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# GENETICS AND BREEDING

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# THE REPEATABILITY STUDY OF CHARACTERS FOR DEVELOPMENT, REPRODUCTION AND MILK PRODUCTION AT THE ACTIVE ROMANIAN BLACK SPOTTED POPULATION FROM PANTELIMON AND MOGOSOAIA FARMS

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***Abstract***

*This paper presents Romanian Black Spotted characters regarding repeatability parameter for the development, reproduction and milk production at the Pantelimon and Mogosoaia farms. The milk production is the main target when we follow the goal of milk production exploitation from those two farms. From the repeatability character point of view, the quantitative characters can be divided in three categories: strong repeatability characters, when the repeatability coefficient is higher than 0.50 (milk production quality acquiring), medium repeatability characters when the repeatability coefficient is between 0.20 and 0.50 (milk production quantitative acquiring), poor repeatability characters when the repeatability coefficient is lower than 0.20 (reproduction characters and general biologic acquiring characters). The repeatability for milk quantity has different values for Pantelimon farm (0.484±0.11) and Mogosoaia farm (0.430±0.15); we found similar values like other authors for Romanian Black Spotted population. The study conduct by us revealed lower values for repeatability at the fat percent (0.430±0.17 - Pantelimon farm and 0.380±0.17 – Mogosoaia farm) comparative with those from the profile literature: Cacula et al .(1968)-0.780, Alexoiu (1983) – 0.690, Murat (1985) – 0.710; exception being the values calculated by Georgescu (1984) – 0.220. The repeatability characters for milk production determined by us at the Romanian Black Spotted cattle population from our study is alike with the values write in the profile literature, exception being the fat percent, which is lower.*

***Key words:*** *repeatability, repeatability coefficient, fat percent, far quantity, milk quantity.*

## INTRODUCTION

This paper studies the characteristics of the Romanian Black Spotted breed in terms of parameter heritability of character development, reproduction and milk production farms from Pantelimon and Mogosoaia.
Milk production is the main objective pursued in milk production operation on the two analyzed farms.
Lash (1937) defined genetic repeatability as a parameter, indicating phenotypic expression of the same character in the same individual at different times of life. In this situation, segregation and independent assortment of the genetic material cannot be considered as a source of fluctuations from one performance to another during the life of the animal.
Alexoiu (1983) highlights that repeatability is a parameter that expresses the upper limit of genetic heritability and while repeatability is a property of each character, being also the property of the population and generation are determinations.

## MATERIALS AND METHODS

Depending on the characters of repeatability, quantitative characters can be divided into three categories (Georgescu, 1988):

- strong-repeatable when CR (coefficient of repeatability) is greater than 0.50 (the case of qualitative milk production);
- repeatable medium when CR characters varies between 0.25 and 0.50 (the case of the quantitative production of milk).

Characters poorly reproducible when CR is less than 0.25 (breeding character and situation of general biological traits).

Repeatability is a genetic parameter considered in genetic evaluation and breeding bulls. Thus, Schaffer (1976) recommends choosing minimum repeatability to ensure objective comparison of bulls at the international level.

## RESULTS AND DISCUSSIONS

Repeatability coefficient values obtained for characters milk production in Romanian Black Spotted cows population from Pantelimon and Mogosoaia farms, is located within the limits of the data presented in the literature.

Table.1. Comparative variation of repetability of milk production in cows from Pantelimon and Mogosoaia farms

| Nr.crt. | Farm | Milk quantity | Fat quantity | Fat % |
|---|---|---|---|---|
| 1. | Pantelimon | 0.484±0.11 | 0.524±0.13 | 0.430±0.17 |
| 2. | Mogosoaia | 0.430±0.15 | 0.520±0.10 | 0.380±0.17 |

The data table 1 is established that the character of milk production in dairy analyzed population have an average repeatability different depending on the nature of the character (quantity of milk, amount of fat, fat percentage). So:

The amount of their milk repeatability genetic this parameter has different values at the two farms in the study (Farm Pantelimon - 0.484 ± 0.11; Farm Mogosoaia - 0.430 ± 0.15) values similar to those found by other authors breed of Romanian Black Spotted cattle.

Samely or closely values were found by others researchers: Thompson and Freeman – 0.501, Mather et al. – 0.440, Johansson -0.400, Syrstad – 0.430, Cacula et al. – 0.428, Barker and Roberthson – 0.430.

Higher values were set by Butcher and Freeman – 0.560, Alps – 0.540, and lower by Hartmann – 0.340, Murat – 0.330 and Muresan – 0.250.

Generally, repeatability amount of milk falls into the values found in literature at Holstein-Friesian cattle breed.

The repeatability of the amount of fat- greater was found on the Pantelimon farm (0.524 ± 0.13), with about 5% higher than on the Mogosoaia farm. Both farms repeatability fat intake registered a value superior to that found for the amount of milk. Repeatability value found by us is similar to those established by Butcher and Freeman (1968) -0.519, Cacula et al., (1968) - 0.510, Forster (1971) - 0.537 and twice that found by Muresan (1984) - 0.260. In general, the repeatability of the amount of fat is highly repeatable character is within the range indicated by the literature.

The repeatability of the percentage of fat - this purpose, the repeatability is found by us (430±0.17 at Pantelimon farm and 0.380±0.17 at the Mogosoaia farm) is lower than those found in the literature: Cacula et al. (1968) - 0.780, Alexoiu (1983) - 0.690, Murat (1985) - 0.710; except that determined by Georgescu (1984) - 0.220.

The repeatability value that we established for this character indicates a strong influence of environmental factors on it, reducing the certainty assessment or improvement value. Instead, repeatability and quantity of milk-fat creates favorable premises for increasing certainty phenotypic assessment to determine the actual production and capacity utilization in the animal improvement.

Table 2.Comparative repetability coeficient variation of the milk production characters in dairy cows from Pantelimon and Mogosoaia farms in the sequence of lactation

| Nr.crt. | Farm | Milk quantity | | | Fat quantity | | | Fat % | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | I | II | III | I | II | III | I | II | III |
| 1 | Pantelimon | 0.64± 0.28 | 0.41± 0.13 | 0.54± 0.22 | 0.53± 0.18 | 0.44± 0.16 | 0.52± 0.17 | 0.27± 0.15 | 0.20± 0.05 | 0.33± 0.13 |
| 2 | Mogosoaia | 0.48± 0.21 | 0.42± 0.18 | 0.33± 0.15 | 0.58± 0.26 | 0.46± 0.20 | 0.42± 0.19 | 0.36± 0.13 | 0.28± 0.12 | 0.30± 0.19 |

Repeatability amount of milk - at both farms studied, the value of this parameter in the sequence of lactations, recorded the highest rates in primiparous (0.64 ± 0.28 at the Pantelimon farm to 0.48 ± 0.21 at the Mogosoaia farm) then declined cows secundiparous tendency is preserved and lactation III to farm Mogosoaia.

Repeatability amount of fat - by age, there is an increasingly high value on breast feeding I, high value being recorded at the primiparous from the Mogosoaia farm (0.58 ± 0.26). Further evolution of this parameter is similar to the entire cow effective registering a downward trend from one lactation to another.

Repeatability fat-percentage - depending on the sequence of lactations, has different values at the two farms studied (between 0.20-0.33 at the Pantelimon farm to 0,28-0.36 at the Mogosoaia farm). **Butcher** and **Freeman** show that the links between successive lactations increases as the animal gets older.

Thus, the coefficient of repeatability of fat percentage increase of 2.35 times on adult cows compared to primiparous.

## CONCLUSIONS

Overall, milk production repeatability characters we established at the Romanian Black Spottedherd breed analyzed is similar to values found in the literature, except fat percentage, which has a lower value.

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# THE ANALYSIS OF PRODUCTION AND REPRODUCTION PARAMETERS OF HOLSTEIN COWS FROM HOLLAND AND GERMANY IN J.-S.C. „AYDYN"

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***Abstract***

*The scientific paper presents the results of research of productive and reproductive parameters of cows of Holstein breed of Dutch and German origin in the herd J-S.C. „Aydyn", Comrat Administrative and Territorial Unit Gagauzia. For the analysis was used performance control for the year 2015: cows on the first lactation (n=122) and the second lactation (n=43) of Dutch origin and cows on the first (n =129) lactation – of German origin. It was established that from cows of the Dutch origin for the first lactation on average was received 7803 kg of milk, which is with 589 kg more than from the cows of German origin, a highly significant difference (P <0.001). It is established by the analysis that 50,8% of the animals of Dutch origin had the productivity from 7501-8000 kg to 9001 kg, whereas 70,5% of the animals of German origin had the productivity from 6000-6500 to 7001-7500 kg and only 29.5% from 7501-8000 to 9001 kg of milk per lactation. The average length of the dry period at cows of Dutch origin was by 6.5 days less than at the cows of German origin, the difference is authentic at P <0.05. Service period at cows of Dutch origin averaged 219.9 ± 12 days, the German origin – 168.2 ± 15.4 days. This affected the increase of the between calving interval at the animals of Dutch origin (483 days), the German origin (446 days), which exceeds the desired duration by 118 and 81 days, respectively.*

***Key words****: lactation, milk index, the service period, Calving interval, the coefficient of reproductive ability.*

## INTRODUCTION

Homeland of Holstein, like and the other related groups of black white cattle, is Holland. It is known that for the first time to North America, black motley frisian cattle was brought by the first Dutch settlers still in 1621. Small parties of these animals were imported in the late XVIII - early XIX century, but the greatest number - more than 100 thousand heads - imported during the period of 1875-1885 years. Bred in the US cattle of this population in 1861 received the name of Holstein-Friesian (Trufanova, 2006, Ulimbashev et al., 2012).

Since 1983, in the US and Canada Holstein-Friesian breed is called Holstein. Currently, the value of this breed is very high, as it is characterized by the high milk yield and is used for improving dairy cattle worldwide. It is distinguished by good adaptability to different climatic and economic conditions, high payment of feed by milk, (Dmitriev et al., 1989)

In XX century the Holstain breed has become dominant in the global dairy cattle breeding. Holstein cattle has the highest genetic potential for milk yield and qualities of the complex, providing a better adaptation to industrial technologies, it is imported in more than 70 countries around the world (Gavrilenco et al.,1994; Kostomahin, 2011; Lazarenko, 1997; Madison, 2007). It is included in breeding programs for the improvement of many breeds of cattle in dozens of countries with developed dairy cattle (Robbins et al., 2000). The world population of Holstein cows is 25 million of heads, or 72% of the 8 most common dairy breeds in the world, (Jansen, 2009).

Holstein breed has high technological potential on further increase in productivity. The average milk yield of cows in the United States and Canada reaches 6500-7000 kg of milk, live weight 600-700 kg, bulls - 1000-1100 kg. Holstain cattle is characterized by strong limbs and aptitude for mechanical milking.

It is characterized by a large body length and considerable height at the withers, milk type addition, adaptable to different climatic zones, capable to high productivity at double milking and and free movement.

To holstein cows belong all world records for milking and yield of milk fat (Ulimbashev et al., 2012). In 1982 the champion on milking among mature cows of holstein breed was Holibenk Medalist 266 300, from which at the age of 8 years, 9 months is received 19245 kg per milk lactation fat content of 3.89%, and the total yield of milk fat per 305 days of lactation was 557 kg (Gavva, 1986). The world record of lifetime productivity is set by cow nr.289 (Calif.). She lived for 19.5 years, and for 5535 days of lactation from her was milked 211212 kg of milk at the output of milk fat 6543 kg (Freeman, 1984).

In 2010, the American Association for breeding of Holstein cattle (Holstain Association US) recorded on the farm Ever-Green-View, (Waldo, Wisconsin, USA.), A new world record: from the cow number 1326 for 365 days of the third lactation was obtained 32804 kg of milk obtained (average 89 kg per day) with 3.86% of fat and 3.12% of protein. Indicators of productivity of this cow in 1934 kg of milk (6.26%) higher than the previous world record (Yanchukov et al., 2011).

Many of the European countries, such as Germany, the Netherlands, Denmark, Switzerland, and others, in the result of long and purposeful use of Holstein, today turned themselves into exporters of high-value genophond of Holstein breed.

The basis of the creation of the German Holstein breeding was also the Dutch Friesian breed of black motley cattle in northern Germany. On the begining, the selection was carried out by milking, and from the second half of the 19th century - on the content of fat and protein in milk. At the same time, great attention has been paid and to meat productivity, (Suleymanov, 2012; Khakimov et al., 2014). As a result, virtually all black motley cattle in Germany, about 95% of previously submitted ostfrizsk breed, was transformed into a Holstein (Shichkin, 2002). During the period from 1984 to 1994 in the western part of Germany milk yield of hybrids of black motley cattle increased from 6082 to 7107 kg, the fat content in the milk increased from 4.02 to 4.29%. German scientist Kalm (Kasperska et al., 1991) notes that due to crossbreeding with Holstein breed happened an increase of genetic variation and genetic progress of black motley cattle. Tozliyan (2007) reported that for 12 years of Holstein cattle in Germany, its productivity has increased significantly: milk yield - 1.4 times, the fat and protein content in milk - by 0.12 and 0.08%.

It should be noted that in the breeding of the Moldovan motley cattle, and then and its improvement was carried out with the use of global genophond of animals of Holstein breed imported from Canada, USA, UK, Germany and other countries (Smirnov et al., 2007).

Holstein cattle breed is characterized by a satisfactory reproductive capacity, due to the physiological characteristics of highly productive animals, which is characterized by early maturity, good fertility and easy calving. With good feeding and keeping of heifers to 15 months of age reach a live weight of 350-380 kg and can be inseminated. Taking into account a high level of milk yield of cows of this breed, the calving period at them most often is 13-14 months.

Currently (beginning with 2010) to the Republic of Moldova are imported Holstein heifers, mainly of Dutch and German origin. According to bonitation for y. 2015, in the republic were 1421 heads of Holstein cattle, including 932 cows. Milk yield per a cow averaged 7612 kg of milk with a fat content of 3.81%.

The aim of the work was to study the productive and reproductive qualities of Holstein cows in the herd J-S.C. "Aydyn".

## MATERIALS AND METHODS

The material for the research was a purebred Holstein cattle imported to J-S.C. "Aydyn", Comrat ATU Gagauzia from Holland and Germany. For the analysis were used data parameters for y. 2015: cows on I-st (n=122) and the II-nd (n=43) of Dutch lactation origin and cows on I-st (n=129) lactation – of German origin.

The assessment and analysis of animals for milk yield was carried out according to conventional techniques based on: milk yield

for 305 days of lactation, the fat content of milk, production of milk fat per lactation. The magnitude of milking cows were divided into The obtained results were processed biometrically (Merkuryeva, 1983; Plohinsky, 1978) with the use of Microsoft classes. Milk index (MI) - the amount of milk based on 100 kg of live weight Reproductive ability was evaluated by indicators: the duration of the service period, the dry and calving (CP) periods, while the animals were divided on the level of milk production. Reproductive capacity coefficient (RCC) was calculated according to the formula of Kramarenko (1974): RCC = 365/CP.

The obtained results were processed Word 2007, Excel 2007, the accuracy of the performance was determined by Student's.

## RESULTS AND DISCUSSIONS

In the analysis of milk production of cows of different selection is established that cows from the Dutch origin for the I$^{st}$ lactation on average is received 7803 kg of milk, which is with 589 kg more that from the cows of the German origin, a highly significant difference (P <0.001) (Table1).

Table 1. Characteristics of cows on milk yield of Dutch origin (I$^{st}$ lactation)

| The level of productivity | Milk, kg | | Fat | | Coefficient of milking |
|---|---|---|---|---|---|
| | Per day | total | % | kg | |
| 6000-6500, n=9 | 21.3±0.34 | 6360.8±42.6 | 3.97±0.04 | 253.1±7.50 | 1010.7±12.4 |
| 6501-7000, n=21 | 22.9±0.32 | 6805.3±24.1 | 3.81±0.01 | 259.2±3.57 | 1081.0±50.5 |
| 7001-7500, n=28 | 23.8±0.23 | 7163±17.1 | 3.76±0.01 | 269.1±2.80 | 1146.1±7.1 |
| 7501-8000, n=6 | 26.1±0.71 | 7641.8±74.6 | 3.60±0.10 | 276.3±7.05 | 1202.3±18.4 |
| 8001-8500, n=19 | 27.7±0.30 | 8251.4±32.2 | 3.65±0.06 | 301.0±4.60 | 1304.9±14.1 |
| 8501-9000, n=19 | 29.1±0.28 | 8796.4±38.4 | 3.73±0.04 | 328.05±3.50 | 1368.3±17.6 |
| > 9000, n=18 | 30.5±0.17 | 9291±54.3 | 3.75±0.04 | 348.4±4.12 | 1436.9±18.6 |
| Average | 25.6±0.30 | 7803.2±90.1 | 3.76±0.02 | 292.5±3.44 | 1228.3±13.9 |

Our results coincide with studies (Lyashenco, 2013) – under the conditions of the Penza region from the Holstein cows of different selection are received high levels of milk production, the best indicators were characterized the animals of Dutch origin.

It should be noted that the variation of milk yield per lactation (305 days) turned out essential for what was conducted the distribution of cows by classes or productivity level.

The analysis of milk production by magnitude of milk yield between cows of different selection showed that in 6 groups of 7 superiority mainly of cows of Dutch origin over the cows of German origin (Table 2). However, by the the magnitude of the productivity 7501-8000 kg of milk of cow of German origin surpassed contemporaries of the Dutch origin over 163 kg of milk at P<0.001.

Table 2. Characteristics of cows on milk yield of German origin (I$^{st}$ lactation)

| The level of productivity | Milk, kg | | Fat | | Coefficient of milking |
|---|---|---|---|---|---|
| | Per day | total | % | kg | |
| 6000-6500, n=30 | 20.8±0.11 | 6289.5±26.9 | 3.86±0.04 | 243.03±2.56 | 988.2±9.02 |
| 6501-7000, n=26 | 22.3±0.11 | 6777±29.7 | 3.88±0.04 | 263.1±2.70 | 1044.4±9.9 |
| 7001-7500, n=35 | 23.5±0.05 | 7162.3±16.1 | 3.76±0.03 | 269.6±2.1 | 1119.5±6.9 |
| 7501-8000, n=9 | 25.6±0.14 | 7804.6±41.9 | 3.76±0.06 | 293.1±4.6 | 1232.8±14.3 |
| 8001-8500, n=12 | 26.9±0.13 | 8222.7±37.9 | 3.6±0.03 | 296.7±3.34 | 1275.2±18.1 |
| 8501-9000, n=14 | 28.9±0.1 | 8812.4±30.8 | 3.65±0.04 | 321.7±3.95 | 1372.9±14.8 |
| > 9001, n=3 | 29.8±0.08 | 9089±30.5 | 3.67±0.09 | 333.3±8.3 | 1418.2±27.7 |
| Average | 23.6±0.32 | 7214±96.3 | 3.77±0.04 | 272.3±3.86 | 1125.4±25.7 |

Cows of Dutch origin outperform their peers of German origin by comparing the average daily milk production on average of 2 kg of milk (P <0,001).

When comparing the average daily milk yield at a magnitude of productivity 6501-7000 kg of milk the difference was 0.6 kg in favor of Dutch cattle origin, with P <0.1.

By comparative detailed analysis of the milk production of cows of different selection is established that 50.8% of the animals of Dutch origin had the productivity from 7501-8000 kg to 9001 kg, whereas 70.5% of the animals of German origin had the productivity from 6000-6500 to 7001-7500 kg and only 29.5% from 7501-8000 to 9001 kg of milk per

lactation. Similar results were obtained on cows, heifers of Holstein breed of australian origin (Khisamov et al., 2012) - most animals are concentrated in classes that have a heightened productivity - 7501 and more (70.6% of the population).

The fat content in the milk is almost the same in the compared populations of cows (3,76-3,77%), but at the exit of milk fat of cow of Dutch origin surpass the cows of German origin by 20.2 kg, (P <0.001).

Considerable importance in dairy cattle breeding has the amount of milk received per each 100 kg of live weight of cow (dairy coefficient). Milk index at cows of Dutch origin on average constituted 1228.3 kg of milk, which is more by 102.9 kg than that of peers – cows of German origin, at td = 3,5, a highly trustworthy difference (P<0.001). Higher coefficient of milk yield was detected at animals of both analyzed populations at the value of productivity >9001 kg of milk, which amounted to 1436.9 kg and 1418.2 kg of milk for cows of the Dutch and German origin, respectively.

It should be noted that the heifers of German origin were acquired later in 2014 and the calving from them started with 8-9 months later than from heifers of Dutch origin. Hence, the preliminary results for the II-nd lactation we present only on animals of Dutch origin (Table 3).

Table 3. Characteristics of cows on milk yield of Dutch origin (II$^{nd}$ calving)

| The level of productivity | Milk, kg | | Fat | | Coefficient of milking |
|---|---|---|---|---|---|
| | Per day | total | % | kg | |
| 6000-6500, n=4 | 21.1±0.05 | 6450.7±15.2 | 3.78±0.06 | 243.8±4.48 | 1006±26.3 |
| 6501-7000, n=7 | 22.2±0.09 | 6758.3±26.5 | 3.73±0.09 | 251.7±5.69 | 1085.9±12.6 |
| 7001-7500, n=9 | 23.2±0.07 | 7086.4±20.3 | 3.81±0.11 | 276.4±3.44 | 1136.8±18.1 |
| 7501-8000, n=2 | 26.1 | 7945.5 | 3.55 | 282.5 | 1251.9 |
| 8001-8500, n=10 | 26.8±0.12 | 8190.2±33.0 | 3.75±0.06 | 307.2±5.51 | 1303.9±16.7 |
| 8501-9000, n=6 | 29±0.53 | 8826.2±65.1 | 3.73±0.06 | 329.5±6.98 | 1386.3±31.9 |
| > 9001, n=5 | 31.0±0.46 | 9455.2±140.6 | 3.72±0.08 | 351.6±5.81 | 1483.3±26.8 |
| Average | 25.5±0.49 | 7746.3±154.1 | 3.76±0.03 | 291.3±5.8 | 1229.8±24.4 |

As it is seen from the data table, milk production for 305 days of lactation decreased by an average for 57 kg of milk compared to the milk production of cows for the I-st lactation. The rest of the analyzed indicators are almost identical to cows of I$^{st}$ lactation.

The analysis showed that 53.5% of the cows on the II$^{nd}$ lactation had a productivity of 7501-8000 kg to> 9001 kg of milk. Thus,by the level of milk production> 9001 kg of milk is observed a tendency of increase in comparison with the animals on the I$^{st}$ lactation: milk yield per lactation increased by 164 kg of milk, the quantity of milk fat by 3.2 kg and lactation coefficient by - 46.4.

As it is known, the achievement of an optimal milk production of cows is possible only with normal reproduction of the herd, in connection with which, by us were studied the reproductive qualities of the analyzed population of Holstein cows of different origin. In the studied groups of animals the average duration of the dried period of cows ranged within normal limits standards - 62.3 days. (Dutch origin) - 68.9 days. (German origin) (Tables 4, 5).

Table 4. Capacity indices of reproduction cows (I$^{st}$ calving) Dutch origin

| The level of productivity | Dry period | Service period, day | Calving interval | Reproductive capacity coefficient |
|---|---|---|---|---|
| 6000-6500, n=9 | 60.4±0.60 | 268±61.4 | 538.4±61.0 | 0.68 |
| 6501-7000, n=21 | 60.2±0.42 | 219.0±34.5 | 475.9±29.8 | 0.77 |
| 7001-7500, n=28 | 60.2±0.42 | 219.0±34.5 | 475.9±29.8 | 0.77 |
| 7501-8000, n=6 | 65.8±6.70 | 272.0±52.9 | 519.5±68.3 | 0.7 |
| 8001-8500, n=19 | 61.2±1.09 | 205.5±33.0 | 493.5±33.2 | 0.74 |
| 8501-9000, n=19 | 63.8±2.20 | 195.0±30.0 | 465.3±31.6 | 0.78 |
| > 9000, n=18 | 62.4±1.70 | 222.9±29.3 | 483.6±29.2 | 0.75 |
| Average | 62.3±0.70 | 219.9±12.3 | 483.6±12.4 | 0.75 |

It should be noted that the average length of the dry period at the cows of Dutch origin cows was with 6.5 days less than at the cows of German origin, the difference is significant at P <0.05.

The greatest length of the dry period was at the level of productivity 8501-9000 at cows of German origin - 75.6 days or with 11.8 days more than at cows of Dutch origin at P <0.05.

As it can be seen, the index rate of the average length of service period at cows of both selections exceeded permissible limits (90-100 days). It should be noted that a long service period was observed at cows Dutch origin (219.9 ± 12 days) that is with 129-119 more than the permissible limits. At the same time it was maximum with the level of productivity of 7501-8000 kg of milk (272 d.), and the minimum-at a rate of 8501-9000 kg (195 days).

Table 5. Capacity indices of breeding cows (I$^{st}$ calving) German origin

| The level of productivity | Dry period | Service period, day | Calving interval | Reproductive capacity coefficient |
|---|---|---|---|---|
| 6001-6500, n=30 | 67.2±2.5 | 157.7±9.1 | 435.0±11.1 | 0.84 |
| 6501-7000, n=26 | 69.1±2.8 | 165.9±10.0 | 445.1±12.3 | 0.82 |
| 7001-7500, n=35 | 66.4±1.98 | 169.2±7.0 | 445.7±8.4 | 0.82 |
| 7501-8000, n=9 | 67.9±4.6 | 166.6±11.3 | 444.4±14.4 | 0.82 |
| 8001-8500, n=12 | 72±4.8 | 181.7±15.7 | 465.3±19.5 | 0.78 |
| 8501-9000, n=14 | 75.6±4.3 | 188.0±12.9 | 473.6±16.7 | 0.77 |
| > 9000, n=3 | 70.7±9.7 | 165.3±17.9 | 446±27.7 | 0.82 |
| Average | 68.9±2.06 | 168.2±15.4 | 446.7±19.7 | 0.82 |

A short service period mentioned in cows German origin (157 days.) at the level of productivity of 6001-6500 kg of milk. If to consider that the optimal service period should be 90 days,then in fact it exceeded this period on the average of 67 days. Similar results were obtained in research (Litvinenko et al., 2014) at the Holstein cows of German origin with duration of the service period 159 days, at cows of Dutch origin it was at the level of 205 days.

In the analyzed population of cows of Holstein breeding the calving interval averaged 483 days (Dutch origin) and 446 days (German origin), which exceeds the desired length with 118 and 81 days, respectively. For cows of Dutch origin the smallest calving period was at the level of productivity of 8501-9000 kg of milk - 465 days for cows of German origin - at the level of productivity of 6001-6500 kg -. 435 days, exceeding the desired length to 100-70 days. Cows of Dutch origin on this indicator exceeded their peers by an average of 36 days, the difference is significant at P <0.01. The results obtained by us coordinate with the data (Nikulin et al., 2011), which point out that the reproductive function at the all imported livestock of Holsteins from Germany and the Netherlands was characterized by a prolonged calving and service periods, the low index of insemination and with the statement (Tekeev, 2015) that a high duration of calving interval at cows after the first calving carries a common pattern for dairy cattle.

