same species in the Aegean Sea revealed that Mysidaceae (42.59%) and Decapod crustacean larvae (22.22%) constituted the most important food source of *H. guttulatus*. (Gurkan et al., 2011). Amphipods and isopods were also present. Based on the number and frequency, the dominant preys of *H. guttulatus* in the Aegean are Decapod crustacean larvae, Mysidaceae and Amphipoda and unidentified prey. These results are consistent with previously published data (Kitsos et al., 2008).

CONCLUSIONS

Summing up, the dominant prey group identified in the gut content of *H. guttulatus* along the Romanian Black Sea coast was represented by *Balanus* cypris larvae (meroplankton) (FO% 70), followed by amphipods (FO% 57.14) Isopoda (FO% 25) and Copepoda (FO% 21.42). The other non-identified prey items could be represented by Mysid crustaceans or Decapod crustacean larvae.

No significant differences between the gut contents of males and females were identified.

Out of the total guts investigated, only two were lacking any prey, while all other contained, in different shares, all the major groups identified.

It is important to point out the fact that the dominance of one group or the other is closely related to its availability in the environment at a certain moment.

Thus, seahorses, as opportunistic predators, will preserve energy by selecting the largest and most available prey items.

This is why it is not by chance that *Balanus* larvae, which are abundant in areas with hard substrate, were identified in large numbers in the guts of seahorses sampled in the southern part of the Romanian coast.

The preference for larger prey (amphipods, for example) was also demonstrated by captive rearing experiments, where adult seahorse always selected the larger brine shrimps *Artemia salina* compared to the rotifer *Brachionus plicatilis*.

Further investigations will be performed on a larger number of specimens, in order to reveal more correlations between prey preference, habitat type and prey availability in the long-snouted seahorse *H. guttulatus* at the Romanian Black Sea coast.

ACKNOWLEDGEMENTS

This study has been carried out with financial support from the GOFORIT project („IntelliGent Oceanographically-based short-term fishery FORecastIng applicaTions"), funded by the Romanian Executive Unit for Financing Higher Education, Research, Development and Innovation (UEFISCDI Contract no. 27/2015) through the ERA-COFASP Programme.

REFERENCES

Banarescu P., 1964. Fauna of the Romanian People's Republic, Pisces - Osteichthyes (Ganoid and bony fish), Vol. XIII, Academy Publishing House, Bucharest, 959.

Caldwell I.R., Vincent A.C.J., 2012. A sedentary fish on the move: effects of displacement on long-snouted seahorse (*Hippocampus guttulatus* Cuvier) movement and habitat use. Environmental Biology of Fishes, 96 (1), 67-75.

Foster S.J., Vincent A.C.J., 2004. Life history and ecology of seahorses: implications for conservation and management. Journal of Fish Biology, 65, 1-61.

Gurkan S., Taskavak E., Sever T.M., Akalin S., 2011. Gut contents of two European seahorses *Hippocampus hippocampus* and *Hippocampus guttulatus* in the Aegean Sea, Coasts of Turkey. Pakistan J. Zool., Vol. 43 (6), 1197-1201.

Hansson S., 1998. Methods of studying fish feeding: a comment. Canadian Journal of Fisheries and Aquatic Sciences, 55 (12), 2706-2707.

Hyslop E.J., 1980. Stomach content analysis - a review of methods and their application Journal of Fish Biology, 17 (4), 411-429.

Kendrick A. J., Hyndes G. A., 2005. Variations in the dietary compositions of morphologically diverse syngnathid fishes. Environmental Biology of Fishes, 72, 415-427.

Kitsos M.S., Tzomos T.H., Anagnostopoulou L., Koukouras A., 2008. Diet composition of the seahorses, *Hippocampus guttulatus* Cuvier 1829 and *Hippocampus hippocampus* (L., 1758) (Teleostei, Syngnathidae) in the Aegean Sea. Journal of Fish Biology, 72, 1259-1267.

Lourie S.A., Foster S.J., Cooper E.W.T., Vincent A.C.J., 2004. A Guide to the Identification of Seahorses.

Project Seahorse and TRAFFIC North America. University of British Columbia and World Wildlife Fund, Washington D.C.

Nenciu M.-I., Rosioru D., Coatu V., Oros A., Rosoiu N., 2013. Characterization of the environmental conditions of the long-snouted seahorse habitat at the Romanian coast. Journal of Environmental Protection and Ecology, Vol. 14 (4), 1695-1702.