The results obtained by us indicate a low coefficient of reproductive capacity of the analyzed livestock of animals of both selections as its optimal value should be within 1-0.95. It was established that the magnitude coefficient of reproductive ability of cows of Dutch origin differed from the cows of German origin that averaged 0.72 and 0.82, respectively. At the level of productivity of 6001-6500 kg of milk, this coefficient was the greatest at the cows of German origin - 0.84, the lowest - 0.68, at cows of Dutch origin.

The results of evaluation of the reproductive ability of cows of the II$^{nd}$ lactation are given in Table 6.

Table 6. Capacity indices of breeding cows (II$^{nd}$ calving) Dutch origin

| The level of productivity | Dry period | Service period, day | Calving interval, days | Reproductive capacity coefficient |
|---|---|---|---|---|
| 6000-6500, n=4 | 61.7±0.75 | 89.0±2 | 367.6±8.5 | 0.99 |
| 6501-7000, n=7 | 83.8±5.96 | 185.9±51.07 | 417.8±18.4 | 0.87 |
| 7001-7500, n=9 | 80.7±3.8 | 74.4±10.6 | 395.6±18.3 | 0.92 |

| | | | | |
|---|---|---|---|---|
| 7501-8000, n=2 | 85.5 | 82.5 | 389.5 | 0.95 |
| 8001-8500, n=10 | 82.4±5.0 | 109.3±17.5 | 418.5±17.7 | 0.87 |
| 8501-9000, n=6 | 69.8±6.4 | 154.2±42.7 | 443.8±21.6 | 0.82 |
| > 9000, n=5 | 66.6±6.37 | 172.8±52.3 | 473.7±59.4 | 0.77 |
| Average | 77.2±2.27 | 123.4±13.3 | 405.6±18.72 | 0.90 |

As it is seen from the data table, it has happened an increase in the dry period of almost 15 days (77.2 days) compared with cows of Dutch origin after the first calving, the difference is highly significant at P <0.001; decrease of service period by 96 days. (123.4 days) at P <0.001 and a decrease with 41 days of calving interval, the difference is unauthentic.

## CONCLUSIONS

1. Dairy efficiency of cows of the Dutch origin for the I$^{st}$ lactation averaged 7803 kg of milk, which is with 589 kg more than from the cows of the German origin, the difference is highly significant (P <0.001).
2. A little more than half of the Dutch breeding animals are concentrated in classes with productivity from 7501-8000 kg to> 9001 kg of milk (50.8% of the population). Most of the animals of the German breeding - 70.5% are concentrated in the classes with the productivity from 6000-6500 to 7001-7500 kg and only 29.5% from 7501-8000 to> 9001 kg of milk.
3. The coefficient of milk yield at cows of Dutch origin averaged 1228.3 kg of milk, which is more by 102.9 kg than that of peers – the cows of German origin, a highly significant difference (P <0.001).
4. The average length of the dry period of cows of both origins fluctuated between standards - 62.3 days (Dutch origin) - 68.9 days (German origin).
5. The service period of cows of Dutch selection averaged 219.9 ± 12 days, the German origin -168.2 ± 15.4 days, which exceeded the maximum recommended duration with 90-100 days. This affected the increase of the calving interval at animals of Dutch origin (483 d.), the German origin (446 d.), which exceeds the desired duration with 118 and 81 days, respectively.

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# GENETIC DIVERSITY IN THE ROMANIAN SHEEP BREEDS QUANTIFIED BY MEANS OF INFORMATIONAL ENERGY

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***Abstract***

*The study makes an analysis of the genetic diversity (**d**) in the Romanian sheep breeds (Palas Merino, Tsigai, Tsurcana and Botosani Karakul) using a concept of informational statistics termed the informational energy (**e**) which measures very precisely the genetic heritage of the taxonomic entities than by common indicators of mathematical statistics (allelic frequency analysis, significance testing etc.). The informational-statistical quantification of genetic diversity used the allelic frequencies within three genetic system types in the calculus algorithm: five biochemical-genetic systems (haemoglobin, transferrin, albumin, amylase, blood potassium), six immunogenetic systems (blood group systems A, B, C, D, M, R-O) and seven genetic-molecular systems (β-lactoglobulin, calpastatin, $\alpha_s$1-casein, prion, SLS, Booroola, Inverdale). The accuracy of the new approach on the genetic diversity resides from a complex analysis of the allele number, their frequencies, inter allelic ratio and genetic polymorphism degree within each system. The paper describes the genetic diversity levels both within each system, as well as in associated systems, and highlights the genetic diversity differences among the Romanian sheep breeds in relation to the systemic parameters mentioned. Also, some comments are made about the heterozygosity degree of sheep breeds in corroboration with genetic diversity. Finally, the paper argues the importance of genetic diversity measured in terms of informational statistics for improvement and conservation programs in the field of farm animal breeding, but makes clarifications also about the danger that comes from increasing the diversity at some loci associated with genetic diseases.*

***Key words:*** *Romanian sheep breeds, genetic marker, genetic diversity, informational energy*.

## INTRODUCTION

Exploration, collection and scientific preservation of genetic resources from all types of agricultural holdings is an urgent action of the utmost importance in which all countries as well as numerous scientific and cultural forums and professional associations of livestock farmers must be involved through coherent state and non-governmental policies (Patterson, 2003). The technical coordinates of the World Programme of FAO, entitled Mo-DAD, has been focused on identification, description, development, use of animal genetic resources, conservation of unique or endangered resources, training, information and participation in the management of animal genetic resources and communication improving through dialogue and international connections to manage these resources. The project The Mo-DAD aims quantifying the genetic variation among the main domestic animal breeds based on molecular genetic evaluation and measuring the genetic distances among breeds, providing the first global information of relations among themselves or about the number and diversity of the breeds (Scher, 2012). Also, within the European Commission for Agriculture and Rural Development there is a committee issuing laws on the conservation, characterization, collection and utilization of genetic resources in agriculture (ECC, 2001; GRFAC, 2011; ECNB, 2011). Thus, it has been created an international scientific current of major attitude known as the management of genetic resources and biodiversity in animals (Galal and Hamond, 1996).

This challenge is achievable by introducing in the animal breeding practice of certain biological indicators expressing the animal inwardness for its genotypic individualization (biochemical and molecular markers) since certain of its external features or productivity traits (morphological markers) have a lower or sometimes even insignificant relevance. The expressions of certain biochemical-genetic (electrophoresis variants of proteins polymorphic or the flam photometric ones of some mineral

elements with discontinuous distribution in the blood), immunogenetic (antigens of blood group factors) and molecular-genetic (DNA sequences, microsatellites) structures, outside any influence of external or internal factors, have the quality of genetic markers that are highly relevant in describing the genetic individuality of an animal (Ordas and San Primitivo, 1986; Sargent et al., 1999; Moioli et al., 2006; Maddox and Cockett. 2007; McManus et al., 2010; Hrincă, 2016*a*).

All approaches regarding the genetic diversity assessment using the genetic markers took into account the comparative analysis of different alleles and genotypes within of some systems with multiple molecular forms, the observed and expected frequencies and testing the genetic equilibrium in animal populations. But, using these indicators of mathematical statistics is not a sufficient condition because they provide rough estimates on the genetic heritage of animal populations. These tools are provided by the informational statistics (Groza and Padeanu, 1999; Hrincă Groza, 2001; Hrincă, 2015, 2016). This is the reason of this paper to quantify as accurately as possible the genetic diversity of the Romanian sheep breeds by means of an informational-statistical concept, named *informational energy*, using various genetic markers in the calculus algorithm.

## MATERIALS AND METHODS

The genetic diversity was analyzed within of some Romanian sheep populations belonging to four indigenous breeds: Palas Merino, Tsigai. Tsurcana and Botosani Karakul. To achieve this goal the allele frequencies from 18 polymorph systems were used: five biochemical-genetic systems (haemoglobin, transferrin, albumin, amylase and blood potassium), six immnunogenetic systems (blood group systems A, B, C, D, M and R-O) and seven molecular-genetic systems (β-lactoglobulin, calpastatin, $\alpha_s$1-casein, prion, Spider Lamb Syndrome, Booroola and Inverdale). The polymorphism of genetic structures was highlighted by specific laboratory methods:

- biochemical-genetic systems - by starch gel electrophoresis methods (for polymorphic proteins) and by flam photometric method (for potassium ions with discontinuous distribution) (Hrincă, 2015);
- immunogenetic systems - by haemolytic assay method (for systems A, B, C, M and R-O) and by haemagglutination method (for system D) (Hrincă, 2015);
- molecular-genetic systems - by PCR-RFLP methods (Kevorkian, 2010; Lazar et al., 2015; Hrincă, 2015).

In terms of mathematical statistics the allelic frequencies were calculated within all genetic systems and the genetic equilibrium of animal populations has been estimated at the level of each locus by means of the $\chi^2$ test.

Quantification of the genetic diversity (*d*) of each sheep breed was performed using the informational energy (*e*) in corroboration with the heterozygosity degree (*Ht*)) of their populations at the determinant loci of all polymorphic systems as a first indicator of diversity (Groza and Pădeanu, 1999; Hrincă and Groza, 2001; Hrincă, 2015).

The complex informational energy ($e_t$) and complex genetic diversity ($d_t$) were calculated for a broader perception of polymorphism level of all genetic systems (Hrincă and Groza, 2001; Hrincă, 2015).

## RESULTS AND DISCUSSIONS

The calculus algorithm of genetic diversity (*d*) by means of informational energy (*e*) was based on the allele frequencies identified in the 18 genetic systems, their number being 70, as follows:

- 17 alleles within the *biochemical-genetic systems*: in the transferrin system nine alleles were found and the other four systems each contain two alleles (Table 1);
- 36 alleles within *immunogenetic systems*: two alleles for each locus of the 13 blood group factors (Table 2);
- 17 alleles within the *molecular-genetic systems*: the prionic system contains five alleles and the other systems are of biallelic type (Table 3).

The number of alleles, their dispersion and especially the distributional ratio among them within each system configure the value of the two indicators of informational statistics. The informational energy and genetic diversity quantified by informational statistics concepts are dimensionless sizes. Being complementary

sizes, both can take values from *0* to *1* depending on the share of all components within a certain system; the more the distributions show a wider variability, the more the informational energy increases and the genetic diversity decreases, and the more balanced these distributions are, the more the informational energy decreases and the genetic diversity increases (Groza and Padeanu, 1999; Hrincă and Groza, 2001; Hrincă, 2015).

All four Romanian sheep breeds are distinguished among them in terms of number of alleles, their distributions and of the polymorphism measure at the analyzed loci, structures that confers to each breed a distinct genetic profile which is reflected on its genetic diversity degree.

***Diversity within the biochemical-genetic systems*** (Table 1)

In the haemoglobin system, the $Hb^B$ allele is prevalent in all sheep breeds compared to its $Hb^A$ codominant. Because of this, the informational energy coefficients record high values, their correspondence being reflected in low or moderate levels of diversity at the Hb locus. The lowest level of haemoglobin diversity occurs in Botosani Karakul (0.16). The Tsigai breed records a decreased haemoglobin diversity, too (0.2). In Tsurcana and especially in Palas Merino, in which the gap between the two Hb alleles are more diminished in comparison with the first two breeds, there is an increase in haemoglobin diversity, reaching 0.35 in Tsurcana and 0.40 in Palas Merino.

In the transferrin system, the richest genetic diversity is found in the Tsigai breed that contains all nine Tf alleles in their genotypic structures (0.98). The diversity is very large in Tsurcana and Palas Merino breeds, too. Although in Turcana there are seven alleles and Palas Merino breed has eight alleles, the transferrin diversity coefficient is slightly higher in Tsurcana (0.96) than in Palas Merino (0.92); this is because the Tf allele distributions of Tsurcana are more uniform than those of Palas Merino. In Botosani Karakul, the transferrin diversity, though it is appreciable, is lower (0.68) than in the previous breeds because there are only six alleles and especially because the share is held by alleles $Tf^C$ (60%) and $Tf^B$ (33%), the other alleles being sporadically encountered in this breed.

The albumin system of the Romanian sheep breeds is characterized by a very limited polymorphism because of the allele $Alb^S$ fixing in an overwhelming proportion, especially by natural selection. Only the Palas Merino and Tsigai breeds present albumin polymorphism of binary type and Tsurcana and Botosani Karakul breeds are monotypic at the Alb locus. In Palas Merino and Tsigai the albumin panel is dominated by the $Alb^S$ allele in a 95-96% proportion and only 4-5% is occupied by $Alb^F$ allele. The albumin monotypism of Tsurcana and Botosani Karakul breeds consists in the existence of homozygous genotype for $Alb^S$ allele only. For these reasons the informational energy coefficients are extremely high, which makes the albumin diversity to be very low, about 0.10 in Palas Merino and Tsurcana and completely absent in Tsurcana and Botosani Karakul (0).

Except the Tsgai breed, the other breeds are monotypic for $Am^B$ allele in the amylase system. Therefore, the genetic diversity of this locus is zero in these three breeds. The amylase diversity is moderately manifested only in the Tsigai sheep (about 0.2) where the polymorphism is owed to the massive presence of $Am^B$ allele (92%) and relatively low incidence of $Am^C$ allele (8%).

Within the blood potassium system the genetic diversity is absent in the Palas Merino breed because the only present allele is the $K^L$ dominant one. In the other three breeds there is kalium diversity because, in addition to $K^L$ allele, its $K^h$ recessive is also present. If in Tsigai sheep the $K^L$ allele is predominant (about 94%), in Botosani Karakul breed the $K^h$ allele has the highest value (about 92%). Although these two alleles occur with diametrically different frequencies in the two breeds, the genetic diversity at this locus is relatively low but almost similar in both breeds because the gap between the two alleles is similar, slightly more reduced in Botosani Karakul than in Tsigai; therefore the blood potassium diversity is slightly higher in Botosani Karakul (0.18) compared to that of Tsigai (0.15). In exchange, in Tsurcana due to very uniform spread of the two alleles, the genetic diversity at Ke locus is quite significant.

***Diversity within the immunogenetic systems*** (Table 2)

The erythrocyte factors belonging to all six blood group systems are well represented having different frequencies for each sheep breed. In most cases, the frequencies of dominant alleles, that control the expression of blood factors, are lower than of their recessive variants that determine the absence of some factors. Can also find some cases when the dominant alleles had higher incidence than their recessive, this situation being met with predilection in Tsurcana sheep at the level of factors Aa, Bb, Bf, Ca, Cb, and Ma, Tsurcana having the richest immunogenetic dowry among all breeds. Also in Tsigai at Cb and Ma loci, in Botosani Karakul at Bb locus and in Palas Merino at Cb locus, the dominant alleles are most common than the recessive alleles. In one case there is an almost unitary ratio between the two allele types at the Bf factor level in Botosani Karakul. Due to the consistent frequencies of erythrocyte antigens within all six blood group systems, the informational energies have such sizes entailing the exteriorization of a considerable genetic diversity at each erythrocyte factor level in all sheep breeds, in most cases the scores ranging between 0.50 and 0.65 (tab. 2). The immunogenetic diversity measure is given by the distributions of one or other of the two allele types and especially by the ratios between their frequencies. As a general rule, for a good score of immunogenic diversity ranged between 0.55 and 0.65, the ratios between the allelic frequencies have to be 35% / 65% or 45% / 55%. If these ratios widen, then the immunogenetic diversity decreases: for example, at a ratio of 20% / 80% the diversity reaches approximately 0.4 and at a ratio of 10% / 90% it decreases to 0.10-0.15.

At the Aa factor level the immunogenetic diversity is significant in Palas Merino, Ţigaie (0.56) and Botosani Karakul (0.61) breeds. In exchange, the high informational energy generated by the large gap between the two alleles in Tsurcana leads to a low diversity.

In the system B, the most diverse locus is the one of factor Bb. In all breeds the diversity exceeds 0.5, especially in Palas Merino and Botosani Karakul. At the level of the other factors within B system the best diversity is recorded in Botosani Karakul, its value being between 0.55 and 0.62. Similar values of immunogenetic diversity are found in Tsurcana too, except for Bf factor (about 0.4). In the other two breeds, the diversity coefficient threshold at the factors of B system is below 0.5, some of them recording relatively low values between 0.2 and 0.25, such as in Palas Merino for Bd and Bg factors or in Tsigai for Bc factor. A more substantial diversity in the two breeds would be signalled at the Bf and even Bi factor level.

Within the system C, the Tsurcana and Botosani Karakul breeds possess a good diversity for Ca factor (around for 0.62); contrary, in Tsigai breed the diversity is moderate (0.19) or relatively low in Palas Merino (about 0.14). Unlike Ca factor, the Cb factor is more diverse in all breeds, the diversity value being more than 0.55, excluding the Tsurcana (0.45).

At the Da factor level, the highest diversity is found in Botosani Karakul and Tsurcana breeds (0.58-0.59). In the other two breeds the diversity is moderate, 0.41 in Tsigai and 0.31 in Palas Merino.

At the Ma factor level the immunogenetic diversity reaches significant levels in Tsurcana (0.55) and especially in Tsigai and Botosani Karakul (0.60-0.62), while in Palas Merino this coefficient is lower (0.4).

Within the R-O system, the O factor is more diverse than the R factor. At the R factor level, the diversity is slightly higher than the 0.5 threshold in Palas Merino and Botosani Karakul; in the other two breeds this coefficient is below 0.5, especially in Tsigai. On the contrary, for the O factor, the immunogenetic diversity value is at the 0.5 limit (in Palas Merino and Tsigai) or more over this limit (in Tsurcana and Botosani Karakul).

***Diversity within the molecular-genetic systems*** (Table 3)

Within the β-lactoglobulin system, the $LGB^A$ allele is more spread than the $LGB^B$ allele in all breeds, but the gap between their distributions are not so large as to generate significant informational energies. Therefore, at the LGB locus we have a consistent diversity being most obvious in Palas Merino and Tsigai (0.62), slightly above the 0.5 threshold in Tsurcana and at the limit of this level in Botosani Karakul.

Table 1. Coefficients of informational energy (*e*), genetic diversity (*d*) and heterozygosity (*Ht*) at the loci of biochemical-genetic systems in the Romanian sheep breeds

| Biochemical-genetic system | Breed | Allele | Allelic frequency | e | d | Ht |
|---|---|---|---|---|---|---|
| Haemoglobin system | Palas Merino $\chi^2$= 0,6014 | $Hb^A$ | 0.1930 | 0.6106 | 0.3894 | 0.3123 |
| | | $Hb^B$ | 0.8070 | | | |
| | Tsigai $\chi^2$= 0,8595 | $Hb^A$ | 0.0850 | 0.8056 | 0.1944 | 0.1559 |
| | | $Hb^B$ | 0.9150 | | | |
| | Tsurcana $\chi^2$= 4,8246 | $Hb^A$ | 0.1667 | 0.6527 | 0.3473 | 0.2785 |
| | | $Hb^B$ | 0.8333 | | | |
| | Botosani Karakul $\chi^2$= 0,4315 | $Hb^A$ | 0.0667 | 0.8444 | 0.1556 | 0.1248 |
| | | $Hb^B$ | 0.9333 | | | |
| Transferrin system | Palas Merino $\chi^2$= 62,2309** | $Tf^I$ | 0.0000 | 0.0785 | 0.9215 | 0.7391 |
| | | $Tf^A$ | 0.3840 | | | |
| | | $Tf^G$ | 0.0010 | | | |
| | | $Tf^B$ | 0.0260 | | | |
| | | $Tf^C$ | 0.0930 | | | |
| | | $Tf^M$ | 0.2290 | | | |
| | | $Tf^D$ | 0.2290 | | | |
| | | $Tf^E$ | 0.0330 | | | |
| | | $Tf^P$ | 0.0050 | | | |
| | Tsigai $\chi^2$= 40,3123 | $Tf^I$ | 0.0080 | 0.0185 | 0.9814 | 0.7871 |
| | | $Tf^A$ | 0.2580 | | | |
| | | $Tf^G$ | 0.0070 | | | |
| | | $Tf^B$ | 0.2420 | | | |
| | | $Tf^C$ | 0.0910 | | | |
| | | $Tf^M$ | 0.1210 | | | |
| | | $Tf^D$ | 0.2580 | | | |
| | | $Tf^E$ | 0.0070 | | | |
| | | $Tf^P$ | 0.0080 | | | |
| | Tsurcana $\chi^2$= 61,7576*** | $Tf^I$ | 0.0000 | 0.0396 | 0.9604 | 0.7703 |
| | | $Tf^A$ | 0.2200 | | | |
| | | $Tf^G$ | 0.0200 | | | |
| | | $Tf^B$ | 0.2250 | | | |
| | | $Tf^C$ | 0.3400 | | | |
| | | $Tf^M$ | 0.1150 | | | |
| | | $Tf^D$ | 0.0500 | | | |
| | | $Tf^E$ | 0.0300 | | | |
| | | $Tf^P$ | 0.0000 | | | |
| | Botosani Karakul $\chi^2$= 57,4509*** | $Tf^I$ | 0.0000 | 0.3182 | 0.6818 | 0.5468 |
| | | $Tf^A$ | 0.0278 | | | |
| | | $Tf^G$ | 0.0000 | | | |
| | | $Tf^B$ | 0.3250 | | | |
| | | $Tf^C$ | 0.5889 | | | |
| | | $Tf^M$ | 0.0278 | | | |
| | | $Tf^D$ | 0.0222 | | | |
| | | $Tf^E$ | 0.0083 | | | |
| | | $Tf^P$ | 0.0000 | | | |
| Albumin system | Palas Merino $\chi^2$= 66,4717*** | $Alb^F$ | 0.0455 | 0.8914 | 0.1086 | 0.0871 |
| | | $Alb^S$ | 0.9545 | | | |
| | Tsigai $\chi^2$= 0,302 | $Alb^F$ | 0.0375 | 0.9098 | 0.0902 | 0.0724 |
| | | $Alb^S$ | 0.9625 | | | |
| | Tsurcana $\chi^2$= 0,00 | $Alb^F$ | 0.0000 | 1.0000 | 0.0000 | 0.0000 |
| | | $Alb^S$ | 1.0000 | | | |
| | Botosani Karakul $\chi^2$= 0,00 | $Alb^F$ | 0.0000 | 1.0000 | 0.0000 | 0.0000 |
| | | $Alb^S$ | 1.0000 | | | |
| Amylase system | Palas Merino $\chi^2$= 0,00 | $Am^B$ | 1.0000 | 1.0000 | 0.0000 | 0.0000 |
| | | $Am^C$ | 0.0000 | | | |
| | Tsigai $\chi^2$=1,4014 | $Am^B$ | 0.9200 | 0.8160 | 0.1840 | 0.1476 |
| | | $Am^C$ | 0.0800 | | | |
| | Tsurcana $\chi^2$= 0,00 | $Am^B$ | 1.0000 | 1.0000 | 0.0000 | 0.0000 |
| | | $Am^C$ | 0.0000 | | | |
| | Botosani Karakul $\chi^2$= 0,00 | $Am^B$ | 1.0000 | 1.0000 | 0.0000 | 0.0000 |
| | | $Am^C$ | 0.0000 | | | |
| Potassium system | Palas Merino | $K^L$ | 1.0000 | 1.0000 | 0.0000 | 0.0000 |
| | | $K^h$ | 0.0000 | | | |
| | Tsigai | $K^L$ | 0.9350 | 0.8481 | 0.1519 | 0.1219 |
| | | $K^h$ | 0.0650 | | | |
| | Tsurcana | $K^L$ | 0.4804 | 0.3760 | 0.6240 | 0.5005 |
| | | $K^h$ | 0.5196 | | | |
| | Botosani Karakul | $K^L$ | 0.0787 | 0.8187 | 0.1813 | 0.1454 |
| | | $K^h$ | 0.9213 | | | |

Table 2. Coefficients of informational energy (*e*), genetic diversity (*d*) and heterozygosity (*Ht*) at the loci of immunogenetic systems in the Romanian sheep breeds

| Blood group system | Blood factor | Breed | Allelic frequency | | | | e | d | Ht |
|---|---|---|---|---|---|---|---|---|---|
| | | | dominant allele | | recessive allele | | | | |
| System A | Aa | Palas Merino | $Aa^+$ | 0.3505 | $Aa^-$ | 0.6495 | 0.4309 | 0.5691 | 0.4564 |
| | | Tsigai | | 0.3390 | | 0.6610 | 0.4398 | 0.5602 | 0.4493 |
| | | Tsurcana | | 0.9458 | | 0.0542 | 0.8718 | 0.1282 | 0.1028 |
| | | Botosani Karakul | | 0.5793 | | 0.4207 | 0.3907 | 0.6093 | 0.4886 |
| System B | Bb | Palas Merino | $Bb^+$ | 0.4261 | $Bb^-$ | 0.5739 | 0.3886 | 0.6113 | 0.4903 |
| | | Tsigai | | 0.2812 | | 0.7188 | 0.4945 | 0.5053 | 0.4053 |
| | | Tsurcana | | 0.6910 | | 0.3090 | 0.4662 | 0.5338 | 0.4281 |
| | | Botosani Karakul | | 0.5488 | | 0.4512 | 0.3809 | 0.6190 | 0.4965 |
| | Bc | Palas Merino | $Bc^+$ | 0.2100 | $Bc^-$ | 0.7900 | 0.5852 | 0.4147 | 0.3326 |
| | | Tsigai | | 0.1215 | | 0.8785 | 0.7332 | 0.2668 | 0.2140 |
| | | Tsurcana | | 0.3422 | | 0.6578 | 0.4372 | 0.5627 | 0.4513 |
| | | Botosani Karakul | | 0.3060 | | 0.6940 | 0.4691 | 0.5309 | 0.4258 |
| | Bd | Palas Merino | $Bd^+$ | 0.1100 | $Bd^-$ | 0.8900 | 0.7552 | 0.2447 | 0.1963 |
| | | Tsigai | | 0.2112 | | 0.7888 | 0.5835 | 0.4165 | 0.3340 |
| | | Tsurcana | | 0.2766 | | 0.7234 | 0.4998 | 0.5002 | 0.4012 |
| | | Botosani Karakul | | 0.4002 | | 0.5998 | 0.3984 | 0.6016 | 0.4813 |
| | Bf | Palas Merinos | $Bf^+$ | 0.3090 | $Bf^-$ | 0.6910 | 0.4662 | 0.5338 | 0.4281 |
| | | Tsigai | | 0.2879 | | 0.7121 | 0.4875 | 0.5125 | 0.4111 |
| | | Tsurcana | | 0.8056 | | 0.1944 | 0.6085 | 0.3915 | 0.3140 |
| | | Botosani Karakul | | 0.4879 | | 0.5121 | 0.3754 | 0.6246 | 0.5010 |
| | Bg | Palas Merino | $Bg^+$ | 0.0890 | $Bg^-$ | 0.9110 | 0.7973 | 0.2027 | 0.1626 |
| | | Tsigai | | 0.1898 | | 0.8102 | 0.6156 | 0.3844 | 0.3083 |
| | | Tsurcana | | 0.2801 | | 0.7199 | 0.4959 | 0.5041 | 0.4043 |
| | | Botosani Karakul | | 0.3729 | | 0.6271 | 0.4154 | 0.5846 | 0.4689 |
| | Bi | Palas Merino | $Bi^+$ | 0.2730 | $Bi^-$ | 0.7270 | 0.5038 | 0.4962 | 0.3979 |
| | | Tsigai | | 0.2595 | | 0.7405 | 0.5196 | 0.4804 | 0.3853 |
| | | Tsurcana | | 0.3508 | | 0.6492 | 0.4306 | 0.5693 | 0.4566 |
| | | Botosani Karakul | | 0.3060 | | 0.6940 | 0.4691 | 0.5309 | 0.4258 |
| System C | Ca | Palas Merino | $Ca^+$ | 0.0590 | $Ca^-$ | 0.9410 | 0.8612 | 0.1388 | 0.1113 |
| | | Tsigai | | 0.0831 | | 0.9169 | 0.8095 | 0.1905 | 0.1528 |
| | | Tsurcana | | 0.5329 | | 0.4671 | 0.3777 | 0.6223 | 0.4991 |
| | | Botosani Karakul | | 0.4369 | | 0.5631 | 0.3849 | 0.6150 | 0.4933 |
| | Cb | Palas Merino | $Cb^+$ | 0.6690 | $Cb^-$ | 0.3310 | 0.4464 | 0.5536 | 0.4440 |
| | | Tsigai | | 0.5303 | | 0.4697 | 0.3773 | 0.6227 | 0.4994 |
| | | Tsurcana | | 0.7634 | | 0.2366 | 0.5484 | 0.4515 | 0.3621 |
| | | Botosani Karakul | | 0.3107 | | 0.6893 | 0.4646 | 0.5354 | 0.4294 |
| System D | Da | Palas Merino | $Da^+$ | 0.1430 | $Da^-$ | 0.8570 | 0.6936 | 0.3064 | 0.2457 |
| | | Tsigai | | 0.2083 | | 0.7917 | 0.5877 | 0.4123 | 0.3306 |
| | | Tsurcana | | 0.3921 | | 0.6079 | 0.4041 | 0.5959 | 0.4779 |
| | | Botosani Karakul | | 0.3593 | | 0.6407 | 0.4245 | 0.5755 | 0.4616 |
| System M | Ma | Palas Merinos | $Ma^+$ | 0.2020 | $Ma^-$ | 0.7980 | 0.5970 | 0.4030 | 0.3232 |
| | | Tsigai | | 0.6680 | | 0.3320 | 0.4456 | 0.5544 | 0.4445 |
| | | Tsurcana | | 0.5969 | | 0.4031 | 0.3985 | 0.6015 | 0.4824 |
| | | Botosani Karakul | | 0.4646 | | 0.5354 | 0.3781 | 0.6219 | 0.4987 |
| System R-O | R | Palas Merino | $R^+$ | 0.3040 | $R^-$ | 0.6960 | 0.4710 | 0.5290 | 0.4242 |
| | | Tsigai | | 0.1780 | | 0.8220 | 0.6342 | 0.3658 | 0.2934 |
| | | Tsurcana | | 0.2546 | | 0.7454 | 0.5255 | 0.4744 | 0.3805 |
| | | Botosani Karakul | | 0.2997 | | 0.7003 | 0.4753 | 0.5247 | 0.4208 |
| | O | Palas Merino | $O^+$ | 0.2830 | $O^-$ | 0.7170 | 0.4927 | 0.5073 | 0.4068 |
| | | Tsigai | | 0.2690 | | 0.7310 | 0.5084 | 0.4916 | 0.3943 |
| | | Tsurcana | | 0.5487 | | 0.4513 | 0.3809 | 0.6191 | 0.4965 |
| | | Botosani Karakul | | 0.3340 | | 0.6660 | 0.4439 | 0.5561 | 0.4460 |

Table 3. Coefficients of informational energy (e), genetic diversity (d) and heterozygosity (Ht) at the loci of molecular-genetic systems in the Romanian sheep breeds

| Molecular-genetic system | Breed | Allele | Allelic frequency | e | d | Ht |
|---|---|---|---|---|---|---|
| β-Lactoglobulin system | Palas Merino $\chi^2$= 64,5945*** | $LGB^A$ | 0.5550 | 0.3826 | 0.6174 | 0.4952 |
| | | $LBG^B$ | 0.4450 | | | |
| | Tsigai $\chi^2$= 9,7537** | $LGB^A$ | 0.5420 | 0.3794 | 0.6206 | 0.4977 |
| | | $LBG^B$ | 0.4580 | | | |
| | Tsurcana $\chi^2$= 1,9213 | $LGB^A$ | 0.6870 | 0.4624 | 0.5376 | 0.4311 |
| | | $LBG^B$ | 0.3130 | | | |
| | Botosani Karakul $\chi^2$= 8,9059** | $LGB^A$ | 0.7350 | 0.5131 | 0.4869 | 0.3905 |
| | | $LBG^B$ | 0.2650 | | | |
| Calpastatin system | Palas Merino $\chi^2$= 4,9392 | $CAST^A$ | 0.7969 | 0.5954 | 0.4046 | 0.3245 |
| | | $CAST^B$ | 0.2031 | | | |
| | Tsigai $\chi^2$= 2,2879 | $CAST^A$ | 0.7684 | 0.5551 | 0.4449 | 0.3568 |
| | | $CAST^B$ | 0.2316 | | | |
| | Tsurcana $\chi^2$= 3,6612 | $CAST^A$ | 0.8624 | 0.7033 | 0.2967 | 0.2379 |
| | | $CAST^B$ | 0.1376 | | | |
| | Botosani Karakul 71.9659*** | $CAST^A$ | 0.8468 | 0.6757 | 0.3243 | 0.2601 |
| | | $CAST^B$ | 0.1532 | | | |
| $\alpha_s$1-casein system | Palas Merino $\chi^2$= 0,00 | $Cn^A$ | 0.0000 | 1.0000 | 0.0000 | 0.0000 |
| | | $Cn^C$ | 1.0000 | | | |
| | Tsigai $\chi^2$= 0,00 | $Cn^A$ | 0.0000 | 1.0000 | 0.0000 | 0.0000 |
| | | $Cn^C$ | 1.0000 | | | |
| | Tsurcana $\chi^2$= 0,00 | $Cn^A$ | 0.0000 | 1.0000 | 0.0000 | 0.0000 |
| | | $Cn^C$ | 1.0000 | | | |
| | Botosani Karakul $\chi^2$= 0,00 | $Cn^A$ | 0.0000 | 1.0000 | 0.0000 | 0.0000 |
| | | $Cn^C$ | 1.0000 | | | |
| Prion system | Palas Merino $\chi^2$ = 4.93 | ARR | 0.4108 | 0.2768 | 0.7232 | 0.5801 |
| | | ARQ | 0.5000 | | | |
| | | AHQ | 0.0270 | | | |
| | | ARH | 0.0297 | | | |
| | | VRQ | 0.0325 | | | |
| | Tsigai $\chi^2$ = 5.12 | ARR | 0.4189 | 0.3191 | 0.6809 | 0.5455 |
| | | ARQ | 0.5279 | | | |
| | | AHQ | 0.0080 | | | |
| | | ARH | 0.0199 | | | |
| | | VRQ | 0.0253 | | | |
| | Tsurcana $\chi^2$ = 10,29 | ARR | 0.3755 | 0.2645 | 0.7355 | 0.5896 |
| | | ARQ | 0.5143 | | | |
| | | AHQ | 0.0306 | | | |
| | | ARH | 0.0082 | | | |
| | | VRQ | 0.0714 | | | |
| | Botosani Karakul $\chi^2$ = 29,74*** | ARR | 0.4025 | 0.3384 | 0.6616 | 0.5306 |
| | | ARQ | 0.5539 | | | |
| | | AHQ | 0.0000 | | | |
| | | ARH | 0.0436 | | | |
| | | VRQ | 0.0000 | | | |
| SLS system | Palas Merino $\chi^2$= 0,00 | FGFR3 | 1.0000 | 1.0000 | 0.0000 | 0.0000 |
| | | $FGFR3^+$ | 0.0000 | | | |
| | Tsigai $\chi^2$= 0,00 | FGFR3 | 1.0000 | 1.0000 | 0.0000 | 0.0000 |
| | | $FGFR3^+$ | 0.0000 | | | |
| | Tsurcana $\chi^2$= 0,00 | FGFR3 | 1.0000 | 1.0000 | 0.0000 | 0.0000 |
| | | $FGFR3^+$ | 0.0000 | | | |
| | Botosani Karakul $\chi^2$= 0,00 | FGFR3 | 1.0000 | 1.0000 | 0.0000 | 0.0000 |
| | | $FGFR3^+$ | 0.0000 | | | |
| Booroola system | Palas Merino $\chi^2$= 0,00 | FecB | 1.0000 | 1.0000 | 0.0000 | 0.0000 |
| | | $FecB^+$ | 0.0000 | | | |
| | Tsigai $\chi^2$= 0,00 | FecB | 1.0000 | 1.0000 | 0.0000 | 0.0000 |
| | | $FecB^+$ | 0.0000 | | | |
| | Tsurcana $\chi^2$= 0,00 | FecB | 1.0000 | 1.0000 | 0.0000 | 0.0000 |
| | | $FecB^+$ | 0.0000 | | | |
| | Botosani Karakul $\chi^2$= 0,00 | FecB | 1.0000 | 1.0000 | 0.0000 | 0.0000 |
| | | $FecB^+$ | 0.0000 | | | |
| Inverdale system | Palas Merino $\chi^2$= 0,00 | FecX | 1.0000 | 1.0000 | 0.0000 | 0.0000 |
| | | $FecX^+$ | 0.0000 | | | |
| | Tsigai $\chi^2$= 0,00 | FecX | 1.0000 | 1.0000 | 0.0000 | 0.0000 |
| | | $FecX^+$ | 0.0000 | | | |
| | Tsurcana $\chi^2$= 0,00 | FecX | 1.0000 | 1.0000 | 0.0000 | 0.0000 |
| | | $FecX^+$ | 0.0000 | | | |
| | Botosani Karakul $\chi^2$= 0,00 | FecX | 1.0000 | 1.0000 | 0.0000 | 0.0000 |
| | | $FecX^+$ | 0.0000 | | | |

The 3/1 representation ratio between the two calpastatin alleles in Palas Merino and Tsigai causes a moderate genetic diversity being slightly below 0.50. The gap widening between the $CAST^A$ and $CAST^B$ allele frequencies leads to decreasing of calpastatin diversity too in the other two breeds (around 0.30).

In the prion system, the larger number of alleles at the PrP locus also influences the diversity by its increasing. In Palas Merino, Tisgai and Tsurcana, where all five prion alleles can be found, the genetic diversity degree is quite high; the differences among the three breeds come from the distribution way of these alleles. In Tsurcana, where the five alleles are more evenly spread, there is the richest diversity (about 0.74), as well as in Palas Merino (0.72). In Tsigai, because the alleles are less evenly spread a decrease of prion diversity occurs. In the Botosani Karakul breed, both the presence of only three alleles and their distribution unevenness have the effect a lower prion diversity coefficient than in the other three breeds although its decreasing is not significant.

Although the $\alpha_s$1-casein, ovine hereditary chondrodysplasia (SLS), Booroola and Inverdale systems are biallelic systems, they are monotypic at respective loci in Romanian sheep breeds. In each of these four systems, only one allele is expressed, respectively $Cn^C$, FRFG3, FecB and FecX, their codominant missing, respectively $Cn^A$, $FRFG3^+$ $FecB^+$ and $FecX^+$. The polymorphism lack at these loci generates maximum informational energies, their counterpart being the complete absence of genetic diversity in these molecular systems.

***Complex genetic diversity***

To get an overview of the genetic diversity quantified within the multiple and associated systems, breeds, etc. (complex genetic diversity - $d_t$), the synthetic indicator is used, namely the complex informational energy ($e_t$).

***Complex genetic diversity on associated system types***

Within the *biochemical-genetic systems*, the large number of alleles and their spread range in the transferrin system generate extremely low informational energies. As a result, the genetic diversity calculated on all breeds throughout transferrin system is very high (about 0.89). In haemoglobin and kalium systems of biallelic type, in which the polymorphism has a middle level, the genetic diversity is moderate (0.27 in haemoglobin system, respectively 0.24 in potassium system). In the albumin and amylase systems, where most breeds are monotypic at the respective loci or in those where there is polymorphism but this is very narrow, the genetic diversity is extremely low (only 0.05). In relation to these coefficients, the total genetic-biochemical systemic diversity is moderate (0.30) (Figure 1).

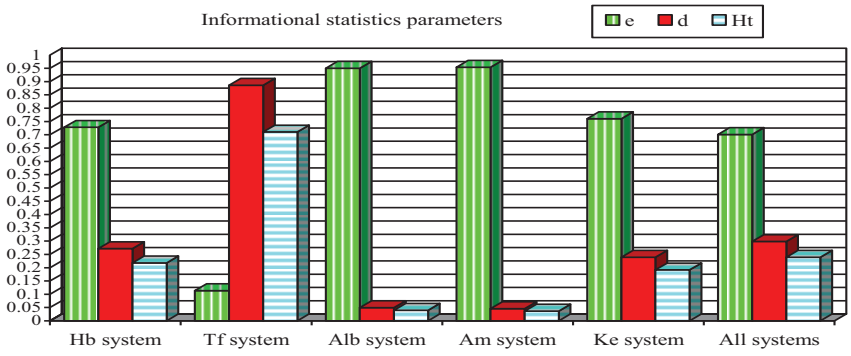


Figure 1. Coefficients of complex informational energy (***e$_t$***), complex genetic diversity (***d$_t$***) and heterozygosity (***Ht***) in the biochemical-genetic systems in the Romanian sheep breeds

Within the *immunogenetic systems* there is a relatively uniform distribution of the two types of alleles (dominant and recessive) at the level of all blood group factors. Even if there are differences in relation to the frequencies of the two allele types, the gaps between them are not exaggerated. For this fact, for most blood factors, the diversity is of middle level, its

framing range being between 0.45 and 0.55. Only at the Ca factor level the variability is lower, however its value have to taken into consideration (0.39). The variability at Bf and Bi blood factors is circumscribed at the 0.5 limit. Higher values than this threshold are achieved by Bb, Cb, Ma and O factors. At the level of factors Aa, Bc, Bd, Bg, Da and R, the immunogenetic variability records scores below the 0.50 median limit. Given the values of these coefficients between the two informational-statistical parameters there is an almost unitary ratio, with a weaker surplus for the informational energy (0.51). As a result, the total immunogenetic diversity is in close proximity to the median threshold (about 0.49) (Figure 2).

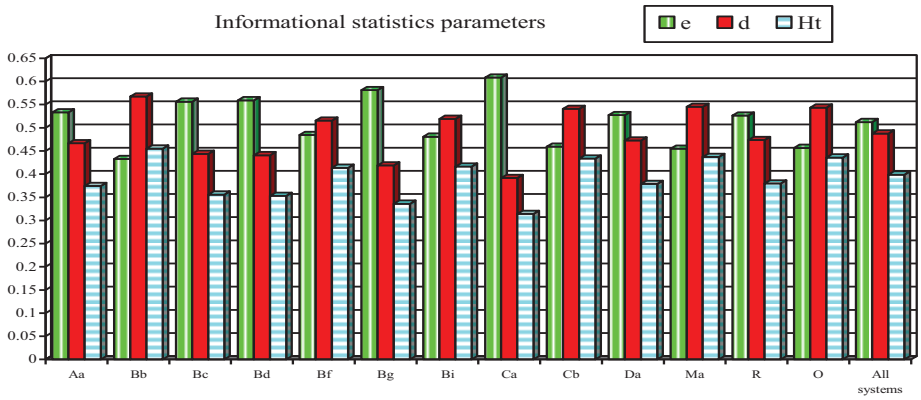


Figure 2. Coefficients of complex informational energy ($e_t$), complex genetic diversity ($d_t$) and heterozygosity (***Ht***) in the immunogenetic systems in the Romanian sheep breeds

The most variable system within the *molecular-genetic systems* is the polyallelic system of prion protein. The five alleles as well as their dispersion in prion panel lead to a high enough diversity (0.70). Within the other six systems of biallelic type, only at LGB and CAST loci there is genetic polymorphism. In the β-lactoglobulin system, due to a more balanced ratio between the two alleles there is a considerable diversity, above the average limit, measuring about 0.56. On the other hand, in the calpastatin system there is a more obvious representation gap of $CAST^A$ and $CAST^B$ alleles, which makes the diversity to be more moderate, too (about 0.37). In the $\alpha_s1$-casein, hereditary chondrodysplasia and fecundity gene (Booroola and Inverdale) systems the Romanian sheep breeds being monomorphic there is not genetic diversity. For all these reasons, the total molecular-genetic diversity is low (about 0.23) (Figure 3).

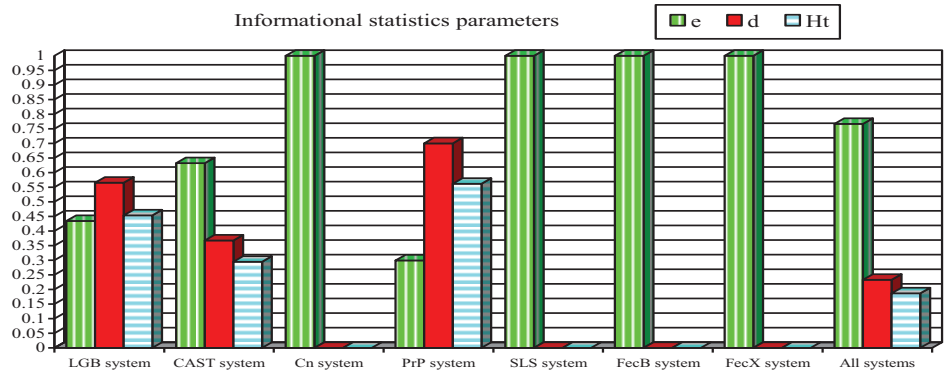


Figure 3. Coefficients of complex informational energy ($e_t$), complex genetic diversity ($d_t$) and heterozygosity (***Ht***) in the molecular-genetic systems in the Romanian sheep breeds

***Complex genetic diversity on sheep breeds***

An informational statistics analysis of the four Romanian sheep breeds on the three types of genetic systems leads to as real as possible quantification of genetic diversity.

Within the *biochemical-genetic systems* the most diverse breed (of middle level) is Tsurcana (0.39) and Botosani Karakul is the breed with the lowest diversity degree (0.20). The moderate diversity of Tsigai (0.32) and Palas Merino (0.28) is situated in this variability range. Overall the Romanian sheep breeds the biochemical-genetic diversity is of moderate level (0.30) (Figure 4).

Within the *immunogenetic systems* the breed with the greatest diversity is Botosani Karakul in which the value of this coefficient is considerable (0.58). The Tsurcana breed performs a diversity of middle level (0.50). In the other two breeds the genetic diversity is below the median threshold, but it has values to be taken into consideration, 0.44 in Tsigai and 0.42 in Palas Merino. Because of these issues, the total immunogenetic diversity for the four Romanian sheep breeds has a medium level (0.49) (Figure 5).

Within the *molecular-genetic systems*, among the four Romanian sheep breeds no significant differences are recorded, the coefficients of this informational-statistical parameter ranging in a narrow enough range from 0.21 in Botosani Karakul to 0.25 in Palas Merino and Tsigai; the Tsurcana diversity (about 0.22) is placed between these limits. On the whole of ovine species from Romania, the molecular-genetic diversity is relatively low (0.23) (Figure 6).

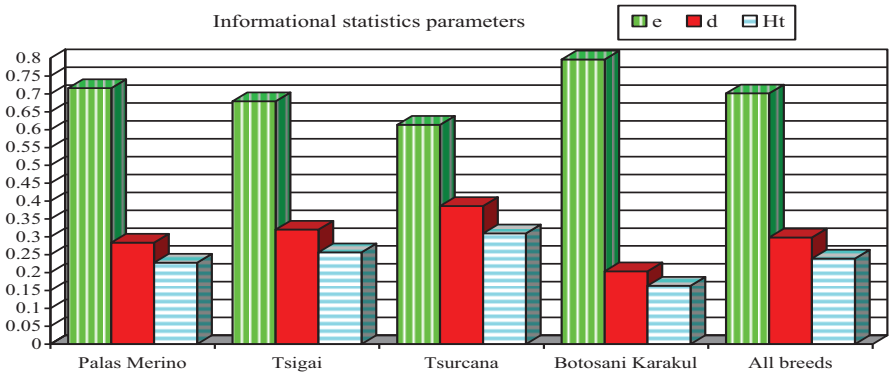


Figure 4. Coefficients of complex informational energy ($e_t$), complex genetic diversity ($d_t$) and heterozygosity (***Ht***) within the biochemical-genetic systems on each Romanian sheep breed

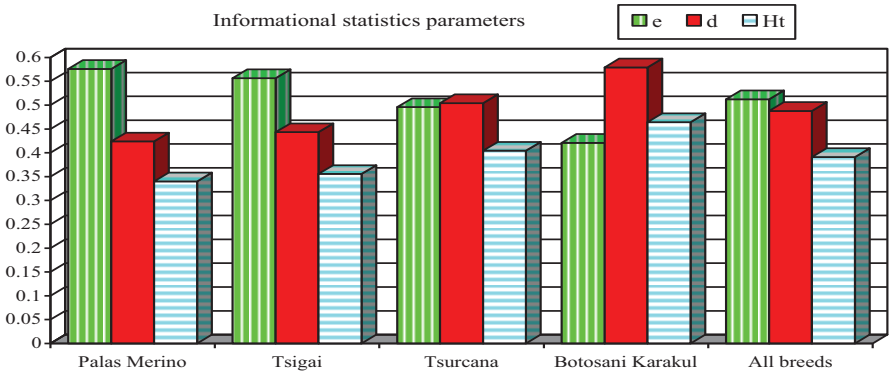


Figure 5. Coefficients of complex informational energy ($e_t$), complex genetic diversity ($d_t$) and heterozygosity (***Ht***) within the immunogenetic systems on each Romanian sheep breed

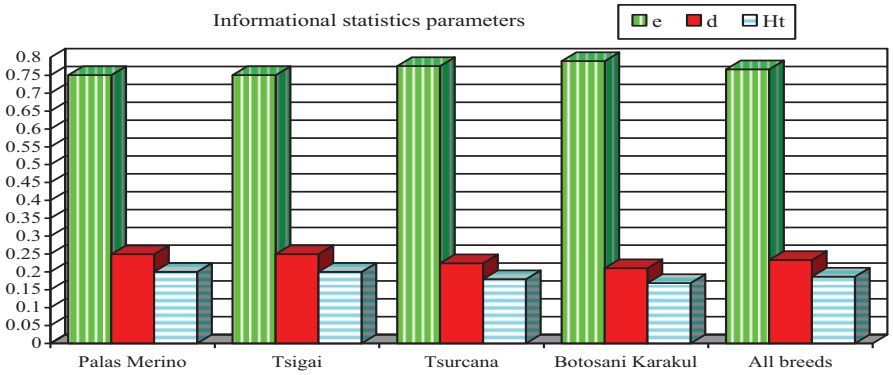


Figure 6. Coefficients of complex informational energy ($e_t$), complex genetic diversity ($d_t$) and heterozygosity (***Ht***) within the molecular-genetic systems on each Romanian sheep breed

Summing *all genetic diversity coefficients* of the three types of polymorphic systems, it is clear that Tsurcana is the most diverse breed, close to the medium level (0.37). A moderate diversity is met in the other three breeds with very small differences among breeds, 0.32 in Palas Merino, 0.33 in Botosani Karakul and 0.34 in Tsigai. On all four Romanian sheep breeds the total genetic diversity recorded a moderate level (0.34) (Figure 7).

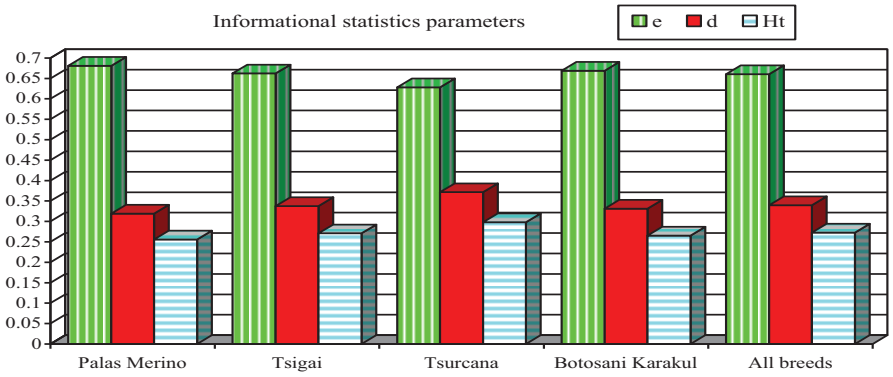


Figure 7. Coefficients of complex informational energy ($e_t$), complex genetic diversity ($d_t$) and heterozygosity (***Ht***) within all genetic systems on each Romanian sheep breed

The coefficient value of informational energy or genetic diversity is a measure of the *polymorphism degree*, both of the genetic systems and of the sheep breeds at all loci levels.

The polyalellic systems are the most allotropic, the highest polymorphism level having firstly the transferrin system and then the prionic system. In the biallelic systems, the polymorphism occurs where both alleles are expressed, either in codominant status or in dominance to recessiveness status. The polymorphism is marked to some of them, such as in the β-lactoglobulin system and at most blood group factors. Within some systems the polymorphism degree is moderate, such as in haemoglobin, potassium, calpastatin and C systems, at the Ca factor level. The polymorphism is barely perceptible in others of them (albumin in Palas Merino and Tsigai and amylase in Tsigai). The biallelic systems in which only one allelic type is expressed are characterized by genetic monomorphism: albumin system in Tsurcana and Botosani Karakul, amylase system in Palas Merino Tsurcana and Botosani Karakul, kalium system in Palas Merino and $\alpha_s$1-casein, SLS, Booroola and Inverdale systems in all sheep breeds.

In the biochemical-genetic respect, the

Tsurcana sheep presents the highest polymorphism degree and the narrowest polymorphism is found in Botosani Karakul. The polymorphism of biochemical-genetic structures of Palas Merino is slightly more emphasized than of the Tsigai.

The immunogenetic structures are highly polymorphic in all sheep breeds, but Botosani Karakul is the most endowed with erythrocyte antigens and Palas Merino is the poorest from the immunogenetically point of view.

The molecular-genetic polymorphism is limited enough in all Romanian sheep breeds and the differences among breeds are minor: the Palas Merino and Tsigai breeds are slightly more polymorphic than Tsurcana and especially than Botosani Karakul.