Nenciu M.-I., Rosoiu N., Petcu C.L., Zaharia T., Nita V., Maximov V., Rosioru D., 2015a. Effects of different live feed diets applied to the long-snouted seahorse (*Hippocampus guttulatus* Cuvier, 1829). Turkish Journal of Fisheries and Aquatic Sciences (TrJFAS), Central Fisheries Research Institute, Trabzon, Turkey, 15, 406-415.

Nenciu M.I., Rosioru D., Zaharia T., Onara D., Taflan E., Holostenco D., Rosoiu N., 2015b. Preliminary genetic analyses for the species *Hippocampus guttulatus* (Cuvier, 1829) in the Romanian Black Sea. Book of Abstracts of the International Symposium Protection of the Black Sea Ecosystem and Sustainable Management of Maritime Activities - PROMARE 2015, 7th Edition, Constanta, Romania, 29-31 October 2015, Editors S. Nicolaev, F.K. Vosniakos, T. Zaharia, M. Golumbeanu, M. Nenciu, 71.

Radu G., Radu E., 2008. Determinator of the main fish species in the Black Sea, Virom Publishing House, Constanța, 558.

Skolka M., Gomoiu M.T., 2004. Invasive species in Black Sea. Ecological impact of invasive species in aquatic ecosystems. Ovidius University Press, Constanța, 180.

Taflan E., Nenciu M.I., Holostenco D., Ciorpac M., Filimon A., Danilov C.S., 2017. Life history of the Black Sea long-snouted seahorse (*Hippocampus guttulatus* Cuvier, 1829): colonization pattern and genetic differentiation". Book of Abstracts of the International Symposium Protection of the Black Sea Ecosystem and Sustainable Management of Maritime Activities - PROMARE 2017, 8th Edition, Constanta, Romania, 17-9 September 2017, CD Press Publishing House, Bucharest, 84.

Totoiu A., Tiganov G., Galatchi M., Nenciu M., 2014. Food array analysis in turbot *Psetta maeotica* (Pallas, 1811) at the Romanian Black sea coast in 2013. Recherches Marines, 44, 164-172.

Van Wassenbergh S., Roos G., Ferry L., 2001. An adaptive explanation for the horse-like shape of seahorses. Nature Communications 2, 164, https://www.nature.com/articles/ncomms1168.

Woodall L., 2012. *Hippocampus guttulatus*. The IUCN Red List of Threatened Species. Version 2018.2, www.iucnredlist.org, downloaded on 3 March 2018.

Scientific Papers. Series D. Animal Science. Vol. LXI, Number 1, 2018
ISSN 2285-5750; ISSN CD-ROM 2285-5769; ISSN Online 2393-2260; ISSN-L 2285-5750

MONITORING OF ALIEN FISH SPECIES PRUSSIAN CARP (*Carassius gibelio*) IN CROATIAN PART OF THE SAVA RIVER AREA FROM 2004 TO 2017

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Abstract

The alien fish species Prussian or gibel carp (Carassius gibelio) was introduced to Croatia from Asia several decades ago. The analysis was performed by the official monitoring data from the Final Report for the Ministry of Agriculture of the Republic of Croatia by the Faculty of Agriculture, University of Zagreb during the last eight years (2010-2017) in combination with the statistical analysis of the fisheries data of the Krapina-Zagorje County and Sisak-Moslavina County in the period from 2004 to 2015. The proportion of Prussian carp catches (in kg) increased at Sisak-Moslavina County from 26.45% to 41.84% from 2004 to 2015, leading to a significant decrease of catches of common carp from 25.59% to 8.70%, respectively (r^2 = 0,936; $p < 0,01$). The catch per unit effort (CPUE) of Prussian carp, defined as annual catch by an average angler, significantly increased, from 6 kg at the beginning of this period to 14 kg at its end. These data show that Prussian carp still enlarges its populations along the Sava River.

Key words*: gibel carp, common carp, CPUE, invasive species, competition.*

INTRODUCTION

The alien fish species Prussian or gibel carp (*Carassius gibelio*) spreads very rapidly and is considered to be one of the causes of the decline of other fish populations, especially due to the possibility of gynogenesis, a process that gives rise to new females. Females spawn with several other species, for example *Cyprinus carpio* (Figure 3) and *Carassius carassius*, but the eggs just develop without being actually fertilized resulting in an only female population.