In terms of all structures with multiple molecular forms, Tsurcana is the most polymorphic breed, much more polymorphic than Botosani Karakul or Tsigai and in particular towards the Palas Merino. But throughout the ovine species from Romania the genetic polymorphism is moderate.

The *heterozygosity degree* of a taxonomic entity for different characters is the true measure of genetic variability. In this regard, it is noted that there is perfect concordance between the genetic diversity and heterozygosity status in the Romanian sheep breeds at the level of all genetic system loci. The differences between these two informational statistics parameters are derived from their values, the heterozygosity being reduced steadily in all situations, with about 22%. Thus, a graphical representation of the two genetic parameters is enlightening as regards the identical allure of their curves. For illustration, we present only the diagram concerning the relationship between diversity and heterozygosity on all genetic systems in the four Romanian sheep breeds (Figure 8).

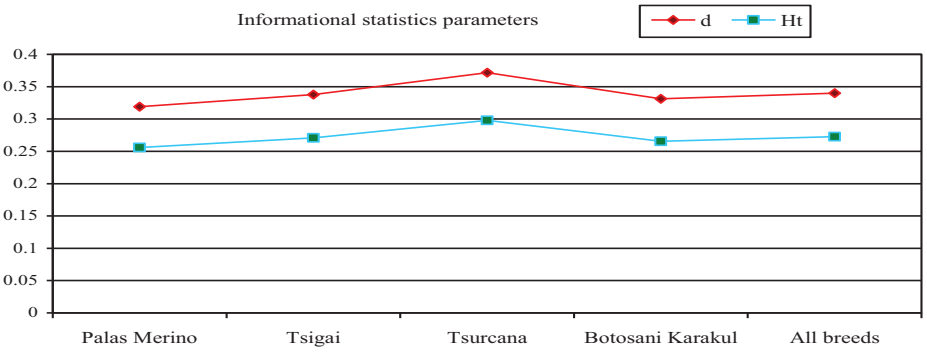


Figure 8. Distributional curves of genetic diversity (**d**) and heterozygosity (**Ht**) within all genetic systems on each Romanian sheep breed

Discussing in cybernetic terms, the values of the two indicators of informational statistics express the *systemic organization degree*, an opposite concept to *entropy* (*H*); when the informational energy is lower and the genetic diversity is more significant, then the organization degree of the system is higher; on the contrary, when the informational energy increases and genetic diversity decreases, then the entropy increases in system (the disorganization degree becomes more emphasized). The highest organization degree is met in the systems of biallelic type, but in which only one allele type is expressed (amylase system in Palas Merino, Tsurcana and Botosani Karakul, potassium system in Palas Merino and $\alpha_s$1-casein, SLS, Booroola and Inverdale systems in all sheep breeds). In the albumin (in Palas Merino and Tsigai) and amylase (in Tsigai) systems the first signs of entropy appear. The disorganization degree increases in haemoglobin, kalium and calpastatin systems and becomes more emphasized in β-lactoglobulin and blood group systems. In the prionic system and especially in the transferrin one it comes out the highest degree of systemic entropy. From this perspective, the breeds can have different levels of entropy with particular reference to each genetic system or system association, but overall the genetic

structures all breeds have roughly the same degree of systemic organization.
From this analysis, it should be mentioned that the polymorphic panel of hereditary heritage of some biological entities acquires greater accuracy if as many genetic systems participate to the construction of the informational-statistical edifice and as they are more diversified like the number of alleles and their distribution.
Determining as correct as possible the genetic diversity structures of sheep breeds contributes to the enriching of cognitive status relating to the genetic variability and genetic resources within this species. This inventory of genetic resources is necessary for development of improvement programs of sheep breeds of economic interest and perfecting of the existing ones, but also to initiate and implement the programs to protect and preserve the genetic potential of breeds in a vulnerability status (Cohen, 1999). The conservation and enhancement of genetic diversity is a key condition in performing the farm animal breeding programs, the diversity becoming, justifiably, one of the most evoked concepts in the economical and environmental policies (Gandini et al., 2007). There are also situations when the genetic diversity increasing at some loci can be dangerous. This issue can be met in the case of genetic diseases and those with hereditary predisposition, by increasing the incidence of lethal alleles, which can produce considerable economic losses and harm effects on animal and human health (Álvarez et al., 2009; Hrincă, 2016b, 2016c).

## CONCLUSIONS

Using the informational energy (*e*) the genetic diversity (*d*) was quantified in four Romanian sheep breeds (Merino Palas, Tsigai, Tsurcana and Botosani Karakul) within three types of genetic systems: biochemical-genetic (haemoglobin, transferrin, albumin, amylase, blood potassium), immunogenetic (blood groups A, B, C, D, M, R-O) and molecular-genetic ($\alpha_s$1-casein, β-lactoglobulin, SLS, Booroola, Inverdale).
The calculus algorithm for determining the size of the two informational-statistical indicators was based on frequencies of 70 alleles: 17 alleles of biochemical-genetic systems, 36 alleles of immunogenetic systems and 17 alleles of molecular-genetic systems.
The genetic diversity level is an informational-statistical function provided by the allele number, their frequencies, the ratio among them within the systems and the polymorphism degree of genetic systems.
The highest diversity occurs in the polyallelic systems (transferrin and prion protein).
In the other systems, of biallelic type, the genetic diversity occurs where both allele types, either in codominant status or in complete dominance relation, are present in the panels of respective systems; in the systems in which the two allele types are more evenly spread, the genetic diversity is more relevant (β-lactoglobulin and blood group systems) and in the systems where the frequency gap gets larger it comes out a decrease of diversity (haemoglobin, blood potassium, calpastatin); in the albumin and amylase systems (in some breeds), where one of allele is prevailing, the diversity is very low.
In the monomorphic systems in which only one allele type is expressed (albumin and amylase systems in some breeds and $\alpha_s$1-casein, hereditary chondrodysplasia protein and FecB and FecX gene fecundity systems in all breeds), the genetic diversity is completely missing.
Among the Romanian sheep breeds there are larger or smaller differences regarding the genetic diversity levels when the analysis takes into account each system or even when diversity is measured on the three system types (biochemical-genetic, immunogenetic and molecular-genetic), but summing all systems the differences among breeds are small and unsignificant regarding the genetic diversity as a whole.
The informational-statistical quantification of genetic diversity in sheep is a very useful tool in breeding programs of economic interest breeds and for preservation of the vulnerable ones.

## ACKNOWLEDGEMENTS

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# EVALUATION OF THE EXTERIOR OF HOLSTEIN AND SIMMENTAL PRIMIPAROUS COWS

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***Abstract***

*The aim of presented research is to study the exterior features and the morphological and functional parameters of the udder of primiparous* cows *of Holstein and Simmental breeds in the herd SLL "Strapit", Kalarash. Research carried out on Holstein primiparous cows (n = 19), Simmental (n = 22) of the Austrian origin. In order to establish the main features of the exterior of the body measurements were taken basic body measurements and defined physique indexes. As a result of research it was established that primiparous cows of Holstein breed is well defined type of dairy cattle, confirming their proportional physique shape, the development of the middle part of the body, strong bone. Simmental primiparous cows for measurements the height at the withers by 3.3% and in the sacrum by 1.2% exceed the minimum requirements of the standard. Chest well developed in depth, wide enough, which is characteristic for the Simmental. Udder of primiparous cows of Holstein breed differs bulkiness, with the developed portions, advantageously tightly attached. Visually, the external structure of the udder of these animals differs by a greater extent along the belly and enough depth than at heifers of Simmental breed.*

***Key words:*** *Holstein, Simmental breed, exterior, index physique, the udder*.

## INTRODUCTION

One of the main problems of modern selection science and zootechnical practice is to obtain animals, combining high productivity with a strength build, suitable for long-term use in industrial technology. Cow of a dairy direction differs with its inherent exterior forms with its peculiar morphofunctional structure of tissue and orientation of physiological processes in the body.

The best specialized dairy breed in the world is the Holstein, which together with high indicators of milk production is characterized by excellent qualities of the exterior thanks to the purposeful selection of animals on the exterior type and has been established this breed (Ackles, 1960).

It should be noted that the genotype of the Austrian Simmental is well selected for the industrial production of milk, which is important for our country.

As it is known a large part of the morphological traits of the udder is the most important and reliable exterior indicators of high milk production and manufacturability of cows (Von Keyserlink et al., 2012; Weary, 2012; Kotendzhi et al., 2007; Kotendzhi et al., 1996).

The study of exterior of animals of Holstein and Simmental breeds grown and used in the same conditions of SLL "Strapit", is quite important from a practical and a scientific point of view. Therefore, the aim of research was to examine the exterior of the body and the morphological and functional parameters of the udder of Holstein heifers and Simmental breeds.

## MATERIALS AND METHODS

The studies were conducted in a herd of pedigree cattle of Holstein and Simmental breeds SRL "Strapit". The exterior of tested animals were studied by the development of the main items of the body structure, measurements of which were taken with dimensional instruments by the standard technique during 2-3 months after calving.

On the foundation of measurements were calculated indices of the build: long-legged, prolixity, hip- breast, chest, consistency, outgrowing, awl back, raw-boned on which

were built exterior profile by the conventional method (Kostomahin, 2007). Morphological and functional properties of the udder were evaluated on the second - third month of lactation according to conventional techniques, (Metodical materials, 1970).

The obtained results of research were processed by methods of variation of statistics and determination of significance of differences according to Merkurev et al., 1983, in Excel, the accuracy of the figures estimated by Student's.

## RESULTS AND DISCUSSIONS

Analysis of body measurements of primiparous cows of different breeds showed that at the animals of Holstein breed is well defined the type of dairy cattle, confirming their proportional body shape, the development of the middle part of the body, strong bones, Table 1.

Table 1. Linear Indicators of measurements of items of the primiparous cows body of Holstein breed

| Measurements | Indicators | |
|---|---|---|
| | M± m, см | Cv, % |
| Estimated animals, heads | 19 | |
| Height at withers | 145.1±0.95 | 2.86 |
| Height at the croup | 151.4±0.86 | 2.48 |
| The depth of the thorax | 70.5±07 | 4.34 |
| The width of the thorax | 45.8±0.35 | 3.37 |
| The width of the croup | 52.0±0.37 | 3.13 |
| The width of the croup at Ischia | 34.7±0.26 | 3.31 |
| Length of the body | 159.2±0.5 | 1.37 |
| The thorax perimeter | 193.4±1.15 | 2.59 |
| The whistle perimeter | 19.5±0.07 | 1.59 |

So, according to high-altitude measurements primiparous cows of Holstein breed were quite tall - 145.1 cm - height at the withers and 151.4 cm - in the sacrum. Chest well developed in depth - 70.5 cm, width - 45.8 cm and girth 193.4 cm.

Primiparous cows of Simmental breed for most of measurements, except metacarpus, exceed the minimum value standard on the given breed (Table 2, Figure 1).

As it is seen, the animals of Simmental breed by altitude measurements also are tall height at the withers by 3.3% and in the sacrum by 1.2% exceed the minimum requirements of the standard. Chest is well developed in depth - 2.6%, is wide enough - by 2.7%, with a girth of 6% above the standard, which is typical for the Simmental breed.

Table 2. Linear Indicators of measurements of items of the primiparous cows body of Simmental breed

| Measurements | Indicators | | Standard |
|---|---|---|---|
| | M±m, cm | Cv, % | |
| Estimated animals, heads | 22 | | |
| Height at withers | 139.5±0.45 | 1.53 | 135 |
| Height at the croup | 146.8±0.58 | 1.85 | 145 |
| The depth of the thorax | 71.8±0.31 | 2.06 | 70 |
| The width of the thorax | 46.2±0.21 | 2.17 | 45 |
| The width of the croup | 55.6±0.2 | 1.73 | 55 |
| The width of the croup at Ischia | 36.4±0.22 | 2.88 | 36 |
| Length of the body | 159.3±0.4 | 1.16 | 155 |
| The thorax perimeter | 203.6±0.96 | 2.21 | 192 |
| The whistle perimeter | 22.6±0.07 | 1.46 | 23 |

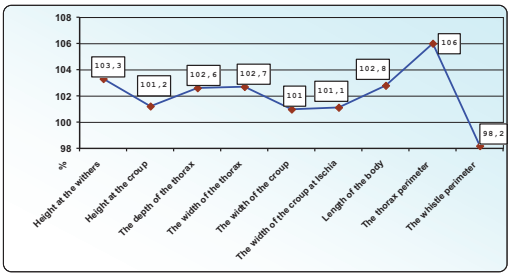



Figure 1. The exterior profile of Simmental primiparous cows

It should be mentioned that the use of body indices allows us to obtain the relative numerical values that characterize the exterior type of dairy cattle in the correlative harmony of all articles of constitution.

Indices of the body of heifers of Holstein and Simmental breeds of investigated animal groups are shown in Table 3.

Table 3. Indices of the body of primiparous cows of Holstein and Simmental breeds, %

| Index | Holstein | Simmental | The breed standard of various productivity directions | |
|---|---|---|---|---|
| | | | Dairy | Dairy and Meat |
| Long-legged | 51.4 | 48.5 | 45.2 | 48.2 |
| Prolixity | 109.7 | 114.2 | 120 | 118.4 |
| Pelvic-thoracic | 88.0 | 83.1 | 80.2 | 85.5 |
| Thoracic | 64.9 | 64.3 | 61.8 | 68.8 |
| Consistency | 121.5 | 127.8 | 118 | 121.3 |
| Outgrown | 104.3 | 105.2 | 100.9 | 102.5 |
| Osseous | 13.4 | 16.2 | 14.6 | 15.4 |

The obtained high leggy index at animals of Holstein breed indicates that they are more long- legged than primiparous cows of Simmental breed, and as a whole the average level of the index 51.4 and 48.5% respectively, characterizes the good development of the organism in a postnatal ontogenesis of animals in both groups. The smaller value of the index

of long-legged animals of Simmental breed (48.5%) is characteristic for the species of milk-meat direction of productivity.

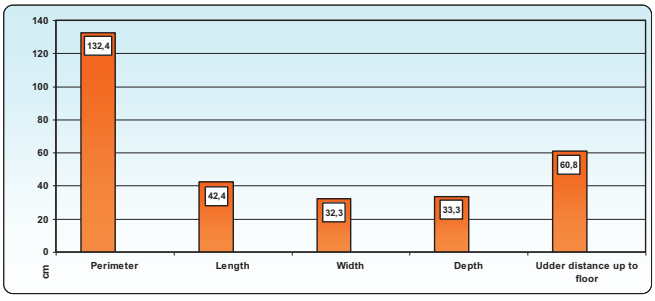
The lower level of the index prolixity, or format, is inherent to dairy cattle with the best characteristic of the quality of exterior type. As the value of indicators of our research, at primiparous cows of Holstein breed the index of stretching amounted to 109.7%, which is with 8.6% less in comparison with the standard for dairy breeds of productivity directions. Index prolixity of Simmental heifers is by 3.5% lower in comparison with the standard for breeds of dairy and meat direction of productivity, which is also characterized by its good quality exterior.

On the overall development of the body and body weight, in particular, can be judged by the index of consistency or compactness. It should be noted that the Holstein primiparous cows and Simmental breeds characterizes compact body with the index of consistency with 127.8 to 121.5%, respectively, which is peculiar to them during the studied period of development.

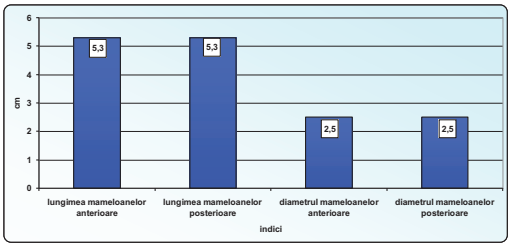
The ratio of height in the sacrum to the height at the withers is characterized by the index of outgrown, which is a good indicator of growth and development in the postnatal period. The corresponding average indices of our research of this given index (104.3 and 105.2%) show an equally good development of the body of animals of both breeds or primiparous cows of Holstein breed the indicator of bony index was with 8.2% below the standard for dairy breeds of productivity direction for primiparous cows of Simmental - with 0.8% lower than the standard for breeds of dairy - meat direction, and the proportion of the body of animals of analyzed breeds are preserved.

Thus, the results of the visual and the index evaluation showed that Holstein primiparous cows had expressed milk type and primiparous cows of Simmental - milk-meat type build. They are characterized by a good body shape and a strong constitution, from which largely depends the level of milk production, health and the duration of the period of productive use.

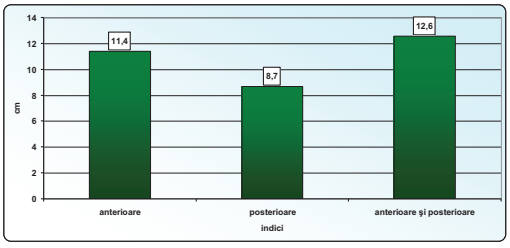
**The form and morphological properties of udder** are one of the important features exterior. Mammary gland with its features that characterize the size, shape, share development, location and size of the nipples, reflects the potential productivity of animals. Indicators of measurements, which are shown in Figure 2, characterize the development of morphological traits of the udder at Holstein primiparous cows of herd SLL "Strapit".




a) size of the udder




b) size of nipples




c) the distance between the nipples

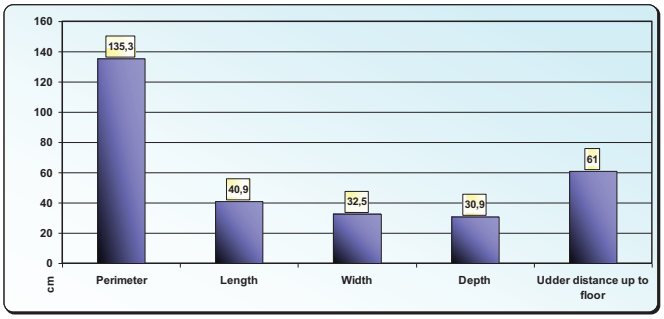
Figure. 2. Morphological signs of udder of primiparous cows of Holstein breed

As it is seen, the average value of measurements of girth (132.4 cm), length (42.4 cm) and width (32.3 cm) were higher than the minimum requirements for the evaluation of the udder at primiparous cows of Holstein breed. Udder is quite deep and has an average of 33.3 cm.

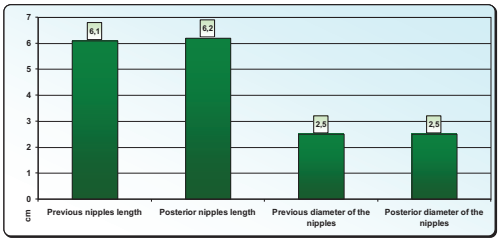
The length of the front nipples were within 5.3 cm, rear - 2.5 cm. The diameter of nipples and the distance between the nipples teats of the udder corresponded to the standard location, convenient for milking. The distance from the bottom of the udder till the ground was within the allowable standards - 60.8 cm.

It is believed that the distance from the bottom of the udder till the ground should be 45-50 cm because too saggy udder prevents the free movement of cows, it is inconvenient for machine milking is more polluted. All measured primiparous cows of Holstein breed had a desired bath-shaped form of the udder, the development of quarters of the udder is symmetrical, uniform, dense attachment to the body, and bottom of the udder is horizontal, nipples of cylindrical shape.

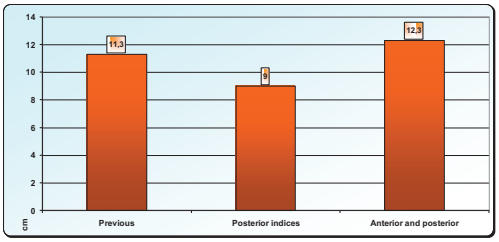
The magnitude of the udder measurements of primiparous cows of Simmental breed exceeded the minimum requirements for the evaluation of the udder and nipples of cows of Simmental breed for the first lactation (Figure 3).




a) size of the udder




b) size of the nipples




c) the distance between the nipples

Figure 3. Morphological signs of udder of primiparous cows of Simmental breed

So udder girth averaged 135.3 cm that is with 35.3 cm bigger the length - 6.9, with - 2.5 and depth - with 5.9 cm bigger than the minimum requirements for cows of Simmental breed (Kobtsev et al., 2011).

The length of the front nipples was in the normal range and the average was 6.1 cm, the diameter of the nipples and the distance between them were within normal limits. The shape of the udder 66.7% of heifers of Simmental breed had a bath form, 33.3% - a bowl form.

Thus, the udder of primiparous cows of Holstein breed differs bulky, with the developed portions, preferably tightly attached. Visually, the external structure of the udder of these animals differs with more length on the belly and enough depth than at heifers of Simmental breed.

## CONCLUSIONS

1. Primiparous cows of Holstein breed are fairly tall: height at the withers on average is 145.1 cm, height at sacrum - 151.4 cm, with a

well-developed breast in depth – 70.5 cm, in width - 45.8 cm, and girth – 193.4 cm.

2. Primiparous cows of Simmental breed on measurements of height at the withers and in the rump exceed the minimum standards for the breed by 3.3 and 1.2%, respectively.

3. Primiparous cows of Holstein and Simmental breeds are characterized by a compact body with an index of consistency at 121.5 and 127.8%, respectively, which is peculiar to them in the studied period of the development.

4. The average values of index outgrown 104.3% (Holstein) and 105.2% (Simmental) indicate an equally good development of the body of animals of both breeds.

5. Udder of primiparous cows of Holstein breed differs bulky, with the developed portions, preferably tightly attached, the external structure of the udder of these animals differs by more length on the belly and enough depth than primiparous cows of Simmental breed.

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# GENETIC CHARACTERISTICS OF HOLSTEIN CATTLE

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***Abstract***

*There are presented the results of the evaluation of animals of Holsteins of Dutch selection in herd SLL „DokSanCom" for the 2 study periods: I$^{st}$ (2012, n = 202) and the II$^{nd}$ (2016, n = 144). During the analyzed periods of research in the population of the assessed animals is observed a high frequency of occurrence of antigens $B_2$, $G_2$, $I_2$, $O_2$, $Y_2$, $E'_2$, G", which is typical for the Holstein breed and other breeds of black motley roots, and also to the Moldovan type of black - motley cattle. In the AEC-system is observed an increase in the frequency of occurrence of antigens $R_1$ (0.0247-0.0833), E (0.5742-0.5903), $X_2$ (0.5891-0.6806), L' (0.0742-0.2083). During the analyzed period of studies were found 21 identical alleles of AEB-locus. The main relative densities in the structure of the allelophond of herd alleles occupy $B_2O_1$, $B_2O_1Y_2D'$, $G_2Y_2E'_1Q'$, $I_2$, D'G'O', O', Q' and G". Low homozygosity ratio (Cα – 5.0-5.2%) shows the high genetic diversity of the population of studied cattle. In the antigenic spectrum and in the allelophond of AEB locus at cattle of SLL „DokSanCom" predominate marker antigens and alleles characteristic to Holstein cattle, to other breeds of black motley roots, and also to Moldovan type of black motley cattle. Further selection and selection of animals in the herd SLL „DokSanCom" expedient to maintain in view of the genetic blood group systems.*

***Key words****: antigen, allele, frequency of occurrence, homozygosity ratio, Holstein breed.*

## INTRODUCTION

The study of genetic polymorphism of blood groups of farm animals allows us to analyze the genetically structure of populations, to identify the individual, group and population features, follow the changes in the genetic structure of herds at the selection (Alimjanov, 1992; Boev, 1990).

Blood groups are constant in the ontogeny of animals, do not depend on changes of external conditions and the state of the organism, it is easily identified on the early stages of animal development and have a co-dominant pattern of inheritance. In dairy farming polymorphic proteins and blood groups are widely used in the study of the genetic structure of populations and the development of ways to manage selection and genetic processes (Marzanov et al.; Mashurov, 1980; Podoba et al., 2007). Comparative study of inbreeding populations by immunogenetic methods is important for the understanding of mechanisms to ensure the relative constancy of the breed and its development.

Of particular significance is the holding of Holstein cattle research on the different types of genetic markers as well as the use of molecular - genetic methods contributes to a more reliable assessment of the genotype of the animals, use it effectively, while maintaining the biodiversity of the population. The greatest number of antigenic factors has EAB-locus, which is mainly used in detecting the correlation of genes of blood groups and economic-useful signs, (Duniec et al., 2002; Morita and al., 1987).

Numerous studies performed on different breeds of animals, proved the existence as statistically significant differences on the frequency of occurrence of certain blood groups as and similarities between species that have a genetic relationship (Popov et al., 2000; Samorukov, 2001; Sivkin et al., 2011; Turbina, 2006). It is known that the more genetically diverse population is, so it is more viable and better adapted to the conditions of keeping.

In recent years, to the Republic of Moldova is imported the livestock of Holsteins from

Holland, Germany, Austria. A Holstein cattle has a high milk yield and plasticity adaptations to different climatic conditions of maintenance and for improvement of other breeds, (Gravert. 1974; Dairy Facts, 1986; Alimjanov, 1992; Buyarov et al., 2011; Galazova, 2004; Izhboldina et al., 1996; Klunduk and al., 1992; Krasnov, 1998; Nikolaev, 2007; Prokhorenko, 2013; Saks et al., 2013).

The purpose of research - to study the genetic polymorphism of erythrocyte antigens at animals of Holstein breed and to assess its genetic structure.

## MATERIAL AND METHODS

As the material for the research served the blood taken from animals of Holsteins breed of Dutch selection in a herd of cattle SLL "DokSanCom" cattle for I$^{st}$ (2012, n=202) and the II$^{nd}$ (2016, n=144) of the study period. Taking blood from animals, staging the reactions of haemolysis of red blood cells, as well as the study of blood groups was performed by the standard procedure (Guidelines, 1983). Blood groups were determined with hemolytic tests with the use of 49 reagents of cattle, unified in international comparative tests, which detected antigens controlled by allelic genes of 9 genetic systems. The frequencies of occurrence of antigens and alleles (q) were determined by a conventional method. The level of homozygosity in locus (Ca) was calculated with the use of Robertson formula (Robertson, 1956). The number of effective alleles (Na) was determined by dividing the unit at the rate of homozygosity. Through the use of homozygosity coefficient was determined also the degree of genetic variation (V). Indicators of immunogenetic similarity (r) and the distance (d) between the two periods of research were determined by the (Serebrovsky, 1970).

The obtained materials were treated on a personal computer.

## RESULTS AND DISCUSSIONS

The most common genetic characteristics of the population serve the data on the number of genetic systems, antigens and alleles in each system. In the study of the population of Holstein cattle, imported from the Netherlands to the Republic of Moldova, are found 49 antigenic factors controlled by allelic genes of 9 chromosome locus. In the antigenic spectrum of blood groups of the evaluated animals is revealed a fairly large range of variation of the frequency of their occurrence and the changes that have occurred in the population in the comparable period of the study. So in the AEA locus antigen Z′ at the animals of the second period of the studies was not revealed. It should be noted that this antigen is extremely rare in most cattle breeds, including Holstein, with the exception of Pinzgau and Sharole. Frequency of occurrence of antigen $A_2$ has increased and amounted to 0.4861.