The milt of male fish is needed to initiate development of the eggs, but when the embryos form, the chromosomes from the males are excluded. The offspring produced are thus copies of the female. This is especially problem with common carp because after spawning stay only Prussian carp females.

In Europe, gibel populations are considered as triploid and only females (Kottelat et al., 2007.). Because of that they have a bad influence on the share of some species in the catch.

The increasing number of Prussian carp individuals over the years can influence the share of common carp (*Cyprinus carpio*) (Gaygusuz et al., 2015). Therefore the aim of this paper is to check such changes analyzing the fisheries information from the Sava river, obtained from three sources (Jakopinac, 2016; Augustin, 2017; Treer et al., 2017).

MATERIALS AND METHODS

Data which were processed are the official monitoring data from the Final Report for the Ministry of Agriculture of the Republic of Croatia by the Faculty of Agriculture, University of Zagreb (2010-2017) (Treer et al., 2017) in combination with the statistical analysis of the fisheries data of the Krapina-Zagorje County (Jakopinac, 2016) and Sisak-Moslavina County (Augustin, 2017) in the period from 2004 to 2015. The scientific sampling by electric gear at several locations along the whole section of the Sava River in Croatia were made in different seasons. The data are interpreted for the period of time from 2004 to 2017. Catch per unit effort (CPUE) is defined as annual catch (kg) by an average angler.

RESULTS AND DISCUSIONS

The proportion of Prussian carp catches (in kg) increased at Sisak-Moslavina County from

26.45% to 41.84% from 2004 to 2015, leading to a significant decrease of catches of common carp from 25.59% to 8.70% (Figure 1).

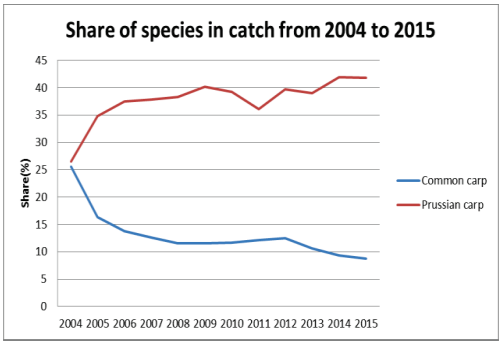

Figure 1. Movement of share of species in total catch in Sisak-Moslavina county from 2004 to 2015

This points to a very pronounced competition between common carp and Prussian carp in this area, which is statistically significant (r^2=0,936; p<0,01), (Figure 2).

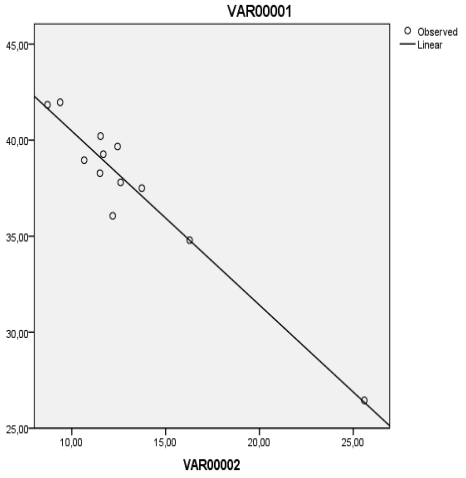

Figure 2. Correlation between the amounts of catches of common carp and Prussian carp through the years (r^2 =0.936, p <0.01) in Sisak-Moslavina County from 2004 to 2015

CPUE of Prussian carp significantly increased, from 6 kg at the beginning of this period to 14 kg at its end. In 2012 it even tripled to nearly 19 kg (Figure 4).

This is also confirmed by the CPUE of common carp, which is in observed period halved. While the average fisherman in 2004 caught nearly 6 kg of carp each year, as in 2012, in 2015 it fell to below 3 kg (Figure 5).

Figure 3. The phenotype difference between common carp (up) and Prussian carp (down)

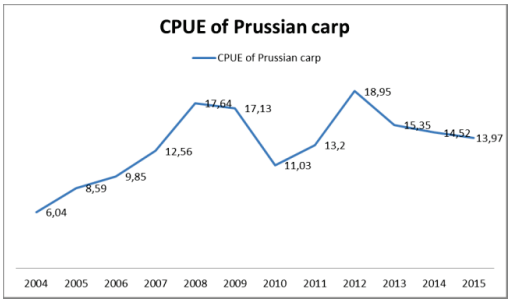

Figure 4. Movement of catch per unit effort of Prussian carp in Sisak-Moslavina County from 2004 to 2015

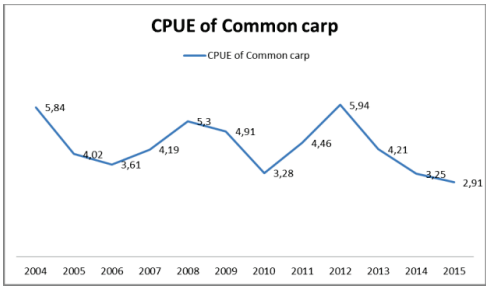

Figure 5. Changes of catch per unit effort of common carp in Sisak-Moslavina County from 2004 to 2015

The significant increase in the number of this species caught has been confirmed by scientific sampling by electric gear at several locations along the whole section of the Sava River in Croatia over several years.

These data show that Prussian carp still enlarges its populations along the Sava River. In the first sampling in 2004 there was only found 4 Prussian carps and in the last sampling in 2017 there was found even 52 Prussian

carps. Research was done on the same locations. Over the years number of Prussian carp in the Sava River area increased and this fish species occupies a share in the total catch of 37.73% (Figure 6).
Average weight of Prussian carp also increased in this part of Sava River area.
Such results in Sisak-Moslavina County are more visible than in Krapina - Zagorje County, but there is also increase of average weight (Figures 7 and 8). These results correspond with the ones find by Gaygusuz et al. (2015).

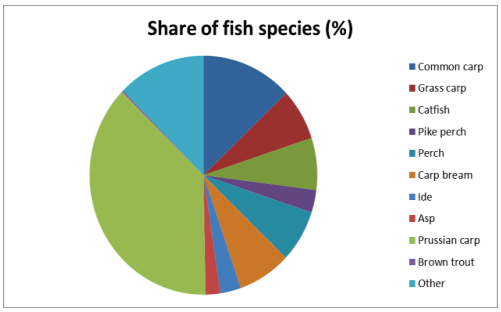

Figure 6. Average mass fraction of species in total catch in Sisak-Moslavina County from 2004 to 2015

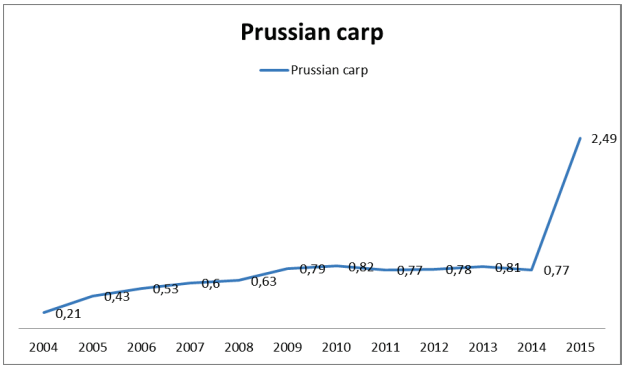

Figure 7. Annual dynamics of the average weight change of Prussian carp in Sisak-Moslavina County from 2004 to 2015

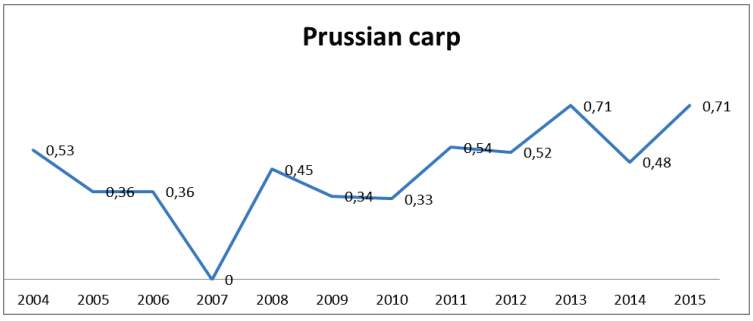

Figure 8. Annual dynamics of the average weight change of Prussian carp in Krapina-Zagorje county from 2004 to 2015

CONCLUSIONS

The proportion of Prussian carp catches (in kg) increased at Sisak-Moslavina County from 26.45% to 41.84% from 2004 to 2015, leading to a significant decrease of catches of common carp from 25.59% to 8.70%.
In the first sampling in 2004 there was only found 4 Prussian carps and in the last sampling by electric gear in 2017 there was found even 52 Prussian carps. The increase in the number of Prussian carp could in the future be endangered the existence of domestic species.
Therefore, this phenomenon should be closely monitored next years. In addition, it is very important to share the catch data to those who are realized in open waters than those realized in closed ecosystems. Therefore, the continuous monitoring of its possible impact on domestic species is important.