In the AEB locus for the comparable periods of the research from 25 studied antigens are detected by 22 antigens. Antigens Q and B″ are not identified in both periods of studies, the antigen $T_1$ - in the first period of research and antigen Q′ - in the second study period, although the frequency of its occurrence in the first period was at the level of 0.5297.

It should be noted that in the analyzed periods of studies at animals of the given population of cattle is observed a high frequency of occurrence of antigens $B_2$, $G_2$, $I_2$, $O_2$, $Y_2$, E'$_2$ and G″ (Figure 1).

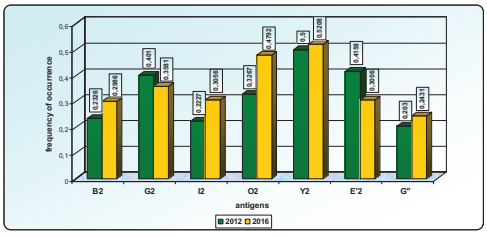


Figure 1. Antigens of AEB locus with a high frequency of occurrence in the dynamics

This is typical for Holstein, as well as to other breeds of black motley root (Popov and al., 2000), the Moldovan type of black motley cattle (Foksha et al., 2001).

During the analyzed period of time has happened an increase in the frequency of occurrence of such antigens as $G_1$ (0.0445-0.6597), B′ (0.1188-0.2500), D′ (0.1732-0.3611), K′ (0.0742-0.2639) and P′ (0.0396-0.4722). It is observed a decrease of the frequency of occurrence of the antigens $I_1$

(0.0841-0.0069), $P_1$ (0.0148-0.0069), G′ (0.2723-0.0556), I′ (0.01287-0.0694), O′ (0.3317-0.0556).

In the AEC- system from 10 studied antigens were identified all antigens, frequencies of occurrence antigens $R_2$, W decreased (0.3564-0.3403) and (0.4703-0.2986) (Figure 2).

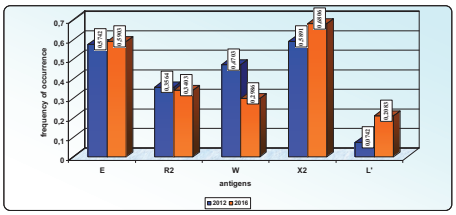


Figure 2. Dynamics of frequency of occurrence of certain antigens of AEC locus

It is observed an increase of the frequency of occurrence of antigens $R_1$ (0.0247-0.0833), E (0.5742-0.5903), $X_2$ (0.5891-0.6806), L′ (0.0742-0.2083). Our data confirm research (Podoba, 1997; Popov et al., 2003). The authors found similar antigens of AEC locus with a high frequency of occurrence inherent to Holstein, Canadian, Dutch and Ukrainian breeding respectively.

In the AEF system the frequency of occurrence of F antigen changed slightly towards increasing – 0.9356-0.9931. The same is observed in AEM- and AAZ- loci, the frequencies of occurrence of antigens M and Z have increased 0.0099-0.0208 and 0.5396-0.5417 (Figure 3).

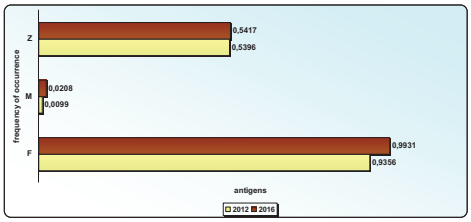


Figure 3. Dynamics of the frequency of occurrence antigens in one-factor loci

According to AES-locus from 6 studied the antigen U″ in the first period of research was not identified, in the second period the bearer of the given antigen was one animal (0.0069).

Most widespread antigen proved to be H′, the frequency of occurrence of antigens $S_1$, U′ increased, and antigens U, H″ - decreased, Figure 4. The saturation with antigenic factors during this period also increased, as the average frequency of antigens in 2016 is 25.0 against 23.7%.

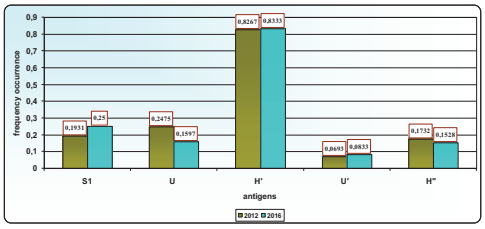


Figure 4. Dynamics of the frequency of occurrence of antigens of AES-locus

Currently the allelic diversity of AEB locus of the blood groups is the most informative and objective criteria for assessing the level of genetic variability. The number of alleles reflects the magnitude of genetic variability. The more alleles are found in a population, the greater is its genetic variability. In the the first period of investigations were identified 79 alleles in the second - 64 allele of AEB-locus.

During the period of studies were found 21 identical alleles, among which the main share in the structure of the allelophond occupy alleles $B_2O_1$ (0.0322-0.0729), $B_2O_1Y_2D'$ (0.0148-0.0243), $G_2Y_2E'_1Q'$ (0.1510-0.1215), $I_2$ (0.1163-0.1458), D′G′O′ (0.0569-0.0208), O′ (0.0223-0.0139), Q′ (0.0544-0.0451) and G″ (0.0421-0.0451) (Figure 5).

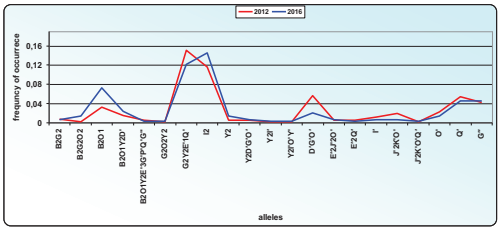


Figure 5. The dynamics of the frequency of occurrence of identical alleles of AEB-locus

Intensive use of the genophond of the black – motley, Dutch and Holstein breeds in a quality of improving in many countries, leads to a common genetic convergence. Thus, the majority alleles found among animals of the herd SLL "DokSanCom" are present in the studies (Litvinenko et al., 2014) in the allelophond of Dutch, Holstein, the German black –motley breeds.

Objective genetic characterization of the analyzed animal populations reflect and such factors as the coefficient of homozygosity (Ca), effective number of alleles of (Na), the degree of genetic variation (V coefficient) (Table 1).

Table 1. Genetic variation of Holstein population of cattle in dynamics (2012-2016)

| No | Indicators | 2012 | 2016 |
|---|---|---|---|
| 1. | In all investigated, heads | 202 | 144 |
| 2. | The number of installed alleles:<br>- basic<br>- rare | <br>255<br>86 | <br>154<br>53 |
| 3. | The total frequency of alleles:<br>- basic<br>- rare | <br>0.6312<br>0.2129 | <br>0.5347<br>0.1840 |
| 4. | homozygosity coefficient, Cα | 0.0501 | 0.0518 |
| 5. | The number of effective alleles, Na | 20 | 19.3 |
| 6. | The degree of genetic variability, V | 95.9 | 95.5 |

As it can be seen, the frequency of occurrence both basic and rare alleles in the first period of studies was higher than in the second and amounted to 0.6312-0.2129 and 0.5347-0.1840 accordingly. In the second period of investigations there was a slight increase of the level of homozygosity (Cα) – 5.0-5.2%, which is reflected in the number of effective alleles - 20 and 19.3 respectively. Low homozygosity coefficient (Cα) shows the high genetic diversity of the population of the studied Holstein cattle. The degree of implementation of the possible genetic variability (V) is fairly high, the differences are minor.

## CONCLUSIONS

1. In the antigenic spectrum and in the allelophond of EAB-locus at cattle of the herd SRL "DokSanCom" prevail marker antigens and alleles typical to Holstein cattle, other breeds of black –motley roots, as well as Moldovan type of black-motley cattle.
2. The allelophond of Holstein breed of the herd SRL "DokSanCom" is quite diverse for conducting selection with the use of blood groups and the homozygosity level (5.0-5.2%) will allow to maintain breeding of 19-20 genetically different from each other structural units and ensure the current genetic variability of the given population of cattle.

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# RESEARCH ON COMPARISON OF BREEDING VALUE METHODS FOR SHEEP MILK PRODUCTION

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***Abstract***

*The aim of this paper is to compare four methods for estimating the breeding value of sheep, for the milk production. The research was conducted at the National Institute of Research and Development for Biology and Animal Nutrition Balotesti, Ilfov. The biological material is represented by a flock of Palas Milk line, consisting of 805 animals: 344 downward, 121 rams and 340 sheep. The character analyzed was the amount of milk in the weaning lamb period to the end of the lactation. Lactation length was between 51 and 230 days. To estimate heritability ($h^2$) and breeding value, BLUP methodology applied to an animal model was used. The heritability value was estimated by the method of single factor analysis of variance, and was 0.73. By the animal model, the heritability was stabilized at 19 1iterations, the value being 0.235. The breeding value was estimated in four ways: a) Performance (PP); b) Average performance of paternal half-sisters (PSS); c) LUSHIndex(IL); d) Individual Animal model (IAM). The best work option was comparatively analyzed through Spearman rank correlation and selection accuracy. The highest rank correlation was obtained with the combination IL –IAM, 0.82 respectively, due to the fact that the methods used commonly a high sources and amount of information. The opposite is the combination of IAM-PSS, where rank correlation is -0.0071. In terms of selection accuracy, the highest value was recorded for the IAM (0.52) and the lowest inbreeding value estimation based on PP (0.48), which indicates a superiority of IAM of 8.33%. In conclusion, to achieve a more precise evaluation of animal breeding, all available sources of information should be use in calculations. Also, the combination of these sources is recommended to be performed by using BLUP methodology, applied to an animal model.*

***Key words****: animal breeding, sheep milk yield, animal model*

## INTRODUCTION

The new and modern conditions of sheep exploitation and the social requirements for milk need further improvement of sheep milk production based on better genetic selection criteria (Mrode, 2014). Success in this direction depends on the material used and on the applied method of breeding value estimation. The improvement of the evaluation methods means to know the level of precision of the implemented methods and their effectiveness. Identifying the best individuals on the genetic merit is the objective of all genetic evaluation (Grosu, 2003). The genetic progress is the criterion for selection of animals for breeding (Grosu et al., 1997). To achieve genetic progress of the sheep populations, those animals with the highest value for the required genetic traits economically important should be selected from the current generation (Draganescu, 1979). Prediction accuracy represents a very important value for the estimation improvement; it depends on the genetic progress in the studied population (Popescu-Vifor, 1990). The purpose of this paper is a comparative analysis of the methods used to estimate the breeding value in sheep, within the context of a more accurate genetic evaluation of the selection candidates for the quantity of milk.

## MATERIALS AND METHODS

*Biologic material.* A flock of Palas Milk Line, consisting of 781 animals, of which 344 offspring, 97 sires and 340 dams. From the 340 dams, 111 appear in the database with their milk production performance, while they also appearing in the position of daughters with associated performance. Therefore, in total, the number of sheep with performance is 455 (111

+ 344). The 455 sheep were born in the period 1991-1999, their performance being registered between1993-2001. Since Palas Milk Line has been selected with priority for milk production, the trait of milk quantity obtained from the lambs weaning (2 months) to the end of lactation was used in the present study. Duration of lactation remaining from weaning the lambs to dry sheep was between 51 and 230 days.

The goals were achieved using a variety of statistical methods, from classical statistics and to BLUP methodology (Henderson, 1963).

Thus, we used two methods to estimate heritability:

a) ANOVA (Analysis of Variance) method in order to obtain start heritability and b) Individual Animal Model, based on start heritability was estimated the final heritability.

For ANOVA was used a two-way model, nested model.

$$P_{ijk} = \mu + A_{i+} B_{j(i)} + e_{ijk}$$

where:

$P_{ij}$ = the trait „j" of a daughter belonging to sire „i" in year „k"; $\mu$ = overall mean of population; $A_i$ = the fixed effect of the year (i=1…12); $B_{j(i)}$ - the genetic effect of sire j (j=1…97), nested within year; $e_{ij}$ = the residual effect.

For Animal Model we used the following equation:

$$y = Xb + Za + e$$

with the Mixt model equation:

$$\begin{bmatrix} X'\cdot X & X'\cdot Z \\ Z'\cdot X & Z'\cdot Z + A^{-1}\cdot k \end{bmatrix} \cdot \begin{bmatrix} \tilde{b} \\ \hat{a} \end{bmatrix} = \begin{bmatrix} X'\cdot P \\ Z'\cdot P \end{bmatrix}$$

where: $k = \dfrac{1-h_0^2}{h_0^2}$

The variance components where estimated as follows:

$$\sigma_e^2 = \frac{P'\cdot P - \tilde{b}\cdot X'\cdot P - \hat{a}'\cdot Z\cdot P}{n - r(X)}$$

(residual variance)

$$\sigma_A^2 = \frac{\hat{a}'\cdot A^{-1}\cdot \hat{a} + \sigma_e^2 \cdot tr(A^{-1}\cdot C_{22})}{q}$$

(additive genetic variance)

where: C22 is the sub-matrix corresponding to random effects in the system of equations obtained after reversed throughout the system of equations (including equation fixed effects):

$$C = \begin{bmatrix} X'\cdot X & X'\cdot Z \\ Z'\cdot X & Z'\cdot Z + A^{-1}\cdot k \end{bmatrix}^{-1} = \begin{bmatrix} C_{11} & C_{12} \\ C_{21} & C_{22} \end{bmatrix}$$

**Spearman Rank Correlation:**

$$\frac{6\cdot \sum d^2}{n\cdot (n^2-1)}$$

**Prediction of the breeding value**. It was done in several versions, then compared with each other in order to identify the best of them. In this context, the breeding value was estimated in next variants:

a) Own Performance;

b) Average performance of paternal half-sister;

c) Performance + Average performance of paternal half-sister(LUSH index);

d) Individual Animal Model.

## RESULTS AND DISCUSSIONS

Estimation of genetic parameters and breeding value prediction of candidate farm animals are essential links of any breeding program, aiming to improve the livestock genetics. Knowing the animal performance allows us to characterize phenotypically the considered population.

### Phenotypic Characterization

Table 1 shows several parameters characterizing the milk production. The yield ranges between 42 and 141.7 kg with an average of 104 kg.

Table 1. Average performance of analyzed sample

| No. | Specification | Unit of measure ment | Value |
|---|---|---|---|
| 1 | Number of animals with performance | number | 455 |
| 2 | $\overline{X} \pm s_{\overline{X}}$ | kg. | 104.09 $\pm$ 0.98 |
| 3 | Lower limit | kg. | 42.005 |
| 4 | Maximum Limit | kg. | 141.70 |
| 5 | S (Standard deviation) | $kg^2$ | 20.87 |
| 6 | CV (Coefficientof variation) | % | 20.05 |

The coefficient of variation (20.05%) shows a good homogeneity of milk yield which is better

than that measured by Creanga et al. (2004), 33.1%.

Since for data processing we used an iterative procedure, a starting value was necessary as input, the so-called start heritability ($h_0^2$). In our study start heritability was obtained by classical two-way analysis of variance.

**Genetic Parameters**

Table 2 shows the analysis of variance (ANOVA nested) estimating the necessary variance to obtain the heritability values for milk yield; we can observe that the variance value had the correct distribution and the heritability obtained (0.276) is normal for milk yield. Puledda et al. (2016) reported a heritability of 0.23 for milk yield in a population of Sarda sheep, while Bittante et al., (2017) reported a lower heritability of 0.16 for Sarda dairy sheep, but all of these values are representative for milk yield.

Table 2. Heritability of milk in the population under study (ANOVA Nested)

| Sources | Degrees of freedom(DF) | Sum of squares(SS) | Average of squares(AS) | Variance |
|---|---|---|---|---|
| Between years (A) | $DF_A$= 11 | $SS_A$ =3379.60 | $AS_A$ =5761.78 | $V_A$ =91.02 |
| Between rams in the years (B:A) | $DF_{B:A}$ =85 | $SS_{B:A}$ =177115.77 | $AS_{B:A}$ =2083.71 | $V_{B:A}$ =117.62 |
| Error (E) | $DF_E$ =388 | $SS_E$ =580289.80 | $AS_E$ =1495.59 | $V_E$ =1495.59 |
| Total (T) | $DF_T$ =484 | $SS_T$ =820785.17 | $AS_T$ =1695.83 | $V_T$ =1704.23 |

$$h^2 = \frac{4 * V_{B:A}}{V_T} = \frac{4 * 117.62}{1704.23} = 0.276$$

**Spearman rank correlation**

The comparison of the methods of selection was done by Spearman rank correlation value (Table 3)

Table 3. Spearman rank correlation case the orderingon different criteria

| Number | The combination of selection methods | $\sum d^2$ | $6 \cdot \sum d^2$ | $n \cdot (n^2 - 1)$ | $r_S$ |
|---|---|---|---|---|---|
| 1 | Animal Model- Performance | 62367 | 374202 | 1685040 | **0.78** |
| 2 | Animal Model- Average performance of paternal half sister | 282826 | 1696956 | 1685040 | **-0.0071** |
| 3 | **Animal Model- Lush index** | 50324 | 301944 | 1685040 | **0.82** |
| 4 | Lush index-Performance | 58449 | 350694 | 1685040 | **0.79** |
| 5 | Lush index- Average performance of paternal half sister | 191072 | 1146432 | 1685040 | **0.32** |
| 6 | Performance - Average performance of paternal half sister | 243803 | 1462818 | 1685040 | **0.13** |

The highest rank correlation was obtained using the combination between *Animal model* and *LUS Hindex*, 0.82 respectively, also due to the multitude of information sources (Performance + Average performance of paternal half-sister) as already mentioned as already mentioned in material and methods.

The opposite is the combination of *Animal model* and *Average performance* of paternal half-sister, whose rank correlation value indicates no correspondence between the two criteria (-0.0071).

The combinations in which Performance is present (ex. Animal model and Performance or LUSH index and Performance) led to higher rank of correlation, which shows that this source of information is the basic piece in relation to information provided by the average performance of paternal half-sister. All the combinations of Average performance of paternal half-sister led to low values of rank correlation.

The second criterion for comparison of the combination considered was the accuracy of selection, which can be analyzed on the basis of the parameters presented in Table 4. It can be observed that the highest accuracy was obtained by using BLUP-Animal Model and on the opposite was by using Average performance of paternal half-sister.

Table 4. Selection accuracy

| No. | Selection Method | Accuracy selection | Relative efficiency of selection methods (%) |
|---|---|---|---|
| 1 | Animal model – BLUP | 0.52 | - |
| 2 | Lush Index | 0.50 | - |
| 3 | Performance | 0.48 | - |
| 4 | Average performance of paternal half sister | 0.19 | - |
| | The combination of selection methods | | |
| 5 | Animal Model-Lush index | - | 4 % |
| 6 | Animal Model – Performance | - | 8.33 % |
| 7 | Lush index-Performance | - | 4.17 % |

## CONCLUSIONS

The main conclusion of this study is that the best results were obtained with the combination: the *Individual Animal Model* and *LUSH index*, resulting in a 0.82 rank correlation. Oppositely was the combination *Animal Model* and *Average Performance* of paternal half-sister which result in a negative rank correlation -0.0071.

To achieve a more accurate evaluation of animal breeding, all available sources of information should be use in calculations. Also, the combination of these sources is recommended to be performed by using BLUP methodology, applied to an animal model.

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# REASONS FOR CULLING AND REPLACEMENT RATE IN DAIRY CATTLE

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***Abstract***

*The replacement rate in cattle breeding has an important effect on the profitability of breeding as well as the success of the breeding program. Culling decisions play an important role whether the herd replacement rate is high or low. The replacement rate in cattle breeding has an important effect on the profitability of breeding as well as the success of the breeding program. Removal decision from herd will play an important role whether the replacement rate high or low. The reasons for culling were reported as low milk yield (29-36%), reproductive problem (15-27%), mastitis (18-23%) and other causes (25%). On the other hand, voluntary and involuntary culling rate are shown as 43,7% is 56.3% respectively. This rewiev focussed on evaluating the reasons for culling of cows, replacement rate, herd life and productive life in dairy cattle enterprises.*

***Key words:*** *cattle, culling, productive life, longevity.*

## INTRODUCTION

In Dairy enterprises, the longest productive lifespan of cows is desirable. However, during the year-round cows are culled from the herd for various reasons or have to be culled. Culling may be defined as removal of cows from the main herd due to different reasons which are usually Involuntary and voluntary culling (Martin 1992; Neerhof, 2000; Weigel and Palmer, 2002). Involuntary culling implies that cows were culled due to disease, injury, bodily defects, mastitis, infertility or death. Low yield or selling of cows are examples of voluntary culling.

Since longevity has played an important role in enterprise profitability in recent years, it has begun to taken into consideration handled specially in breeding programs.

The survival period is the productive period between the date when the first calf of the cow was born and the date of culling. In other words, number of calves that the cow gives birth or lactation number completed during the life-span of the cow. Therefore, longevity is expressed as productive life (Martin, 1992; Powell, 1997; Kumlu and Akman, 1999).

Herd life is a low heritability trait. In studies carried on this traits, 10% of the phenotypic variation due to the genetic effects only has been reported (Martin, 1992; Faust, 2003). For this reason, optimization of environmental conditions is the most important factor increasing the cow longevity (Savaş et al., 1999).

### Productive Life

The productive life can also be defined as the life-span of a cow. Life-span of cows is the time from birth to culling time or died. This criterion includes growth, production and dry period.

Keeping cattle in herd as stable, healthy and productive form for a long time will in particular benefit the enterprises and the country in general. It will be possible to reduce the cost of veterinary and medicines, decreasing of replacement cost, increasing the proportion of cows removed from herds voluntarily, increasing of selection intensity as a result increasing of genetic improvment by staying in the herd a long time (Setati et al., 2004).

It was found the mean duration of staying in herd 36.8 ± 2.60 months (Kara et al., 2010). This value indicates that cows are used for breeding average of 3 years. It is considered to be ideal staying in herd for 4 years in cattle breeding. Because, it is possible to obtain enough breeding heifers to replace the cow removed during this period (Kumlu, 2003).

**Longevity**

Most dairy farmers are fully aware of the importance of achieving a low herd replacement rate. High replacement rate increases the cost. The most important things to do is to decrease the replacement rate in the herd. It will beas long as possible to keep cows alive till die. In order to keep animals they must maintain their efficiency in the desired scale. For example, there is no reason to keep a cow which is not getting pregnant or reduced milk production due to mastitis. For this reason, it is more appropriate to use the concept of productive life rather than life-span expression for a production animal. Productive life is the time from first calving to culling. Long productive life means is reduction in replacement rate.

When productive life is known, the herd replacement rate can be calculated. If the productive life (PL) is expressed in months, the herd replacement rate; HRR = 12 / PL, if the productive life is expressed in years, it is calculated by the equations of HRR = 1 / PL. For example, if the productive life is 40 months, the rate of replacement will be 12/40 = 0.30 = 30% (Akman, 2003).

When the culling rate increases, the number of pregnant heifers will be increased for keeping the herd size. At this stage, it may be necessary to calculate the number of pregnant heifers to be produced from this herd and how many heifers will be sold in a year to keep herd size. The number of pregnant heifers to be produced depends on birth rate. If the the calving interval in one herd is 14 months, The highest value for birth rate is calculated as 86% (12/14). However, this rate should not be considered for the heifers calving for the first time.

**Reasons for culling**

Cows are removed from the herd for various reasons. In many studies, it has been observed that cows removed from the herd for involuntary (forced) reasons are between 50% and 80% of all cows removed from the herd (voluntary + forced) (Bascom and Young, 1998; Seegers et al., 1998a; Stevenson and Lean, 1998; Beaudeau et al., 2000; Yaylak, 2003).

Causes of cow removal from the herd are possible under two category; voluntary (mastitis, foot-leg problems, disability, reproductive problems, sickness, old age and death) and forced (low milk yield, external appearance characteristics, behavioral problems.

However, Fetrow et al. (2005) rejected this grouping and they argued that it would be more appropriate to collect the reasons for removed from herd under two category: biological and economic reasons as an alternative (Table 1).

Table 1.Traditional removal reasons and category recomended by Fetrow et al.

| Traditional | Reasons for Removal | Fetrow et al. (2005) |
|---|---|---|
| Voluntary | Low yield<br>Overstock | Economic |
| Involuntary | Mastitis<br>Udder structure<br>Lameness<br>Reproduction Problems (except infertility)<br>Aged | |
| | Serious disability<br>Disease<br>Infertility<br>Death | Biological |

According to the traditional category, only low yield and overstock breeding or butchery sales are considered to be the reason for voluntary removal, and all of the remaining reasons are shown among the involuntary while Fetrow et al. (2005) considered as the biological removal reasons only that led to loss of the possibility of being productive in the future.

Biological causes were included as death, completely sterile, seriously disabling, compulsory slaughter and incurable diseases. In contrast, they put mastitis is not an involuntary but among the economic reason. They claimed that the breeder do not remove every cows suffering from mastitis from the herd, however replace it when they finds better one or when they meet economically unacceptable yield loss. Therefore, this is not a forced removal such as death, seriously disabling or infertility, but for economic reasons.

Bascom and Young (1998) found that cows were removed for involuntary reasons as 78%, Seegers et al. (1998) 71%, Yaylak (2003) 56%

and Light (2006) 69%. In the study done by Işık (2006) to show the rate of removal from herd, fertility problems took place in the first with 31%, it follows the overstock breeding sales and the milk yield decrease. In the first three ranks of research conducted in this respect, infertility , udder problems and low productivity or overstock sales were reported (Martin 1992; Bascom and Young, 1998; Seegers et al., 1998; Yaylak 2003).

## CONCLUSIONS

In Dairy cattle enterprises, the rate of herd replacement and removal which are important indicators of herd management and breeding should be determined by making yearly calculations to determine the number of cows to be needed in terms of enterprises, region and country and concrete suggestions and solution should be taken.