REFERENCES

Augustin K., 2017. Analiza podataka ribolova Sisačko-moslavačke županije. Agronomski fakultet Sveučilišta u Zagrebu, 22.

Gaygusuz O., Tarkan A. S.; Aydin H., Dorak Z., Top N., Karakus U., Vilizzi L., 2015. Stocking of Common Carp (*Cyprinus carpio*) into Some Newly-Established Reservoirs of North-West Anatolia May Enhance the Spread of Non-Native Fish. Turkish journal of fisheries and aquatic sciences, 15, 833-840.

Jakopinac T., 2016. Statistička analiza ribolova Krapinsko-zagorske županije. Agronomski fakultet Sveučilita u Zagrebu, 38.

Kottelat M., Freyhof J., 2007. Handbook of European freshwater fishes. Publications Kottelat, Cornol and Freyhof, Berlin, 646.

Piria M., Simonović P., NikolićV., Ristovska M., Apostolou A., Adrović A., Povž M., Zanella D., Mrdak D., Milošević D., Vardakas L., Koutsikos N., Kalogianni E., Gregori A., Kostov V., Škrijelj R., Korro K., Bakiu R., Tarkan A.S., Joy M.K., 2017. Alien freshwater fish species in the Balkans – vectors and pathways of introduction. Fish and fisheries, DOI:10.1111/faf.12242

Treer T., Piria M., Tomljanović T., Šprem N., Matulić D., Rezić A., 2017. Završno izvješće o provođenju programa praćenja stanja u slatkovodnom ribarstvu u 2017. godini (Final Report of implementation monitoring program of the freshwater fisheries in 2017). Agronomski fakultet, Zagreb, 94 (in Croatian).

Scientific Papers. Series D. Animal Science. Vol. LXI, Number 1, 2018
ISSN 2285-5750; ISSN CD-ROM 2285-5769; ISSN Online 2393-2260; ISSN-L 2285-5750

THE EFFECTS OF FODDER ADDITIVES (ACTIGEN, SELPLEX, YEA-SACC 1026) ADMINISTERED IN THE DIET OF THE SIBERIAN STURGEON (*Acipenser baerii*) ON MEAT QUALITY

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Abstract

The research has followed the effects of the feeding bio-additives Actigen, SelPlex and Yea-Sacc-1026 on the quality of the Siberian sturgeon (Acipenser baerii) meat. The experiments were carried out on a number of 108 Siberian sturgeons distributed in four groups, each with 27 individuals per group. In the experimental group 1 (1E), probiotic Actigen was added 0.08% to the fodder in the combined fodder, 0.03% organic selenium (SelPlex) was added to the experimental group 2 (2E), and to the third experimental group (3E) probiotic Yea-Sacc-1026 in proportion of 0.2% was added. The experimental period lasted 68 weeks, from 1st of July 2016 to 2nd of November 2017. At the end of the experiment control slaughters were made, 5 sturgeons from each group, the meat quality being determined but also its chemical composition. In terms of slaughter yield, equal values have been found between the experimental and control groups, the differences being not statistically represented. The administration of bio-additives (Actigen, SelPlex and Yea-Sacc 1026) resulted in a decrease in water content in experimental groups, 1E (Actigen) 28.71%, 2E (SelPlex) 26.39% and 3E (Yea-Sacc 1026), while the control (C) has 31.20%. The average percentage of crude protein of the 3E (47.31%) increased by 11% compared to the control group (36.29%) and by 6.5% compared to the experimental groups 1E and 2E. The highest gross fat value was observed in 2E group (31.75%) exceeding the control group score (30.27%), while the 3E group has shown the lowest value (23.3%) followed by experimental group 1E with 26.33%. The crude ash content did not show statistically represented differences between the experimental groups and the control group.

Key words*: Siberian sturgeon, Actigen, SelPlex, Yea-Sacc 1026, meat quality, meat composition.*

INTRODUCTION

As an alternative strategy for antibiotic use, in aquaculture, prebiotics and probiotics are more recently used in feeding fish species of culture through their beneficial effects on production and health performance.

In a series of tests, one of the firsts dating from 2011 (Hung et al.), it appear that the effect of Actigen prebiotic on survival rate in pangasius (*Pangasianodon hypophthalmus*) artificially infected with *Edwardsiella ictaluri* bacteria was evaluated.