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# NUTRITION

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# NUTRIENT CONCENTRATE FERMENTATION BASED SHRIMP WASTE AND EFFECT ON PRODUCTION PERFORMANCE PHASE LAYER NATIVE CHICKEN

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***Abstract***

*Efforts toward improving the quality of waste containing high chitin through bioprocess shrimp waste utilizing Bacillus licheniformis, Lactobacillus sp. and Saccharomyces cereviseae, in order to obtain the product (Nutrient Concentrate) quality in order to meet the nutritional needs of local poultry (native chicken). Biological test products in the ration to determine its effectiveness towards achieving the optimal performance needs to be done. Native chicken has an important role as a provider of meat and eggs to be relied. The study was conducted using a laboratory experiment using a completely randomized design, consisting of 6 treatments rations and each repeated four times. Ration treatment: R0 = control diet (15% protein and ME 2,750 kcal/kg); R1 = rations containing 5% Nutrient Concentrate (Protein 15% and ME 2,750 kcal/kg); R2 = ration containing 10% Nutrient Concentrate (Protein 15% and ME 2,750 kcal/kg); R3 = rations containing 15% Nutrient Concentrate (Protein 15% and ME 2,750 kcal/kg); R4 = rations containing 20% Nutrient Concentrate (Protein 15% and ME 2,750 kcal/kg); and RS = standard ration (Protein 18% and ME 2,750 kcal/kg). Variables observed that the performance of native chicken layer phase (egg weight, number of eggs, daily egg production and feed efficiency) and hematological values chicken blood (erythrocytes, leukocytes, and blood hematocrit). Data were analyzed by analysis of variance and differences between treatments were tested by Duncan's multiple range test. The results obtained by the performance of native chicken layer phase with the use Nutrient Concentrate at the rate of 20% in the ration equivalent to the standard ration, the weight of the eggs ranged from 40.51 to 43.46 g/grain, the number of eggs from 32.37 to 33.16 grains/2 months, han-day 53.95% - 55.26% and feed efficiency of 54.02% - 58.95%. ). Values range chicken blood hematological phase layer in the normal range, the number of erythrocytes ranged from 2.06 to 2.16 $\times 10^6$ /mm$^3$; leukocytes from 36.42 to 37.27 $\times 10^3$ /mm$^3$; and hematocrit 33.25% - 34.25%. Nutrient Concentrate can be used as a source of animal protein in the ration formulation native chicken layer phase and use up to the level of 20%.*

***Key words****: nutrient concentrate, shrimp waste, bioprocess, layer phase, native chicken.*

## INTRODUCTION

Industrial waste material processing frozen shrimp is the potential to serve an alternative feed ingredients for poultry. It is based on nutritional content, ie: 43.41% crude protein, 18.25% crude fiber, 7.27% fat, 5.54% calcium, 1.31% phosphorus, 3.11% lysine, 1.26% methionine and 0.51% cystine, and gross energy 3,892 kcal/kg (Abun, 2008). Factors limiting the use of waste materials such as poultry feed is the presence of chitin in the amount of about 15-20%. Chitin bind strongly to protein, fat and mineral covalent bond ß (1-4) so difficult to be digested by the digestive enzymes of poultry (Leeson and Summers, 2001). Poultry do not have enzymes that can break the glycosidic bond β-(1-4), so that before used as feed material, the waste must be processed first. One of the efforts to transform organic material into useful new products and nutritional value better is to exploit microbes through bioprocess.

Bioprocess shrimp waste can be done in two phases, namely deproteination using *Bacillus licheniformis*, and demineralization with *Lactobacillus* sp. and *Saccharomyces cerevisiae. Bacillus licheniformis* is a bacteria that can produce protease and chitinase in relatively large quantities (Williams and Shih, 1989; Rahayu et al., 2004). *Lactobacillus* sp. a microbial decomposers glucose, sucrose,

maltose and lactose into lactic acid, causing mineral deposits (Lee and Tan, 2002). *Saccharomyces cerevisiae* is yeast that can produce the enzymes amylase, lipase, protease and other enzymes that can petrify digestion of nutrients in the digestive organs (Wagstaff, 1989).

The optimal performance of native chicken can only be realized if given rations of quality that meets the requirements in sufficient quantities and balanced. Fulfillment of nutrients in the diet can be done by adding the feed additives. It needs to be considered, as feed additives can improve the quality and value of benefits in native chicken rations. Thus, it is necessary to find alternative feed additives are inexpensive, easy to obtain, the quality is good, as well as non-food. One of them is the use of shrimp waste processed with fermentation technology and hereinafter referred Nutrient Concentrate.

Indonesia is the country's third largest shrimp producer in the world, annually produced about 0.08 million tons from an area of 380,000 hectares of shrimp ponds. Approximately 80-90% of the shrimp is exported in frozen form without heads and skins. Skin, head, and tail shrimp is industrial waste from factories freezing of shrimp, this waste can reach 30 - 40% of the weight of the whole (Krissetiana, 2005). The use of shrimp waste processed product is a source of alternative feed ingredients that can be used in the preparation of chicken rations.

Experts have conducted research to test the product bioprocess. Measurement of metabolizable energy product of fermentation residue oil palm by *Aspergillus niger* in broiler chickens, and the result is an increase in metabolizable energy by 14% from 1,844 kcal/kg to 2,103 kcal/kg (Simanjuntak, 1998). Increasing the value of shrimp waste protein digestibility fermented with *Bacillus licheniformis* and *Aspergillus niger* amounted to 13.27% (from 63.44% to 71.86%) in broiler chickens (Abun, 2008). Improving the quality of nutrition bioprocess resulted in complex molecules or organic compounds such as proteins, carbohydrates and fats into molecules that are simpler and easier to digest (Darana, 1995).

Native chicken has long been known by the people of Indonesia as the local chicken, chicken vegetable, or chicken, in Latin is known *Gallus domesticus*. Range chicken accounts for 20 to 40% of eggs and 25% of meat consumed in the country (Directorate General of Livestock, 2014). Native chicken is more likely to be developed as farm people, given the domestic poultry does not require substantial capital investment, easy maintenance, high adaptability, as well as meat and eggs are more favored by the public.

In general, native chicken reared traditionally-extensive (production is low and the mortality rate is high enough), causing the population to fluctuate from time to time. According to Rasyaf (1990), the potential and prospects of native chicken is very good but to date information and research on the development of native chicken is still small. The low level of productivity of native chicken is influenced by genetic and environmental factors. Genetic factors are poorly coupled with means and feeding the still traditional is the cause of low production of native chicken, both growth and egg production. Egg production reached only 30-60 eggs per year with an average egg weight of 37.5 grams per egg (Kingston, 1982). Rations containing Nutrient Concentrate bioprocess products sought a positive influence on the performance of native chickenlayer phase. It is understood by paying attention to the health of livestock is to know the condition of hematologic. Hematologic circumstances and in accordance with animal health standards causing the transport of oxygen and nutrients into the body to be smooth so the metabolic processes in the body, the better and, in turn, can increase the productivity of native chicken.

## MATERIALS AND METHODS

(a) Preparation of "Nutrient Concentrate"

Deproteination. Starter inoculum *B. licheniformis* cultivated in 50 ml broth and incubated for 2 days at a temperature of $50^0$C, then inoculated on shrimp waste substrate with a dose of 2% (v/w). Liquid substrate fermentation is done using auto-shakerbath for 2 days at a temperature of $45^0$C with a rotation of 120 rpm (Abun et al., 2012).

Demineralization. Starter inoculum *Lactobacillus* sp. cultivated, mixed standard

solution (0.5% (w/v) yeast extract, 0.5% $NH_4NO_3$; 0.05% KCl; 0.05% $MgSO_4$; 0.01% $FeSO_4$; and 0.001% $CuSO_4$. inoculum of *Lactobacillus* sp. added to the product deproteination a dose of 2% (v/w) for 2 days at a temperature of $45^0$C with a rotation of 120 rpm (Abun et al., 2012).

Fermentation with *Saccharomyces cerevisae*. *Saccharomyces cerevisiae* pure cultures were incubated for 3 days, then made inoculum to prepare a standard solution ($NH_4NO_3$ 0.5%; 0.05% KCl; $MgSO_4.7H_2O$ 0.05%; $FeSO_4.7H_2O$ 0.01%; and $CuSO_4.5H_2O$ 0,001 %) and then fermented in auto-shakerbath. Product demineralization, then added inoculum of *Saccharomyces cerevisiaese* lot of 3% (v/w), then incubated for 2 days at a temperature of $35^0$C (Haetami et al., 2010).

(b) Feeding Trial

Research using native chicken (*chicken Sentul*) of the Research Institute of Poultry Jatiwangi, Majalengka-West Java, Indonesia, grower phase. Chickens were randomly divided in 24 units cages, each cage contains one tail and on each cage are numbered. The chicken used in the study has a coefficient of variation of the initial weight of 4.07%.

Cages used in this study is a cage cages are divided into 24 cages with a capacity of 1 fish per cages, one unit cage measuring 50 cm long, 50 cm wide and 80 cm high. The enclosure has insulation made of bamboo, each bulkhead are numbered treatment. Each group comes with a cage where food and drink are made of plastic.

Feed materials making up the ration consists of: yellow corn, fine bran, soybean meal, coconut meal, fish meal, grit (flour shells), $CaCO_3$, coconut oil, and Nutrient Concentrate.

Experiment ration. Control diet (R0) and the standard ration (RS) is based on ISO (1995) and Zainuddin, et al. (2004). The content of protein and energy to the control diet (R0) is 15% and 2,750 kcal / kg, and the standard ration (RS) is 18% and 2,750 kcal/kg.

Ration treatment is as follows:

R0 = control diet (15% protein and ME 2,750 kcal/kg); R1 = rations containing 5% Nutrient Concentrate (Protein 15% and ME2,750 kcal/kg); R2 = ration containing 10% Nutrient Concentrate (Protein 15% and ME 2,750 kcal/kg); R3 = rations containing 15% Nutrient Concentrate (Protein 15% and ME 2,750 kcal/kg); R4 = rations containing 20% Nutrient Concentrate (Protein 15% and ME 2,750 kcal/kg); RS = standard ration (Protein 18% and ME 2,750 kcal/kg).

(c) Experimental Procedure

Include the maintenance of native chicken layer phase for 8 weeks (2 months) began laying hen (hand day 5%). Chickens were placed in cages of 24 units, each enclosure consists of one chicken. The stage of collecting and recording data, starting with chicken weighed to determine the initial weight. Measurement of feed intake and the number of eggs and egg weight do every day.

(d) Variable Observed

Variables measured: (1) egg weight (g/grain); (2) The number of eggs (item); (3) daily egg production (hand-day) (%); (4) Efficiency ration (%); (5) The number of erythrocytes ($mm^3$) is calculated using Haemocytometer; (6) Number of leukocytes ($mm^3$) was calculated from the total white blood cells; and (7) The hematocrit value (%) to calculate the volume of cells in the blood.

(e) The experimental design and statistical analysis

The experiments were performed using the experimental method in the laboratory. The experimental design used was a completely randomized design, consisting of 6 treatments ration (R0 = ration low in protein / 15%; R1 = rations containing 5% Nutrient Concentrate / protein 15%; R2 = rations containing 10% Nutrient Concentrate / protein 15%; R3 = rations containing 15% Nutrient Concentrate / protein 15%; R4 = rations containing 20% Nutrient Concentrate / protein 15%; and RS = high ration protein18%) and each repeated four times. Data were analyzed with Fingerprint Car (Test F) and the differences among the treatments tested using Duncan's Multiple Range Test.

## RESULTS AND DISCUSSIONS

The average egg weight, number of eggs, the daily production and feed efficiency of native chicken layer phase for 8 weeks (2 months) experiment is shown in Table 1.

Table 1. Egg weight, number of eggs, eggs daily production and efficiency phase native chicken layer rations for 8 weeks (2 months) trial

| Variables | Treatments | | | | | |
|---|---|---|---|---|---|---|
| | R0 | R1 | R2 | R3 | R4 | RS |
| Egg Weight (g/item) | 44.21$^{A}$ | 43.46$^{A}$ | 43.00$^{A}$ | 43.43$^{A}$ | 40.51$^{A}$ | 44.65$^{A}$ |
| Number og Eggs (grains) | 32.37$^{A}$ | 33.16$^{A}$ | 33.16$^{A}$ | 33.16$^{A}$ | 32.37$^{A}$ | 33.16$^{A}$ |
| Eggs Daily Production (%) | 53.95$^{A}$ | 55.26$^{A}$ | 55.26$^{A}$ | 55.26$^{A}$ | 53.95$^{A}$ | 55.26$^{A}$ |
| Efficiency Ration (%) | 59.53$^{A}$ | 58.95$^{A}$ | 57.33$^{A}$ | 57.90$^{A}$ | 54.02$^{A}$ | 57.95$^{A}$ |

The average weight range chicken eggs experimental results ranged from 40.51 to 44.65 grams/grain. The analysis showed that the treatment ration showed no significant differences (P>0.05) to the weight range chicken eggs. Use of Nutrient Concentrate up to the level of 20% in native chicken ration phase layer does not affect the egg weight.

The average number eggs of native chicken layer phase for 8 weeks of the experiment results ranged from 32.37 to 33.16 grains. The analysis showed that the treatment ration showed no significant differences (P>0.05) to the number eggs of native chicken layer phase. Use of Nutrient Concentrate up to the level of 20% in native chicken ration phase layer does not affect the number of eggs.

The mean hand day production range native chicken eggs for 8 weeks of the experiment results ranged from 53.95 to 55.26%. The analysis showed that the treatment ration showed no significant differences (P>0.05) on hand day production range native chicken eggs. Use of Nutrient Concentrate up to the level of 20% in native chicken ration phase layer does not affect the hand day egg production.

The average efficiency feed of native chicken trial results for 8 weeks ranged from 54.02 to 59.53%. The analysis showed that the treatment ration showed no significant differences (P>0.05) on the efficiency of feed utilization of native chicken. Use of Nutrient Concentrate up to the level of 20% in native chicken ration phase layer does not affect the efficiency of feed utilization.

Use of Nutrient Concentrate up to the level of 20% in the diet did not negatively impact performance in native chicken egg production, and the results were equivalent to a standard diet (18% protein ration). Chicken body weight during the study are relatively homogeneous with a coefficient of variation of body weight ranged between 3.80% - 4.07%, with each chicken feed consumption of 75 g/day.

The mean value of erythrocytes, leukocytes, and blood hematocrit range native chicken layer phase containing rations Nutrient Concentrate for two months trial are presented in Table 2.

Table 2. Values of erythrocytes, leukocytes, and blood hematocrit native chicken phase layer rations containing nutrient concentrate for two month trial

| Variables | Treatments | | | | | |
|---|---|---|---|---|---|---|
| | R0 | R1 | R2 | R3 | R4 | RS |
| Erythrocytes($\times 10^6$/mm$^3$) | 2.18$^{AB}$ | 2.16$^{AB}$ | 2.13$^{AB}$ | 2.06$^{B}$ | 2.09$^{AB}$ | 2.22$^{A}$ |
| Leukocytes($\times 10^3$/mm$^3$) | 36.07$^{AB}$ | 36.42$^{AB}$ | 36.65$^{AB}$ | 36.79B$^{B}$ | 37.27$^{AB}$ | 36.53$^{A}$ |
| Hematocrit (%) | 33.00$^{B}$ | 33.25$^{B}$ | 33.38$^{B}$ | 33.63$^{AB}$ | 34.25$^{A}$ | 33.38$^{B}$ |

The average value range chicken blood erythrocyte experiment results for 8 weeks ranged from 2.065 to 2.221$\times 10^6$ /mm$^3$.

The analysis showed that the treatment ration significant effect (P<0,05) to native chicken blood erythrocytes.

Use of Nutrient Concentrate in the ration at the rate of 20% (R4 /15% protein) did not show

significant differences (P>0.05) with standard ration (RS / 18% protein) against the value of the phase range chicken blood erythrocyte layer. Normal red cell count in chickens according to Smith (1987) ranged from 2.0 to 3.2 × $10^6$ grains/$mm^3$, mean blood erythrocyte value of native chicken are still in the normal range. The number of erythrocytes per $mm^3$ of blood varies according to species and also between individuals within a species. According to Swenson (1977), the number of erythrocytes is influenced by several factors, including age, sex, diet quality, disease and environmental temperature.

The mean value of native chicken blood leukocyte 8-week experiment results ranged from 36.07 to 37.27 ×$10^3$ / $mm^3$. The analysis showed that the treatment ration significant effect (P <0.05) on blood leukocytes range chicken. Use of Nutrient Concentrate in the ration at the rate of 20% (R4 / 15% protein) did not show significant differences (P>0.05) with standard ration (RS / 18% protein) against the value of the phase range chicken blood erythrocyte layer. The content of leukocytes from the research results within the normal range according to Smith (1987) that is between 16-40 ×$10^3$ grains/$mm^3$. Chicken means not impaired in their blood because of physiological systems across flats are within the normal range. According to Brown, et al (1989), the number of erythrocytes and leukocytes far below varies depending on the type of animal. Fluctuations in the number of leukocytes in each individual is quite large on certain conditions such as: stress, physiological activity, nutrition, age, and others. Frandson (1993), stated that increasing the number of leukocytes is generally a sign of infection or injury.

The mean blood hematocrit values range chicken for 8-week results of the experiment ranged from 33.00 to 34.25%. The analysis showed that the treatment ration significant effect (P <0.05) on blood hematocrit values range chicken. Use of Nutrient Concentrate in the ration at the rate of 20% (R4 / 15% protein) significantly (P <0.05) higher than the standard ration (RS / 18% protein) against native chicken blood hematocrit valuesphase layer. Normal hematocrit values in chickens according Sturkie (1986) ranged from 29-40%, meaning chicken not impaired in their blood because of physiological systems across flats are within the normal range. The high hematocrit value caused by the tendency of the red cell count is high. According to Swenson (1977), hematocrit value has a positive relationship with the number of erythrocytes. Frandson (1993), adding that the hematocrit value is the percentage of blood that consists of red blood cells (erythrocytes). Hematocrit value of all treatments on the results of this study are in the normal range.

Native chickenby layer phase containing rations Nutrient Concentrate 20% during the study had hematologic value good (healthy) and decent as test animals. The fact that the value of the erythrocytes obtained ranged from 2.06 to 2.2 ×$10^6$ grains/$mm^3$ in the normal range (poultry ranged between 2.0 to 3.2 ×$10^6$ grains/$mm^3$). Leukocyte values ranged from 36.07 to 37.27 ×$10^3$ grains/$mm^3$ in the normal range (poultry ranged between 16-40 ×$10^3$ grains/$mm^3$). Hematocrit values ranged from 33.00 to 34.25% in the normal range (poultry ranges between 29-40%).

## CONCLUSIONS

Production of the optimal range native chicken eggs for 60 days of maintenance is the treatment of rations containing 20% Nutrient Concentrate with the average egg weight 40.51 g/grain; the number of eggs 32.37 grains; and the production of hand-day 53, 95%; the feed efficiency of 54.02%.

Nutrient Concentrates can be used up to a level of 20% in native chicken ration phase layer without affecting the health of chickens (erythrocytes = 2.06 to 2.22 × $10^6$/$mm^3$; leukocytes = 36.07 to 37.27 × $10^3$/$mm^3$; and hematocrit = 33.00 to 34.25%).

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# EFFICACY OF HERBAL EXTRACTS ON GROWTH PERFORMANCE, SERUM BIOCHEMISTRY AND INTESTINAL SELECTED BACTERIAL POPULATION IN BROILERS

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***Abstract***

*This study was conducted to evaluate the effects of two herbal extracts (Emerald and Gundelia tournefortii L. Seed) on growth performance, serum biochemistry and intestinal bacterial population in broilers. A total of 280 Ross 308 1-day-old male broiler chicks were distributed into 5 groups of 7 in each consisting 8 replicates per treatment for 42 days. Five treatments were used; control group received basal diet (without any herbal extract supplementation), the Emerald at 100 mg/kg diet and G. tournefortii L. seed extracts (GTE) at 2, 4 and 8 g/kg diet were added to basal diet. The addition of 4 and 8 g GTE to the diet resulted in significantly higher body weight compared with control group (P<0.05). Moreover, supplementing the highest level of GTE (8 g/kg diet) significantly increased intestinal lactic acid bacteria counts (P<0.05).However, no differences were observed among treatments for feed intake and feed conversion rate at the end of the study (P>0.05). In addition, serum uric acid (UA) and glucose (Glu) concentrations and aspartate amino transferase (AST) and alkaline phosphatase (ALP) activities were not affected by any treatment (P>0.05). In conclusion, our results showed that different dietary levels of GTE may improve the growth performance by increasing intestinal lactic acid bacteria counts.*

***Key words****: Gundelia tournefortii L., growth performance, serum biochemistry, bacterial population, broiler.*

## INTRODUCTION

Various types of antibiotics have been widely used as growth promoters in animal production for a large number of years worldwide. Following the banning of the antibiotics using as growth promoters in animal feeds many researchers started to research novel approach for alternative feed additives in poultry production (Denli et al., 2016). Different types of probiotics, prebiotics, plant extracts, aromatic herbs, spices, essential oils have been applied as feed additives to improve growth performance of livestock animals (Fulton et al., 2002). Many types of them have been tested within varying levels and receiving great interest as replacement of antibiotics in animal production (Akyildiz and Denli, 2016). During the last decade, various herbs, spices, plant extracts, essential oils and phytogenic compounds have been used in animal nutrition due to their potential beneficial effects or active compounds. Many of them have been approved in poultry as growth promoters (Alloui et al., 2011; Hafeez et al., 2016) and due to their antimicrobial effects in poultry by stimulating appetite and digestion (Kamel, 2001; William and Losa, 2001).

In this study we aimed to evaluate the effects of different levels of *Gundelia tournefortii L. seed extracts* (GTE) and a commercial plant extract (Emerald) on growth performance, serum biochemistry and intestinal bacterial population in broilers.

## MATERIALS AND METHODS

This study was performed at the Dicle University, Animal Research Center according to the guidelines for animal experimentation of Dicle University and approved by the Ethical Committee (DUHADEK- No: 01.12.2016-36).A total of 280 Ross 308 1-day-old male broiler chicks were distributed into 5 groups of 7 in each consisting 8 replicates per treatment for 42 days. Five treatments were used; chicks

were fed by basal diet as control group, basal diet plus 100 mg Emerald/kg diet, three levels of GTE (2, 4 and 8 g GTE/kg diet).

Two basal diets; grower (1 to 21 day) and finisher (22 to 42 days) were formulated according to the NRC (1994) recommendations to meet the nutrient requirements of broilers. The composition of the basal diets is presented in Table 1. Chickens received the feed and water as *ad libitum* throughout the experiment. Emerald is a commercial product which has developed by Igusol Advance SA, Spain. Emeraldis a combination of essential oils designed to be gradually released into the digestive tract improving animal's well-being. *Gundelia tournefortii* L. seeds were collected from Bismil town in Diyarbakir in Turkey. The *Gundelia tournefortii* L. seeds was dried then ground through 1.0 mm mesh and incorporated into the experimental diets.

Table 1: Composition of experimental diets (%)

| Ingredients | Starter (1-22 day) | Finisher (23-42 day) |
|---|---|---|
| Maize | 57.0 | 58.0 |
| Soybean meal (46 % CP) | 25.5 | 22.3 |
| Full fat soybean | 13.7 | 13.0 |
| Sunflower oil | - | 2.9 |
| Dicalcium phosphate[a] | 2.00 | 2.0 |
| Limestone | 0.90 | 1.0 |
| NaCl | 0.30 | 0.35 |
| Vitamin premix[b] | 0.10 | 0.10 |
| Mineral premix[c] | 0.15 | 0.15 |
| L-Lysine HCl | 0.20 | - |
| Dl-Methionine | 0.15 | - |
| **Calculated composition** | | |
| Crude Protein | 22.0 | 20.4 |
| ME (kcal/kg) | 3,010 | 3,197 |
| Calcium | 0.97 | 0.99 |
| Available phosphorus | 0.47 | 0.43 |
| L-lysine | 1.36 | 1.26 |
| Methionine+cystine | 0.90 | 0.76 |

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

[c]Provided (per kg of diet): Iron, 80 mg; zinc 40 mg; manganese 60 mg; iodine 0.8 mg; copper, 8 mg; selenium, 0.2 mg; cobalt, 0.4 mg.

Chickens were weighed individually and feed intake determined by pen from 7 to 42 d (n=8). Mortality was checked daily and recorded throughout the experimental period. Body weight gain and feed intake were determined weekly then feed conversion rate (FCR) (g:g) was calculated and recorded.

At the end of the experiment, blood samples (2 mL per bird) were collected from 10 chickens per treatment for serum biochemical determination. Within 1 h, the serum was obtained by centrifugation (2,500 × g for 15 min) and stored at −80°C until further analysis. Serum biochemical parameters were measured by using Architect System Reagents and an automatic clinical chemistry analyzer. The concentration of total protein (TP) was measured by following the Biuret method; uric acid (UA) by following the uricase method; cholesterol by following the cholesterol esterase-peroxidase method; respectively; triglyceride by following the glycerol phosphate oxidase method; and the enzymatic activities of alkaline phosphatase (ALP), aspartate aminotransferase (AST) by using the recommended International Federation of Clinical Chemistry and Laboratory Medicine reference methods. After taking blood samples, chickens were euthanized with an intravenous injection of sodium pentobarbital and immediately intestinal tract, liver, gizzard and abdominal fat pad were removed and weighed (data expressed as relative organ weight; grams of organ per 100 g of BW). Small intestine was immediately removed and digesta contents (from final part of small intestine) from 50 chickens (10 chickens per treatment) were collected separately, cooled at once used for microbial assays *(Escherichia coli, Enterococcus, Colostridium* and *Lactobacillus).*

The data were analyzed by using the one-way ANOVA with the General Linear Model (GLM) procedure of SPSS 16.0 (2011). Treatment means were also partitioned into linear, quadratic, and cubic effects of dietary supplementation of GTE level with orthogonal polynomial contrasts. Statistical significance was considered at $P < 0.05$. Differences among means were evaluated using Tukey's test.

## RESULTS AND DISCUSSIONS

The effects of dietary Emerald and different levels of GTE supplementation on growth performance and internal organ weights are shown in Table 2. In the present study, body weight gain was correlated with the level of GTE (P=0.009) and maximized by the supplementation level of 4 g/kg of diet (Table 2). Besides, body weight gain and feed conversion rate were showed a linear response

to GTE (P<0.05). Our study clearly indicates the positive effects of the tested GTE on body weight gain and feed conversion rate in broilers.

In our previous study, dietary supplementation of Emerald resulted in useful effects as enhancer of growth performance by reducing by reducing the number of *Escherichia coli* count in the intestines of broiler chickens (Akyildiz et al., 2016).This study confirmed the useful effects of Emerald in broilers. These results are in agreement with those reported by Ocak et al. (2008) in that dietary supplementation of peppermint and thyme significantly improved body weight gain in broilers. Similarly, Halle et al. (2004) reported that graded oregano extracts and essential oil collected from oregano significantly improved feed conversion rate in broilers. Our results contrast with those of Calıslar et al. (2009) who found that phytogenic additives containing extracts from *Origanum vulgare ssp. hirtum* had no effects on the body weight gain in broilers.

All groups had similar feed intake and feed conversion rate at 42 day. However, chickens were fed diets supplemented 2 and 8 g of GTE/kg diet had an improved feed conversion rate compared with control chickens at 42 day.

The supplementation of Emerald and GTE had no significant effect on the weights of liver, gizzard, or spleens and abdominal fat pad (P>0.05). However, the intestine weight of chickens supplemented with GTE at 8 g had a higher (P<0.05) compared with the control groups and linear effects of GTE on intestine weight were observed. Differences between study results may be attributable to different composition of the plant extracts, used levels and the active substances and their biological activity, respectively (Amad et al., 2011).