There was observed a high immunity rate to this bacterium, so the Actigen prebiotic increased the survival rate from the control group. The positive effects of Actigen administration at a dose of 0.04% in Nile Tilapia (*Oreochromis niloticus*) feeding on growth rate improvement were highlighted by Yutharaksanukul P. (2011, cited by Ringo et al., 2010), and 0.08% and 0.12% concentrations were found to reduce feed conversion, to improve the immune status and health, and reduce sleep mortality (Hung et al., 2012).

A new trend in aquaculture is to use probiotics with positive effects on growth and health such Yea-Sacc-1026. The use of this probiotic in aquaculture is relatively new, but in terrestrial animals Yea-Sacc-1026 has been used with good and very good results to improve the growth rate. Mohsen Abdel (2010) shown that using yeast as a probiotic in fish fodder increases their resistance to bacterial infections and increases digestive enzyme activity in the intestine. The administration of organic selenium (Sel-Plex) to carp (*Cyprinus carpio* L.) has positively influenced the survival rate and the accumulation of biomass (Ani et al., 2008; Ani et al., 2009). When feeding the fountain trout (*Slvelinus fontinalis* M.) organic selenium (Sel-Plex) had positive effects on growth performance and survival rate (Şara et al., 2009b; Şara et al., 2010a, b; Barbu et al., 2009).

In the literature we have not found data on the influence of some additives on the quality of

the meat in the sturgeon. In the present paper, we suggested that the effects of the mentioned bioadditives on some meat quality indices in the Siberian sturgeon should be highlighted.

MATERIALS AND METHODS

Research has been done at the Forestry Research Institute in Gilău, Cluj county, Romania between July 2016 and November 2017 on a number of 108 sample individuals distributed in three experimental groups (Actigen 0.08%, Sel-Plex 0.03% and Yea-Sacc 0.2%) and a control one. The species of interest was represented by the Siberian sturgeon (*Acipenser baerii*) by the age of 10 months at the beginning of the experiment. At the beginning of the experiment, the fish had an average body weight of 400 g/specimen and 50 cm body length. All four groups were maintained under the same environmental conditions in identical concrete basins (4/1/1.3 m basins) with a surface area of 4 m^2 and a water flow rate of 20 l/min/basin. Recorded chemical parameters of the water were dissolved oxygen, pH, sulphates, ammoniacal nitrogen, nitrites and nitrates (Table 1).

Table 1. Chemical parameters of the water from the experiment

Specification	Unity measure (U.M.)	Values
O_2	mg/l	8.74
pH	pH	7.45
Sulphates	mg/l	32
NO_2	mg/l	0
NO_3	mg/l	0
NH_3	mg/l	0.09

The fodder used was the Skretting grain fodder without addition of bio-additives for the control group, respectively with addition of Actigen 0.08% for experimental group 1, Sel-Plex 0.03% for experimental group 2 and Yea-Sacc 0.2% for experimental group 3.

The nutritional characteristics of the fodder are shown in Table 2.

The fodder was administered in two servings/day at 9 am and 18 pm. Distributed fodder availability varied based on water temperature and total biomass. At the end of the experiment, control slaughters were made, five individuals of each group, whose body weight has the average values of the group they were part of.

Table 2. Nutritional characteristics of Skretting fodder

Specification	Unity measure (U.M.)	Values
Crude proteins	%	41
Crude ash	%	7.8
Crude fat	%	12
Crude cellulose	%	2.5
Phosphorus	mg	1.1
Copper	mg	6
Vitamin A	U.I.	10000
Vitamin E	mg	150

Slaughter yield, meat quality and chemical composition were established as a result of the slaughters. To determine the slaughter yield, specific operations have been performed on this parameter, such to measure various anatomic regions. Meat samples were collected from each individual to determine the chemical composition of the meat. Laboratory analyzes determined dry matter, crude fat, crude protein and raw ash in meat. Raw experimental data were statistically analyzed by T test using GraphPad InStat 3.

RESULTS AND DISCUSSIONS

The proportion of different body parts (expressed by weight and percentage) as well as the slaughter yield are shown in Tables 3, 4 and 5, respectively.

Analyzing Tables 4 and 5 we can conclude that the administration of additives (Actigen, SelPlex and Yea-Sacc) did not significantly affect the slaughter yield, its values being relatively equal. The chemical composition of the Siberian sturgeon meat at the end of the experimental period is shown in Table 6.