Table 2. Effects of Emerald and *Gundelia tournefortii* L. seed extracts (GTE) on growth performance and internal organ weights in broilers at 42 d of age

| Measurements | Control | Emerald (100 mg/kg) | (GTE) (g/kg) | | | SEM | P | Contrasts | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | 2 | 4 | 8 | | | L | Q | C |
| BWG, g | 2152.9[b] | 2232.3[ab] | 2265.4[ab] | 2297.6[a] | 2282.2[a] | 14.07 | 0.009 | ** | * | NS |
| FI, g | 3778.1 | 3682.9 | 3713.9 | 3886.7 | 3669.6 | 35.50 | 0.291 | NS | NS | NS |
| FCR | 1.75 | 1.71 | 1.69 | 1.70 | 1.67 | 0.009 | 0.148 | * | NS | NS |
| Intestine weight[2] | 5.71[b] | 5.85 | 5.92[ab] | 5.97[ab] | 6.37[a] | 0.088 | 0.160 | ** | NS | NS |
| Liver weight[2] | 2.10 | 2.07 | 2.08 | 2.18 | 2.26 | 0.03 | 0.213 | * | NS | NS |
| Gizzard weight[2] | 2.87 | 2.92 | 2.88 | 2.77 | 3.01 | 0.06 | 0.852 | NS | NS | NS |
| Abdominal fat pad[2] | 1.56 | 1.25 | 1.17 | 1.23 | 1.47 | 0.05 | 0.092 | NS | * | NS |

SEM: Pooled standard error of mean- L: Linear, Q: Quadratic, C: Cubic effects.
[1]Each value represents the least square mean from 8 pens per each treatment: [2](g/100 g body weight)
[a-d]Means within a column without a common superscripts differ statistically (P < 0.05).
NS: No significant (P>0.05), *: P<0.05, **: P<0.01

In our study, ileo-cecal microflora populations and serum biochemistry were determined to explore a possible mechanism for the improvement of performance in broilers with GTE supplementation. Effects of Emerald and GTE on serum biochemistry in broilers are shown in Table 3. Liver enzymes are indicators for many diseases and they are a marker of damage to live cells. ALP and AST activities and serum glucose and uric acid concentrations were not affected by treatments (P>0.05). Albumin and globulin are main component of total amount of protein in serum help growth and healing. Low total protein concentration in serum can imply a liver disorder, a kidney disorder, or a disorder in which protein is not digested or absorbed properly. Serum cholesterol concentrations responded quadratically (P < 0.01) with increasing levels of dietary GTE. These results are consistent with those of Mohan et al. (1996) and Jin et al. (1998) who conducted that intestinal microbial population affect the serum cholesterol concentration in broilers. In present study, decreases of serum cholesterol concentration may be in resulting of the effects of dietary GTE on intestinal microbial population in broilers.

Serum triglyceride concentrations were decreased in chickens were fed the diet supplemented with 4 and 8 g GTE/kg feed (P<0.05).

Table 3. Effects of Emerald and *Gundelia tournefortii* L.seed extracts (GTE) on serum biochemistryin broilers at 42 d of age

| Measurements | Control | Emerald (100 mg/kg) | (GTE) (g/kg) | | | SEM | P | Contrasts | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | 2 | 4 | 8 | | | L | Q | C |
| ALP (U/L) | 1391.4 | 1500.2 | 1270.6 | 1247.5 | 1156.7 | 50.71 | 0.235 | NS | NS | NS |
| AST (U/L) | 250.7 | 297.1 | 252.5 | 250.2 | 239.7 | 8.70 | 0.263 | NS | NS | NS |
| CHOL (mg/dL) | 109.5$^{b}$ | 112.1$^{ab}$ | 124.6$^{a}$ | 104.1$^{a}$ | 112.6$^{ab}$ | 1.82 | 0.004 | NS | NS | ** |
| Glucose (mg/dL) | 227.2 | 258.2 | 244.2 | 225.12 | 227.9 | 4.64 | 0.098 | NS | NS | NS |
| TP (g/dL) | 2.78$^{ab}$ | 3.25$^{a}$ | 2.83$^{ab}$ | 2.57$^{b}$ | 2.88$^{ab}$ | 0.07 | 0.036 | NS | NS | NS |
| TRG (mg/dL) | 60.87$^{b}$ | 80.62$^{a}$ | 65.12$^{ab}$ | 50.5$^{b}$ | 57.37$^{b}$ | 2.51 | 0.001 | NS | NS | * |
| UA (mg/dL) | 8.65 | 9.96 | 9.71 | 8.56 | 10.41 | 0.35 | 0.189 | NS | NS | NS |

SEM: Pooled standard error of mean,ALP:Alkalinephosphatise, AST: Aspartate amino transferase, CHOL:Cholesterol, UA: Uric acid, TP: Total protein,TRG: Trigliseride- L: Linear, Q: Quadratic, C: Cubic effects.
$^{a-d}$Means within a column without a common superscripts differ statistically (P < 0.05).
NS: No significant (P>0.05), *: P<0.05, **: P<0.01

In our study, the colony numbers of *Lactobacillus* was increased in the ileal gut contents of chickens fed diet supplemented Emerald and GTE levels at the 42 day (P<0.05). Our results are supported by the findings of Franciosini et al. (2016), who showed that *Lactobacillus* count increased in ileo-cecal content in broilers fed diet supplemented with oregano *(Origanum vulgare* L.*)* and rosemary *(Rosmarinus officinalis* L.*)* aqueous extracts. On the other hand the *Escherichia coli* counts was decreased in chickens fed diet Emerald and GTE supplemented with compared to control. Both types of *Clostridium perfringens* (A and C) may cause necrotic enteritis in many species of birds (Crespo et al., 2007). Kaldhusdal and Hofshagen (1992) reported that increasing of numbers of *Clostridium perfringens* in the gut of broilers may lead to lower growth rate and feed efficiency.

Table 4. Effects of Emerald and *Gundelia tournefortii* L. seed extracts (GTE) on serum biochemistry in broilers at 42 d of age

| Measurements | Control | Emerald (100 mg/kg) | (GTE) (g/kg) | | | SEM | P | Contrasts | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | 2 | 4 | 8 | | | L | Q | C |
| *Escherichia coli (log CFU g-1)* | 4.84$^{a}$ | 3.63$^{b}$ | 4.35$^{ab}$ | 4.30$^{ab}$ | 4.29$^{ab}$ | 0.13 | 0.086 | NS | NS | NS |
| *Enterococcus (log CFU g-1)* | 1.00 | 3.41 | 1.05 | 2.04 | 1.55 | 0.34 | 0.148 | NS | NS | NS |
| *Colostridium spp. (log CFU g-1)* | 3.74$^{a}$ | 3.50$^{ab}$ | 3.46$^{ab}$ | 3.23$^{b}$ | 3.44$^{ab}$ | 0.05 | 0.070 | * | * | NS |
| *Lactobacillus spp. (log CFU g-1)* | 2.97$^{b}$ | 4.61$^{a}$ | 4.31$^{ab}$ | 4.20$^{ab}$ | 4.69$^{a}$ | 0.18 | 0.014 | NS | NS | NS |

SEM: Pooled standard error of mean- L: Linear, Q: Quadratic, C: Cubic effects.
$^{a-d}$Means within a column without a common superscripts differ statistically (P < 0.05).
NS: No significant (P>0.05), *: P<0.05, **: P<0.01

## CONCLUSIONS

In conclusion, results of this study showed that different dietary levels of GTE may improve the growth performance by increasing intestinal lactic acid bacteria counts. However, more experiments are needed to explain whether GTE may affect antimicrobials or antioxidants in poultry diets.


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# CHEMICAL AND NUTRITIONAL CHARACTERIZATION OF RAW MATERIALS USED TO PRODUCE EGGS AS A FUNCTIONAL FOOD AND THEIR IMPACT ON THE BIO-PRODUCTIVE PERFORMANCE OF LAYING HENS

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***Abstract***

*Nutritional manipulation is relatively simple in the poultry field as long as the raw material of the laying hens is identified that ensures the bioavailability of the desired nutrient to be enriched in the egg.*
*The purpose of this study is to characterize the nutritional and chemical ingredients of the recipe for laying hens and its effect on egg quality and the bio-productive performance of the hen. Thus, with regard to the chemical composition of the recipe used which was improved with 2 carotenoid additives with 2% lutein and zeaxanthin, respectively with 5% lutein and zeaxanthin compared to the control recipe, and the administration of these recipe to hens gives a better specific consumption compared to group M; With regard to the quality indices of the eggs obtained, a weight of 65.47 g was observed in the group of birds fed with AC1 2% and 65.25 g in the group in whose recipe AC1 was used 5% compared to the control group Where the average weight of the eggs was 64.13g. In terms of yolk colour, it had average values of 3.78 for group E1 and 4.14 for group E2. Thus, due to the use of raw materials enriched in lutein and zeaxanthin the colour intensity of the yolk was significantly higher compared to the M group (3.61%).*

***Key words****: nutrition, quality indicators, egg, lutein, zeaxanthin.*

## INTRODUCTION

Knowing that on a national and international level there is a special emphasis on superior capitalization of crop plants and of the spontaneous flora, of the natural resources in general, depending on their chemical composition, knowledge of the biologically active substances is a priority for those who want to produce functional foods. Only by knowing the chemical composition of the plants, the biochemical mechanisms underlying the biological phenomena will be obtained feedings for poultry with high content in biologically active substances, which increase the biological value of egg yolk by the high intake of lutein and zeaxanthin (Breithaupt et al., 2007; Hadden et al., 1999; Herke et al., 2014). There are studies that demonstrate that lutein and zeaxanthin help to: maintain heart health by reducing the risk of atherosclerosis; Reduce the development of conditions that compromise immunological status; Can inhibit cellular proliferation, cellular transformation, and modulate expression of genetic determinants in the prevention of certain cancers (Leeson et al., 2004; Martinez et al., 2012; Moros et al., 2002).

Given that a laying hen is a true "egg factory" (Al-Shami Mutahar și colab., 2011) which produces an egg almost daily, the food ration must be elaborated according to rigorous scientific principles, because a small deficit of one or more food components, in within a few days, unbalance the functions of the entire organism, making it receptive to disease and diminishing its production capacity (Al-Haweizy et al., 2007; Perry et al., 2009). The concentration of lutein in eggs is dependent both on the content of carotenoids in the feed administered and on the growing conditions, as it is shown that hens grown on the ground have twice the yolk in yolk than those grown in batteries (Güçlu et al., 2004; Hesterberg et al., 2012; Marin et Pogurschi, 2006). Alfalfa is an rich ingredient in xanthophylls with a high content of protein and vitamins, also corn-rich diets can contain up to 10 mg of lutein and about 5 mg of zeaxanthin. Thus, alfalfa and red corn diets are improving the concentration of

lutein and zeaxanthin in egg yolk (Laudadio et al., 2014; Lokaewmanee et al., 2011; Navid et al., 2011).

## MATERIALS AND METHODS

The experiment was conducted for 5 weeks on 170 hens, 35 weeks old. Birds were weighed individually at the beginning of the experiment, being weight-based, in three groups (M, E1, E2). Husked animals were housed with 2 birds / cage in a 3-layer structured battery, allowing for daily logging and food scraps. Water and mixed fodder were administered *ad libitum*. The incandescent lighting of the hall was done according to the growth guide. Birds of the 3 batches were fed with compound feeds that had the same basic structure. In addition to the conventional raw materials used to prepare a mixed feed for the control group (M), a herbal mixture (AC1) was used in the 2 experimental recipes (E1, E2). The rate of inclusion of the two mixtures was 2% and 5% respectively.

A number of accredited methods have been used in line with current standards.

The dry matter, crude protein, crude fat, cellulose and ash content of the fodder were determined in accordance with the provisions of Regulation (EC) No. 152/2009 .

The content of lutein and zeaxanthin was determined by high performance liquid chromatography (HPLC).

Average daily consumption (g NC / head / day), specific consumption (kg NC / kg egg), mean weight of eggs (g), laying rate (%), calculated on the basis of daily production records eggs. In terms of egg analyzes, these included weight of the eggs and their components, colour of the yolk using La Roche scale, thickness of the shell was determined using Egg Shell Thickness Gauge and shear breaking force of shell using Egg Force Reader. The data obtained were statistically processed to assess significance of the statistical differences.

## RESULTS AND DISCUSSIONS

Table 1 shows the structure of the carotenoid additive used, with 40% alfalfa being used in its composition, which is used at low concentrations in poultry feed due to its fibre content (2), red corn red corn is found with an 20%, pumpkin and tagetes are in concentration of 15% and marigold 10%.

The experiment was aimed at testing the capacity of the added vegetable mixture in different proportions (2% and 5%), enriching egg yolk in lutein and zeaxanthin.

Table 1. Structure of the carotenoid additive AC-1

| Carotenoid additive AC-1 | Rate |
|---|---|
| Alfalfa - meal (dry-milled) | 40% |
| Red corn (dry, milled) | 20% |
| Pumpkin - fruit pulp (dry, ground) | 15% |
| Tagetes - flower (dry milled) | 15% |
| Marigold - flowers (dry milled) | 10% |

The structure of the experimental recipes is presented in Table 2, being set up in accordance with NRC nutritional requirements and the hybrid growth guide.

Table 2. Structure of experimental recipes used

| Specification | M1 | E1 (AC1 2% ) | E2 (AC1 5%) |
|---|---|---|---|
| Maize, (%) | 53.23 | 51.23 | 49.67 |
| Soybean meal, (%) | 15.71 | 15.98 | 15.56 |
| Sunflower seed, (%) | 15 | 15 | 15 |
| AFC 5, (%) | - | 2 | 4 |
| Oil, (%) | 3.21 | 3.18 | 3.17 |
| Lysine, (%) | 0.09 | 0.08 | 0.09 |
| Methionine, (%) | 0.12 | 0.11 | 0.11 |
| Calcium, (%) | 9.85 | 9.64 | 9.64 |
| Phosphat, (%) | 1.34 | 1.33 | 1.32 |
| Salt, (%) | 0.4 | 0.4 | 0.39 |
| Colina, (%) | 0.05 | 0.05 | 0.05 |
| Premix A6, (%) | 1 | 1 | 1 |
| Total raw materials | 100 | 100 | 100 |

It can be seen in table 2 that quality parameters have been ensured in all 3 combined fodder that have been produced, which allowed the health status and the production process to be unaffected during the experiment. From the combined feed samples, determinations were made on the chemical composition, with slight differences in crude protein content, respectively, the content of lutein and zeaxanthin, and it was found that the recipe of the 5% AC1 group had the highest xanthophyl content, followed by those of the 2% AC1 and M1 groups, as shown in table 3.

Table 3. Chemical composition of recipes used

| Specification | M | E1 | E2 |
|---|---|---|---|
| Dry matter, (%) | 92.27 | 91.22 | 92.28 |
| Crude protein, (%) | 16.99 | 16.45 | 17.56 |
| Crude fat (%) | 5.01 | 4.91 | 4.98 |
| Cellulose, (%) | 6.14 | 6.48 | 6.62 |
| Ash, (%) | 14.33 | 13.79 | 14.14 |
| Lutein + zeaxanthin, (mg / kg) | 5.257 | 13.268 | 19.791 |

Bio-productive performances (table 4) show that in E1 group (AC1 2%) the eggs had, on average/experiment, a significant weight (P≤0.05) lower than the other groups. Of the experimental groups, the eggs harvested from the chickens of the E2 group (AC1 5%) had a weight of eggs comparable to that recorded in group M. The data on the laying intensity indicates 96.19% in the control group (M) while in experimental groups E1 and E2 it was 97.06% and respectively 95.37%.

Table 4. Bio-productive performances

| Specification | M | E1 | E2 |
|---|---|---|---|
| Daily average consumption (g MF / head / day) | 120.63±5.50 b | 116.64±6.17 a,d | 118.32±4.22 |
| Specific consumption (kg MF / kg egg) | 2.02±0.26 | 1.97±0.23 | 2.00±0.21 |
| Average egg weight (g) | 63.09±0.96 b,e | 61.98±0.55 a,c,e | 63.13±3.48 b,e |
| Laying intensity (%) | 96.19±11.3 | 97.06±10.91 | 95.37±8.58 |

* Where: a, b, c, d are significant differences (P≤0.05) from M, E1, E2

Regarding physical parameters, respectively, increase of the yolk colour in the experimental group compared to control, it was made under the influence of addition of carotenoid additive in the combined feed, so that it was 3.61 for the group M, respectively 3.78 for E1 group, and 4.14 for E2 group. The colour of yolk showed proportional increases for the batches analyzed for both the thickness of the shell and its bursting force (table 5).

Table 5. Eggs quality parameters (mean values / experiment)

| Specification | M | E1 | E2 |
|---|---|---|---|
| Yolk color | 3.61±0.65 | 3.78±0.42 | 4.14±0.64 |
| Egg shell thickness (mm) | 0.343±0.029 | 0.344±0.028 | 0.354±0.026 |
| Shell breaking force (Kgf) | 3.898±0.519 | 3.964±0.522 | 4.042±0.562 |

Regarding variation in weight of egg components, we notice an increase in white proportion 2.05% and a decrease in yolk proportion by 0.3% (fig. 1).
The results obtained from xanthophylls studies in fresh yolk samples indicate mean values of 14.66 mg/kg for group E2, 9.41 mg/kg for group E1 and only 6.65 mg/kg for control group.

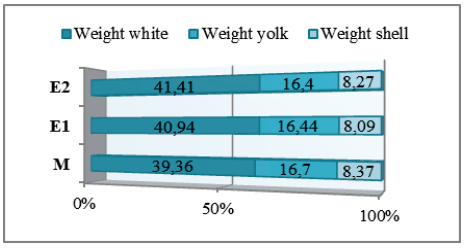


Figure 1. Weight of components in structure of eggs obtained in experiment (average values / experiment)

The administration of compound feed enriched in xanthophylls by the inclusion of carotenoid additives resulted in an increase in xanthophylls content of yolk as well.

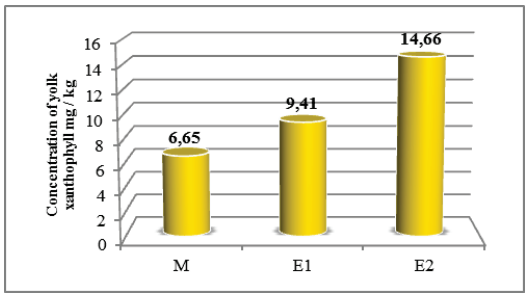


Figure 2. Concentrations of xanthophylls in fresh yolk samples

As expected, the colour of yolk has influenced the decision of consumers who, in an increasing crisis, are looking for quality products with improved nutritional characteristics that help maintain health and provide functionality through daily consumption.

## CONCLUSIONS

- carotenoid additives (AC1) used in structure of the compound feed fed to the hens brought a significant improvement in lutein and zeaxanthin;
- the advantages obtained by using AC1 having a high concentration of xanthophylls in compound feed have intensified the colour of the yolk of obtained eggs;
- the use of AC1 in compound feeds administered to laying hens has affected some bio-productive performances and some physical quality parameters, laying strength and egg weight.

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# EFFECTS OF SUMAC POWDER *(Rhus coriaria* L.**) ON GROWTH PERFORMANCE, SERUM BIOCHEMISTRY AND INTESTINAL MICROBIOATA IN BROILERS AT DIFFERENT STOCKING DENSITIES**

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***Abstract***

*This study aimed to evaluate the effects of dietary supplementation of sumac powder (Rhus coriaria) on growth performance, serum biochemistry and intestinal microbiota in broilers reared at different stocking densities. A total of 378 one-day-old Ross 308 male broiler chicks were subjected to a 2 stocking densities (10 and 20 chicks/m$^2$ floor area; normal and high stocking density) x 3 sumac powder levels (0.0, 0.75 and 1.5 g/kg feed) factorial arrangement of treatments. Body weight and feed intake were significantly lower in chickens reared at high stocking density than normal stocking density groups (P<0.05). Moreover, feed conversion rate was negatively affected by high stocking density (P<0.05). Dietary addition of sumac powder had no effect on these variables in both stocking densities (P>0.05). Both stocking densities and the supplementation of sumac powder to the feeds had a reducing effect on intestinal weight (P<0.05). Serum total protein concentration of chickens reared at high stocking density was higher than those of the control normal groups (P<0.05). There was interaction between stocking density and dietary sumac powder supplementation for only body weight and abdominal fat pad variables at the end of the study (P<0.05). However, no differences were observed for the relative weight of liver, spleen, gizzard and proventriculus (P>0.05). In addition, alkaline phosphatase (ALP), aspartate amino transferase (AST) activities and high density lipoprotein (HDL), cholesterol and triglyceride concentration in the serum were not influenced by the any stocking density and sumac powder supplementation (P>0.05). In conclusion, our results showed that broilers exhibited low performance when reared at high stocking density and dietary supplementation of sumac powder has not prevented this decline.*

***Key words**: s**tocking density, sumac powder, growth performance, serum biochemistry, broiler.*

## INTRODUCTION

Stocking density is the numbers of chickens per unit area reared during grow out. Stocking density has been one of the principal concerns in the welfare of poultry production practices. Presently, there is an ongoing debate at the ideal density for chickens reared on the floor and in the different types of cages. Sorensen et al. (2000) reported that lower growth and fattening performance and higher mortality and prevalence of leg weakness in animals reared in high stocking density. Environmental conditions may have different effects on the productivity of poultry. During the summer season stocking density plays an important in broiler production and low growth performance and high mortality may occur at higher stocking densities in broilers (Türkyilmaz, 2008). High stocking density may cause decreases of growth performance of poultry and can lead to disease or death due to changes in the immune system of animal (Rotllant et al., 1997). Puron et al. (1995) reported that the reduction of feed intake of broiler chickens at high density because of the stress caused by environmental conditions.

Recently, several plant extracts have received considerable attention because of their natural antioxidants effects as feed additives in poultry nutrition.

Sumac *(Rhus coriaria* L.) is a plant, grows widely in Asian countries and it uses as tradetional medicine (Shidfar et al., 2014). Tannins and flavonoids are the main compounds of sumac extracts (Jung, 1998) and it has gallic acid and several group B vitamins (EL Sissi et al., 1972). Some researchers reported that dietary sumac powders improved growth performance in broilers (Gulmez et al, 2006; Ghasemi et al., 2014) and enhanced intestinal

characteristics on broiler chicks (Ghasemi et al., 2014).

The aim of this study was to investigate the effects of dietary supplementation of sumac powder on growth performance, serum biochemistry and intestinal microbiota in broilers reared at different stocking densities.

## MATERIALS AND METHODS

This study was performed at the Dicle University, Animal Research Center Unit according to the guidelines for animal experimentation of Dicle University and approved by the Ethical Committee (DUHADEK- No: 01.12.2016-5).

Totally, 378 one-day-old Ross 308 male broiler chicks were randomly divided into 6 experimental groups. The 6 experimental treatments subjected to a 2 x 3 factorial arrangement, in which the 2 variation factors were the 2 stocking densities (10 and 20 chicks/$m^2$ floor area; normal and high stocking density) and 3 sumac powder levels (0, 0.75 and 1.5 g/kg feed).

Sumac powder was purchased from a local market in Mardin province and it added to experimental diets after grinding.

Diets were formulated based on NRC (1994) recommendations to meet the nutrient requirements of broilers from d 1 to 21 (grower diet) and from d 22 to 42 (finishing diet). The composition of the basal diets is presented in Table 1.

Table 1. Composition of experimental diets (%)

| Ingredients | Starter (1-22 day) | Finisher (23-42 day) |
|---|---|---|
| Maize | 58.2 | 57.0 |
| Soybean meal (48 % CP) | 22.0 | 23.0 |
| Full fat soybean | 11.0 | 12.0 |
| Fish meal (60 % CP) | 5.2 | - |
| Sunflower oil | - | 3.7 |
| Dicalciumphosphate[a] | 1.75 | 1.60 |
| Limestone | - | 1.0 |
| NaCl | 0.30 | 0.35 |
| Vitamin premix[b] | 0.10 | 0.10 |
| Mineral premix[c] | 0.15 | 0.15 |
| L-Lysine HCl | 0.15 | - |
| Dl-Methionine | 0.15 | - |
| **Calculated composition** | | |
| Crude Protein | 22.9 | 20.1 |
| ME (kcal/kg) | 2,996 | 3,213 |
| Calcium | 0.90 | 0.98 |
| Available phosphorus | 0.45 | 0.37 |
| L-lysine | 1.43 | 1.10 |
| Methionine+cystine | 0.92 | 0.75 |

[a] Contains 240 g Ca and 17.5 g P/kg;

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

[c] Provided (per kg of diet): Iron, 80 mg; zinc 40 mg; manganese 60 mg; iodine 0.8 mg; copper, 8 mg; selenium, 0.2 mg; cobalt, 0.4 mg.

Feed and water were provided *ad libitum* throughout the experiment. The experiment lasted 42 d, including 21 d on the grower diet and from d 22 to 42 on the finishing diet.

Chickens were weighed individually and feed intake determined by pen from 7 to 42 d (n=7). Mortality was checked daily and recorded throughout the experimental period. Feed conversion rate (FCR) was calculated by pen with dividing total feed intake to body weight.

At the end of the experiment, blood samples (2 mL per bird) were collected from 10 chickens per treatment for serum biochemical determination. Within 1 h, the serum was obtained by centrifugation (2,500 × g for 15 min) and stored at −80°C until further analysis. Serum biochemical parameters were measured by using Architect System Reagents and an automatic clinical chemistry analyzer. The concentration of total protein (TP) was measured by following the Biuret method; uric acid (UA) by following the uricase method; cholesterol by following the cholesterol esterase-peroxidase method; respectively; triglyceride by following the glycerol phosphate oxidase method; and the enzymatic activities of alkaline phosphatase (ALP), aspartate aminotransferase (AST) by using the recommended International Federation of Clinical Chemistry and Laboratory Medicine reference methods. After taking blood samples, chickens were euthanized with an intravenous injection of sodium pentobarbital and immediately intestinal tract, liver and spleen were removed and weighed (data expressed as relative organ weight; grams of organ per 100 g of BW). Small intestine was immediately removed and digesta contents (from final part of small intestine) from 60 chickens (10 chickens per treatment) were collected separately, cooled at once used for microbial assays *(Escherichia coli* and *Lactobacillus).*

The data were analyzed by using the ANOVA with the General Linear Model (GLM) procedure of SPSS 16.0 (2011) by using the following model: $Yijk = \mu + \alpha i + \beta j + (\alpha\beta)ij + \varepsilon ijk$, where Yijk is the dependent variable, μ is the overall mean, αi is the effect of stocking density (i = 1,2); βj is the effect of sumac powder (j = 1, 2, 3); (αβ)ij is the interaction between stocking density and sumac powder levels; and $\varepsilon \sim N(0,\sigma 2\varepsilon)$ represents the

unexplained random error. The α-level used for determination of significance for all the analyses was 0.05. Differences between means were tested by Tukey's least significant difference when an interaction between stocking density and sumac powder was significant. Data are presented as means and SEM.

## RESULTS AND DISCUSSIONS

Results of the effects of sumac powder *(Rhus coriaria* L.*)* on growth performance and intestinal microbiota in broilers at different stocking densities are given in Table 2. Body weight gain and feed intake were significantly lower in chickens reared at high stocking density than normal stocking density groups (P<0.05). These results are in agreement with in previous studies by Dozier et al. (2005), who reported that, the negative effect of high stocking density on cumulative body weight gain in broilers. Negative effects of high density on live performance in broilers might be broilers faced an access difficulties to feed and water difficulties (Sørensen et al., 2000).