Table 3. The proportion of different body parts of fish

Specification	Group							
	Control		1E (Actigen 0.08%)		2E (SelPlex 0.03%)		3E (Yea-Sacc 0.2%)	
	g	%	g	%	g	%	g	%
Body weight	870	100	955	100	890	100	1165	100
Head	134	15.40	148	15.49	124	13.93	151	12.96
Viscera	51	5.86	50	5.23	66	7.41	87	7.46

Table 4. The slaughter yield at the four batches at the end of the experimental period (trunk)

Specification	Control	1E (Actigen 0.08%)	2E (SelPlex 0.03%)	3E (Yea-Sacc 0.2%)
Slaughter yield	77.40%	78.01%	78.08%	78.97%

Table 5. Slaughter yield at the four batches, trunk and head

Specification	Control	1E (Actigen 0.08%)	2E (SelPlex 0.03%)	3E (Yea-Sacc 0.2%)
Slaughter yield	93.05%	93.50%	93.06%	93.73%

Table 6. The chemical composition of Siberian sturgeon meat in the four batches

Specification	Control	1E (Actigen 0.08%)	2E (SelPlex 0.03%)	3E (Yea-Sacc 0.2%)
Dry matter (%)	68.8± 3.38	71.29±2.19	73.61± 0.74 *	70.3± 3.09
Crude protein (%)	36.29±2.95	40.88± 1.14*	40.72± 1.39*	47.31 ± 2.15 ***
Crude fat (%)	30.27±4.32	26.33± 3.99	31.75±3.66	23.33 ± 5.75 *
Crude ash (%)	2.83± 0.19	3.44± 0.65	3.07± 0.17	2.83± 0.69

*** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$

Lower water content was observed in E3 group by comparing with control group; significant differences were observed between groups 3E and 2E, the highest dry matter value was recorded in group 2E which was fed with organic selenium (SelPlex) (Table 7).

Table 7. The statistical significance of the differences between the four batches regarding the chemical composition of Siberian sturgeon meat (Student test)

Comparison	Average differences	q value	*P* value
Dry matter			
Control vs 2E	-4.812	4.189	*<0.05
3E vs 2E	3.319	4.712	*<0.05
Crude protein			
Control vs 1E	-4.598	5.046	*<0.05
Control vs 2E	-4.436	4.868	*<0.05
Control vs 3E	-11.020	12.094	***<0.001
1E vs 3E	-6.422	7.048	***<0.001
2E vs 3E	-6.584	7.225	***<0.001
Crude fat			
Control vs 3E	8.716	4.509	*<0.05
2E vs 3E	8.422	4.357	*<0.05

* significant differences; ** distinct significant differences; *** highest significant differences

When meat protein content is analyzed, there are very significant differences between group 3E (Yea-Sacc) and group 1E (Actigen), also between 2E group (Selplex) and control group. Significant differences can be seen between batches 1E and control group as well as between group 2E and control group. All three experimental groups showed an increase in protein content, the highest value being recorded in group 3E (Yea-Sacc).

Analyzing crude fat, significant differences were observed between group 3E (Yea-Sacc) and control group, respectively between group 2E (SelPlex) and group 3E (Yea-Sacc); the highest crude fat value was observed in experimental group 2E (31.75%), exceeding the control group (30.27%), whereas experimental group 3E had the lowest value (23.3%).

Crude ash content of Siberian sturgeon meat analysis revealed that no significant differences among all experimental groups were recorded.

Overall, there is an improvement in meat quality as a result of the protein content increase and fat reduction in experimental groups 1E and 3E.

CONCLUSIONS

Administration of various bio-additives to Siberian sturgeon did not favorably influence the slaughter yield, the differences between experimental and control groups being insignificant.

The water content of Siberian sturgeon meat was lower in all 3 experimental groups compared to the control group.

Administration of bio-additives has led to an increase in the crude protein content of Siberian sturgeon meat in all three experimental groups compared to the control group. 3E Yea-Sacc group recorded very significant differences based on meat protein content analysis of 47.3%, 11% higher than the control group.

The highest crude fat value in Siberian sturgeon meat was observed in the experimental group 2E (SelPlex) (31.75%), exceeding the values recorded in the control group (30.27%), while the experimental group 3E (Yea-Sacc) has recorded the lowest value (23.3%).

Crude ash content of Siberian sturgeon meat analysis revealed that no significant differences among all experimental groups were recorded.

The results obtained demonstrate the beneficial effects of Actigen, Yea-Sacc-1026 bio-additives and organic selenium on the quality of Siberian sturgeon meat.

REFERENCES

Ani Alina, Sara A., Barbu A., 2008. The influence of organic selenium on carp fry rearing. Buletin USAMV-CN Cluj-Napoca, 65(1-2), pISSN 1843-5262.

Ani Alina, Sara A., Barbu A., 2009. Effects of Sel-Plex on performance and survival of carp fry, Alltech USA.

Barbu A., Sara A., Ani Alina, Bentea M., 2009. The effects of some fodder bioadditives on the production performances of brook trout (*Salvelinus fontinalis* M.), Scientifical Papers Animal Sciences and Biotechnologies, 42(2), 8-13, Ed. Agroprint Timisoara.

Hung L., Kim T.N.T., 2007. Reducing fish meal utilization in Pangasius catfish feeds trough application of enzymes, Aian-Pacific Aquaculture, Meeting abstract 27.

Hung L.T., 2011. Building new aquafeeds: Feeding for health and performance in Tra catfish (*Pangasiaodon hypophtalamus*). The Alltech 28th annual International Symposium, Lexington, Kentucky.

Hung L.T., Trung N.L., Thuy D.N., 2012. Effects of Actigen on growth performances and fish health improvement of Tra catfish (*Pangasiaodon hypophtalamus*). The Alltech 28th annual International Symposium, Lexington, Kentucky.

Hung S.S.O., Storebakken T., Cui Y., Tian L., 1997. High energy diets for white sturgeon Acipenser transmontanus Richardson, Aquaculture Nutrition, vol. 3, Blackwel Science Ltd.

Mohsen Abdel-Tawwab, 2012. Interactive effects of dietary protein and live bakery yeast, Saccharomyces cerevisiae on growth performance of Nile tilapia, *Oreochromis niloticus* (L.) fry and their challenge against Aeromonas hydrophila infection, Aquaculture International, 20, 317-331.

Parker R.O., 2000. Aquaculture Science, Ed. Delmat Thomson Learning.

Ringo E., Olsen R.E., Gifstad T.O., Dalmo R.A., Amlung H., Hemre G.I., Bakke A.M., 2010. Prebiotics in aquaculture: a review. Aquaculture Nutrition, 16, 117-136.

Sturgeon hatchery manual, 2013. Food and Agriculture organization of the United Nations, Ankara 2013, FAO.

Surai P.F., 2004. New valences of selenium supplementation. Exploring New Horizons, The 18th Alltech Conference Tournament for Europe, the Middle East and North Africa.

Sweetman J., Davies S., 2006. Improving growth performance and health status of aquaculture stocks in Europe through the use of Bio-Mos. In: Nutritional Biotechnology in the Feed & Food Industries: Proceedings of Alltech's 22nd Annual Symposium (T.P. Lyons, K.A. Jacques, and J.M. Hower Eds), Nottingham University Press, UK, 445-452.

Sweetman J, Dimitroglou A., Davies S., 2008. Nutrient uptake gut morphology a key to efficient nutrition, International AquaFeed, January-February 08.

Şara A., 2008. Rational feeding of farm animals, Ed. Risoprint, Cluj-Napoca.

Şara A., Barbu A., Pantă L., Benţea M., 2009a. Effects of Bio-Mos, NuPro, or SelPlex in diets for Brook trout juvenils (*Salvelinus fontinalis*). The sustainability principle the next agricultural imperative. The Alltech 25th Annual International Symposium, May 17-20, Lexington, Kentucky.

Şara A., Alina Ani, Pantă L., Benţea M., 2009b. Influence of Sel-Plex organic selenium on the performance of Common carp (*Ciprinus carpio*) juveniles. The sustainability principle the next agricultural imperative, The Alltech 25th Annual International Symposium, May 17-20, Lexington, Kentucky.

Şara A., Barbu A., Alina Ani, Benţea M., 2010a. The effect of some fodder aditives on the growth and consumption indices of Brook trout (*Salvelinus fontinalis*). Bulletin USAMV- Animal Sciences and Biotechnologies, 67.

Şara A., Barbu A., Alina Ani, Benţea M., 2010b. The effects of some additives on the bioproductive indices and meat quality of Brook trout (*Salvelinus fontinalis*). Scientific papers on Animal Husbandry and Biotechnologies, USABT, vol. 43.

*** www.aller-aqua.com

*** www.altech.com

*** www.fao.org

*** www.iubmb.org