On the other hand, dietary supplementation of sumac powder had no significant effect on body weight gain, feed intake and feed conversion rate (P>0.05). In contrast, Ghasemi et al. (2014) reported that addition of different levels of sumac extract (1, 2 and 3 % of diet) at had significant effects on the feed intake. Similarly, Lee et al. (2003) showed that the improvement of feed efficiency in broilers fed diet supplemented sumac extracts. Different results might be partially explained by differences in the sumac extract level supplemented to diet.

*Lactobacillus* spp. may be considered main microorganisms in the gut of broilers 6 weeks of age (Dumonceaux et al., 2006). Our results indicated that an interaction was existed between stocking density and sumac powder supplementation for body weight gain, total aerobs, *E. coli* and *Lactobacillus* population in gut (Table 2).

Table 2. Effects of sumac powder *(Rhus coriaria* L.*)* on growth performance and intestinal microbiota in broilers at different stocking densities

| **Treatments**[1] | | **Growth performance**[1] | | | **Bacteriacolony** | |
|---|---|---|---|---|---|---|
| Stocking density (bird/m$^2$) | Sumac powder (g/kg feed) | BW gain (g) | Feed intake (g) | Feed conversion rate | *E. coli* (logCFU$^{g-1}$) | *Lactic acid bacteria* (log CFU$^{g-1}$) |
| 10 | 0 | 2773.0$^{ab}$ | 4536.8$^{a}$ | 1.66 | 4.73 $^{a}$ | 3.56$^{b}$ |
| 10 | 0.75 | 2863.7$^{a}$ | 4529.4$^{a}$ | 1.63 | 4.08$^{ab}$ | 3.97$^{ab}$ |
| 10 | 1.5 | 2842.9$^{a}$ | 4569.5$^{a}$ | 1.65 | 3.68$^{b}$ | 3.98$^{ab}$ |
| 20 | 0 | 2650.2$^{bc}$ | 4452.9$^{ab}$ | 1.68 | 4.88$^{a}$ | 3.30$^{b}$ |
| 20 | 0.75 | 2481.6$^{d}$ | 4205.3$^{b}$ | 1.66 | 3.87$^{b}$ | 4.14$^{a}$ |
| 20 | 1.5 | 2596.0$^{cd}$ | 4364.8$^{ab}$ | 1.66 | 3.64$^{b}$ | 4.22$^{a}$ |
| SEM | | 16.92 | 30.41 | 0.004 | | |
| Main effect | | *Probability* | | | | |
| Stockingdensity | | ** | ** | NS | * | NS |
| Sumacpowder | | NS | NS | NS | ** | * |
| StockingdensityxSumacpowder | | ** | NS | NS | * | * |

SEM: Pooled standard error of mean

[1]Each value represents the least square mean from 7 pens per each treatment

[a-d]Means within a column without a common superscripts differ statistically (P < 0.05).

NS: No significant (P>0.05), *: P<0.05, **: P<0.01

[1]Results are reported as means for 5 replicates of 3 broilers each.

The effects of stocking density and dietary sumac powder supplementation on serum biochemistry in broilers are presented in Table 3. At the end of the experiment, none of the serum biochemistry variables was affected by dietary sumac powder (P>0.05) (Table 3). Although there was no interaction between stocking density and sumac powder in serum biochemistry variables (P>0.05) Serum uric acid, total protein and albumin concentrations were affected by stocking density (P<0.05). However, ALP, and AST activities, serum HDL, cholesterol and triglyceride concentration of broilers were not affected by any treatment (P>0.05).

Table 3. Effects of sumac powder *(Rhus coriaria* L.*)* on serum biochemistry in broilers at different stocking densities

| **Treatments**[1] | | **Measurements** | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Stocking density (bird/$m^2$) | Sumac powder (g/kg feed) | ALP (U/L) | AST (U/L) | CHOL (mg/dL) | UA (g/dL) | TP (g/dL) | HDL (mg/dL) | ALB (mg/dL) |
| 10 | 0 | 1744.6 | 392.5 | 121.1 | 3.46 | 3.65 | 64.8 | 0.56 |
| 10 | 0.75 | 1757.0 | 344.1 | 126.7 | 4.28 | 3.78 | 69.65 | 0.53 |
| 10 | 1.5 | 1470.0 | 362.6 | 122.0 | 3.63 | 3.65 | 64.33 | 0.48 |
| 20 | 0 | 1467.3 | 313.5 | 129.7 | 4.17 | 4.13 | 73.08 | 0.48 |
| 20 | 0.75 | 1626.8 | 274.0 | 127.2 | 4.93 | 4.24 | 69.68 | 0.47 |
| 20 | 1.5 | 1811.0 | 311.2 | 131.0 | 5.06 | 4.11 | 74.03 | 0.49 |
| SEM | | 68.71 | 14.92 | 1.91 | 0.21 | 0.62 | 1.20 | 0.008 |
| Main effect | | *Probability* | | | | | | |
| Stocking density | | NS | NS | NS | * | ** | NS | * |
| Sumac powder | | NS | NS | NS | NS | NS | NS | NS |
| Stocking density x Sumacpowder | | NS | NS | NS | NS | NS | NS | NS |

SEM: Pooled standard error of mean,ALP:Alkaline phosphatise, AST: Aspartate amino transferase, CHOL:Cholesterol, UA: Uric acid, TP: Total protein: HDL: High density lipoprotein, ALB: Albumin

[1]Each value represents the least square mean from 7 pens per each treatment

[a-d]Means within a column without a common superscripts differ statistically (P < 0.05).

NS: No significant (P>0.05), *: P<0.05, **: P<0.01

[1]Results are reported as means for 5 replicates of 3 broilers each.

Our findings demonstrate that stocking density had significant effects on spleen, proventriculus, intestine weight and abdominal fat pat (P<0.05) (Table 4). However, internal organ weights of broilers were not affected by any levels of supplemental sumac powder. A higher stocking density significantly decreased percentage gizzard, intestine weight and abdominal fat pad at 42 days (P<0.05). Thaxton et al. (2006), similarly, observed that lower internal organ weight of broilers reared at high stocking density. However, the interaction of sumac powder supplementation and stocking density was not significant for the internal organ weights checked.

Table 4. Effects of sumac powder *(Rhus coriaria* L.*)* on internal organ weights in broilers at different stocking densities

| **Treatments**[1] | | **Measurements** (g/100 g body weight) | | | | | |
|---|---|---|---|---|---|---|---|
| Stocking density (bird/$m^2$) | Sumac powder (g/kg feed) | Liver weight | Spleen weight | Gizzard weight | Proventriculus weight | Intestine weight | Abdominal fat pad |
| 10 | 0 | 2.27 | 0.136 | 1.91 | 0.38 | 4.69 | 0.81 |
| 10 | 0.75 | 2.18 | 0.129 | 1.70 | 0.42 | 4.59 | 1.20 |
| 10 | 1.5 | 2.26 | 0.127 | 1.92 | 0.41 | 4.18 | 0.95 |
| 20 | 0 | 2.20 | 0.136 | 1.72 | 0.39 | 3.94 | 0.72 |
| 20 | 0.75 | 2.42 | 0.126 | 1.57 | 0.36 | 3.95 | 0.75 |
| 20 | 1.5 | 2.11 | 0.112 | 1.56 | 0.32 | 3.44 | 0.77 |
| SEM | | 0.51 | 0.005 | 0.05 | 0.01 | 0.08 | 0.04 |
| Main effect | | *Probability* | | | | | |
| Stocking density | | NS | NS | * | NS | ** | * |
| Sumac powder | | NS | NS | NS | NS | NS | NS |
| Stocking density x Sumac powder | | NS | NS | NS | NS | NS | * |

SEM: Pooled standard error of mean

[1]Each value represents the least square mean from 7 pens per each treatment

[a-d]Means within a column without a common superscripts differ statistically (P < 0.05).

NS: No significant (P>0.05), *: P<0.05, **: P<0.01

[1]Results are reported as means for 5 replicates of 3 broilers each.

## CONCLUSIONS

In conclusion, our results showed that broilers exhibited low performance when raised in high stocking density and dietary supplementation of sumac powder has not prevented this decline.


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# STUDY ON THE CHEMICAL COMPOSITION AND NUTRITIONAL VALUE OF THE *GALEGA ORIENTALIS* LAM. AND THE PROSPECTS OF ITS VALORIFICATION IN THE REPUBLIC OF MOLDOVA

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***Abstract***

*The results of the research on biological features, productivity, chemical composition and forage value of Galega orientalis Lam., variety Speranţa, are presented in this paper. The research was focused on the chemical composition and the nutritional value of Galega orientalis Lam., which is a non-traditional fodder plant for the Republic of Moldova, and on the fodder obtained from it. The dry matter of this plant contains 15-20% crude protein, 2.7-3.9% crude fat, 32.2-37.8% crude fibre, 7.3-8.8% ash and 30.6-39.5% nitrogen-free extract. The possibility of producing high quality hay and haylage of this plant under laboratory, semi-production and production conditions has been demonstrated.*

***Key words:*** *chemical composition, Galega orientalis, hay, haylage, nutritional value.*

## INTRODUCTION

Forages play a significant role in livestock nutrition and approximately 85% of all feed units are from forages. In recent years, in many countries, more non-traditional fodder crops are cultivated and used as sources of protein, essential amino acids, biologically active substances, to provide a balanced diet for animals, increasing their productivity, as well as to improve soil fertility, to restore degraded soils etc. (Uteush, 1990; Kshnikatkina et al., 2005;).

Broadening the range of fodder plants is necessary because, in the Republic of Moldova, only a few basic fodder crops are traditionally used: alfalfa, sainfoin and soybean – as sources of protein and corn – as a source of energy. These fodder crops are highly effective and well studied, but in some years, have a poor harvest because of natural hazards (drought, heat waves). Therefore, it is necessary to diversify the sources of fodder by studying the chemical composition and the nutritional value of new and non-traditional crops (Bahcivanji et al., 2012; Teleuţă and Ţîţei , 2012; Coşman, 2014).

The introduction, acclimatization and implementation, in Moldova, of fodder plants from other floristic regions of Earth, rich in nutrients and biologically active substances, is one of the possibilities to broaden the range of fodder sources, to diversify animal nutrition, to enhance the quality of animal feed, to increase the productivity and the quality of animal products. The species *Galega orientalis* Lam., family *Fabaceae* Lindl., is distinguished by high and stable yields over several years, accumulating biomass with a high content of protein and essential amino acids (lysine, methionine).

*Galega orientalis*, eastern galega, fodder galega, is an herbaceous perennial, native to the Caucasus. It forms a solid shrub of 10 to 18 leafy stems, 0.8-2.0 m tall; has alternate, odd-pinnate, 15-30 cm long leaves, which have a good feature to remain undamaged while drying hay. The tap-root system is

composed of combined lateral rhizomes; at a depth of 7 cm, the main roots produce 2–18 lateral offspring – rhizomes; they grow horizontally over 30 cm in length and form buds, which sprout shoots. It has been introduced to many other regions for use in agriculture (Uteush, 1990; Nommsalu, 1994; Stjepanović et al., 2007; Darmohray, 2009; Pikun, 2011).

## MATERIALS AND METHODS

The local cultivar *Speranta* of fodder galega *Galega orientalis* (Photos 1, 2) created in the Botanical Garden (Institute) of the ASM, registered in the in the Catalogue of plant varieties of the Republic Moldova, served as subject of study, the traditional leguminous fodder crop *Medicago sativa* – as control variant. The samples, necessary for determining the chemical composition of *Galega orientalis*, were taken at the first mowing, in the budding (27.04.2016), early flowering (03.05.2016) and flowering periods (18.05.2016), and the samples of *Medicago sativa* – in the budding period (18.05.2016).

Photo 1. *Galega orientalis* in budding period

Photo 2. *Galega orientalis* in flowering period

The analyses were performed in the Laboratory of Nutrition and Feed Technology of the Institute of Biotechnology in Animal Husbandry and Veterinary Medicine and included the determination if the following indices: initial and hygroscopic moisture content, nitrogen, crude protein, crude fat, crude fibre, ash, nitrogen-free extract (NFE) and carotene.

Haylage and hay were prepared from the green mass harvested in the flowering period. The haylage was prepared from wilted green mass (2 days after mowing). In the obtained haylage, the chemical composition was determined according to the above-mentioned indices and the following indices: pH index, concentration of organic acids in free and fixed state (lactic, acetic and butyric) and the organoleptic characteristics (smell, colour and consistency) were assessed. The content of neutral detergent fibre (NDF), acid detergent fibre (ADF), acid detergent lignin (ADL) and matter digestibility were evaluated using the near infrared spectroscopy (NIRS) technique in the Laboratory for Determining Feed Quality of the Research-Development Institute for Grassland Brasov, Romania.

## RESULTS AND DISCUSSIONS

Studies were conducted on the evolution of the content of nutrients in *Galega orientalis*, depending on the development stage. The data are presented in Table 1. The obtained results demonstrate that *Galega orientalis* is primarily characterized by high moisture content, 87.48% in the budding period, 86.43% in the early flowering period, gradually decreasing to 81.93% in the flowering period. Yet, the dry matter content increased gradually from 12.52% in the budding period, up to 18.07% in the flowering period. As compared with alfalfa, the moisture content of *Galega orientalis* was with 6.79% higher in the budding stage. The crude protein content was also quite high and constituted 20.56% dry matter in the budding period, decreasing gradually to 15.13% in the flowering period, that was, over 5%. These data are particularly important when determining the best harvest time for *Galega orientalis*. The data on the crude protein content of *Galega orientalis* in the budding stage were comparable with the

data on alfalfa in the same phenological stage, which contained 20.44% crude protein.

The crude fat content in the dry matter of *Galega orientalis* varied from 3.51% in the budding period to 3.01% in the flowering period and was somewhat lower than in alfalfa (4.4%). The amount of ash was also higher in alfalfa – 9.7% as compared with 7.02-7.98% in *Galega orientalis*. No substantial differences were found in the content of nitrogen-free extract (NFE), this index varied from 29.98% to 35.13% in *Galega orientalis* and 32.92% in alfalfa. These two plant species were also comparable in the crude fibre content, which reached 29.01% in *Galega orientalis* and 25.34% in alfalfa, in the budding period. It was found that the amount of crude fibre in *Galega orientalis* increased and reached 34.45% in the flowering period.

Fibre refers to the cell wall constituents – hemicellulose, cellulose and lignin. The data on the constituents of cell walls demonstrated that, as the plants aged, from the budding period until the flowering period, the amount of indigestible fractions, such as ADL, increased from 3.9% to 6.3%, or, 1.6 times. The fractions of ADF and NDF also increased, ADF – from 28.8%, in the first stage, to 37.7%, in the last stage, or with 8.9%, and NDF – from 46.5% to 57.9%, or with 11.4 %, respectively. The hemicellulose content gradually changed from 17.7%, in the budding period, to 20.5%, in the early flowering period, and decreased to 20.2%, in the flowering period, while the cellulose content increased essentially from 24.9% to 31.4%.

Table 1. Dynamics of the chemical composition of *Galega orientalis* depending on the harvest time

| Indices | | *Galega orientalis* | | | *Medicago sativa* |
|---|---|---|---|---|---|
| | | Budding period | Early flowering period | Flowering period | Budding period |
| Moisture content,% | initial | 86.1 | 85.3 | 80.67 | 79,19 |
| | hygroscopic | 9.96 | 7.70 | 6.51 | 7,2 |
| | totals | 87.48 | 86.43 | 81.93 | 80,69 |
| Absolutely dry matter, % | | 12,52 | 13.57 | 18.07 | 19.31 |
| Nitrogen ,% | dry matter | 3.29 | 2.80 | 2.42 | 3.27 |
| | abs. dry matter | 3.65 | 3.03 | 2.59 | 3.52 |
| | natural forage | 0.46 | 0.41 | 0.47 | 0.68 |
| Crude protein , % | dry matter | 20.56 | 17.50 | 15.13 | 20.44 |
| | abs. dry matter | 22.84 | 18.96 | 16.18 | 22.02 |
| | natural forage | 2.86 | 2.57 | 2.92 | 4.25 |
| Crude fat ,% | dry matter | 3.51 | 3.01 | 3.19 | 4.40 |
| | abs. dry matter | 3.90 | 3.26 | 3.41 | 4.74 |
| | natural forage | 0.49 | 0.44 | 0.62 | 0.92 |
| Crude fibre , % | dry matter | 29.01 | 29.49 | 34.45 | 25.34 |
| | abs. dry matter | 32.22 | 31.95 | 36.85 | 27.31 |
| | natural forage | 4.04 | 4.33 | 6.66 | 5.27 |
| Acid Detergent Fibre, % | | 28,8 | 32.6 | 37.7 | - |
| Neutral Detergent Fibre, % | | 46,5 | 53.1 | 57.9 | - |
| Acid Detergent Lignin,% | | 3,9 | 5.0 | 6.3 | - |
| Ash ,% | dry matter | 7.98 | 7.17 | 7.02 | 9.70 |
| | abs. dry matter | 8.86 | 7.77 | 7.51 | 10.45 |
| | natural forage | 1.11 | 1.05 | 1.36 | 2.02 |
| NFE ,% | dry matter | 29.98 | 35.13 | 33.71 | 32.92 |
| | abs. dry matter | 32.18 | 38.06 | 36.05 | 35.48 |
| | natural forage | 4.03 | 5.16 | 6.52 | 6.85 |
| Dry matter digestibility | | 82.0 | 72.2 | 64.0 | - |
| Organic matter digestibility | | 77.9 | 69.9 | 58.2 | - |
| Relative Feed Value | | 133 | 112 | 97 | |

Digestibility is an important factor of the nutritive value of feed. Digestibility determines the relation between the content of nutrients and the energy that is available to ruminants. Thus, the organic matter digestibility (OMD) from the plants harvested in the budding period was 77.9%, a very high index, in the early flowering period – 69.9% and in the full flowering period this index decreased to 58.9%. The dry matter digestibility changed similarly: from 82.0% to 72.2% and 57.9%., according to the phenological stage.

The most widely accepted measure of the quality is Relative Feed Value. It was found that Relative Feed Value *Galega orientalis* decreased from 133 to 97.

The obtained data show that *Galega orientalis* needs to be collected in the early stages of development. In this period, it is more valuable as a fodder crop with a high level of crude protein and high digestibility of organic matter and, because of this, it can help solving some problems in the livestock sector by providing a balanced diet for animals with an appropriate amount of protein and fibre.

As for the organoleptic properties, the haylage prepared from *Galega orientalis* is green-brown leaves and yellowish-green stems; has a pleasant smell of fruits and pickled vegetables; the texture of the plants stored as haylage was preserved well, without mould and mucus (Photo 3). Thus, by the organoleptic characteristics, the haylage made from the non-traditional fodder plant *Galega orientalis* belongs to Class I, according to the Moldovan quality standards.

The data from table 2, indicate that the total moisture content in the freshly mowed green mass of *Galega orientalis* constituted 81.93%, in the green mass wilted for two days – 64.73%, in the prepared haylage – 61.53% and in hay – 12.75%. The content of absolutely dry matter constituted: in freshly mowed plants – 18.07%, in the wilted plants – 35.27%, in haylage – 47.81% and in hay – 87.25% respectively.

The dry matter of the freshly mowed plants for making haylage contained 2.59% of nitrogen,

Photo 3. Haylage of *Galega orientalis*

after two days of wilting – 2.62%, the haylage – 2.65% and the hay – 2.76%. The crude protein content was at almost the same level in the green mass, wilted mass, haylage and hay of *Galega orientalis*, and ranged from 16.18 to 17.23%. In the dry matter of freshly mowed plants, there was 3.41% crude fat, in the wilted plants 2.68%, in haylage 3.22% and only 1.49% - in the hay produced from the non-traditional fodder crop.

We assume that in the process of dehydration of the initial mass to 12.75% of moisture in hay, the fat content suffered considerable loss.

The crude fibre in the dry matter of fresh plants constituted 36.85%, in wilted plants – 37.85%, in haylage – 40.61% and in hay – 39.85%. The ash content in dry matter was of 7.51% in freshly mowed plants, 8.57% in wilted plants, 8.54 % in haylage and 7.41% in hay.

The nitrogen-free extract in freshly cut plants constituted 36.05% of the dry matter, in wilted plants – 34.51%, in haylage – 31.29% and in the hay of *Galega orientalis* – 34.03%.

Table 2. The content of nutrients in the freshly mowed and wilted plants, haylage and hay of *Galega orientalis*

| Indices | | Green mass | Wilted green mass | Haylage | Hay |
|---|---|---|---|---|---|
| Moisture content,% | initial | 80.67 | 62.7 | 58.23 | 8.22 |
| | hygroscopic | 6.51 | 5.45 | 7.90 | 4.94 |
| | totals | 81.93 | 64.73 | 61.53 | 12.75 |
| Abs. dry matter,% | | 18.07 | 35.27 | | 38.47 |
| Nitrogen ,% | dry matter | 2.42 | 2.48 | 2.44 | 2.62 |
| | abs. dry matter | 2.59 | 2.62 | 2.65 | 2.76 |
| | natural forage | 0.47 | 0.93 | 1.02 | 2.40 |
| Crude protein ,% | dry matter | 15.13 | 15.50 | 15.25 | 16.38 |
| | abs. dry matter | 16.18 | 16.39 | 16.56 | 17.23 |
| | natural forage | 2.92 | 5.78 | 6.37 | 15.03 |
| Crude fat ,% | dry matter | 3.19 | 2.53 | 3.43 | 1.42 |
| | abs. dry matter | 3.41 | 2.68 | 3.72 | 1.49 |
| | natural forage | 0.62 | 0.94 | 1.43 | 1.30 |
| Crude fibre ,% | dry matter | 34.45 | 35.79 | 37.40 | 37,88 |
| | abs. dry matter | 36.85 | 37.85 | 40.61 | 39.85 |
| | natural forage | 6.66 | 13.35 | 15.62 | 34.77 |
| Ash ,% | dry matter | 7.02 | 8.10 | 8.73 | 7.04 |
| | abs. dry matter | 7.51 | 8.57 | 9.48 | 7.41 |
| | natural forage | 1.36 | 3.02 | 3.65 | 6,46 |
| NFE ,% | dry matter | 33.71 | 32.63 | 27.29 | 32.35 |
| | abs. dry matter | 36.05 | 34.51 | 29.63 | 34.03 |
| | natural forage | 6.52 | 12.17 | 11.40 | 29.69 |
| nutritive units | natural forage | 0.16 | 0.29 | 0.26 | 0.53 |
| Carotene, mg/kg | | 39.99 | 33.8 | 24.0 | 30.12 |

Table 3. The content of organic acids in haylage of *Galega orientalis*

| | pH | Organic acids,% | | | | | | | | | Sum of lactic+ butyric + acetic | Correlation of lactic acids in % of the total | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Free | | | Fixed | | | Totals | | | | | | |
| | | acetic | butyric | lactic | acetic | butyric | lactic | acetic | butyric | lactic | | acetic | butyric | lactic |
| Haylage | 5.18 | 0.04 | 0 | 0.64 | 0.37 | 0 | 2.28 | 0.41 | 0 | 2.92 | 3.33 | 12.31 | 0 | 87.69 |

The fresh mass of *Galega orientalis* was characterized by a rather high content of carotene – 39.99 mg/kg, gradually, during the process of producing haylage, this index decreased to 33.8 mg in the wilted mass and 24.0 mg in the obtained haylage. In the hay made from *Galega orientalis*, the carotene content was higher in comparison with the haylage, reaching 30.12 mg/kg.

The pH of the haylage from *Galega orientalis* was 5.18 units and met the superior quality standards (Table 3).

The amount of organic acids accumulated during the process of lactic acid fermentation was 3.33%. It is significant that of all the organic acids, lactic acid in the haylage of *Galega orientalis* constituted 87.69%, mostly being accumulated in fixed form. Acetic acid was accumulated almost entirely in fixed form, butyric acid was not detected, which is indicative of the high quality of the obtained forage.

The green mass of *Galega orientalis* used for preparation of hay, leaves remain on the stem,

which helps ensure higher forage value. The content of nutrients in the absolutely dry matter of the hay prepared from *Galega orientalis* is characterized by: 87.25% of absolutely dry matter, 2.76% of nitrogen, 17.23% of crude protein, 7.41% of ash and 34.03% of NFE. One kg of hay contains 0.53-0.60 NU.

In conditions of semiarid continental east Croatia fodder galega hay had higher leaf portion (44.90% against 33.05% in lucerne), protein concentration (20.85% against 17.61% in lucerne hay) and concentration of Mg, K and P, whreas lucerne hay had higher fibre content (39.67% ADF and 45.21% NDF against 39.09% ADF and 43.98% NDF in fodder galega hay) and Ca concentration than fodder galega hay.

Relative feed value of fodder galega hay was a little higher than that of lucerne hay (124 against 119), and calcium/phosphorous ratio in fodder galega hay was more favorable than in lucerne hay (Stjepanović et. al. 2007).

## CONCLUSIONS

*Galega orientalis*, in the Republic of Moldova, is characterized by rapid growth and development rates, the budding stage starts 3 weeks earlier as compared with alfalfa, but the fodder harvested in this period is poorer in dry matter.

The nutritional value of the non-traditional crop *Galega orientalis* is almost at the same level as the traditional fodder crop – alfalfa. The dry matter of the harvested fodder contains 15-20% crude protein, 2.7-3.9% crude fat, 32.2-37.8% crude fibre, 7.3-8.8% ash and 30.6-39.5% NFE.

The haylage prepared from *Galega orientalis* is characterized by organoleptic and biochemical indices such as green-brown leaves and yellowish-green stems, pleasant smell of fruits and pickled vegetables, pH 5.18, lactic acid constituted 87.69%, butyric acid not detected.

For preparation of hay leaves remain on the stem which helps ensure higher forage value.

The non-traditional fodder plant *Galega orientalis* is fully suitable for preparing high quality feed, can be a good additional fodder to lucerne.

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# EVALUATING ISOFLAVONES ON CHOLESTEROL AND FAT DEPOSITION IN EGG YOLK DURING LAST FAZE OF EGG

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***Abstract***

*The average content of cholesterol per egg varied from 153.45 to263.90 mg and it varies depending on genotype and, mainly,on the diet. During past decades, there are a lot of experiments with different supplementation of the diet (mineral, probiotic, vegetable oil) of laying hens to decreased the level of the cholesterol in the egg yolk. This experiment was performed to evaluate dietary daidzein and genistein on cholesterol and fat deposition in egg yolk during late phase of egg production. A total of 80 ISA Brown Laying hens 63-wks old were randomly assigned to 4 treatment groups containing 20 hens each. Birds were fed commercial feed diet containing: 0 (BF), 1000 (BF +1000 mg SI), 2000 (BF +2000 mg SI) and 3000 (BF +3000 mg SI) supplemented isoflavones. Water was offered for ad libitum consumption throughout the experiment. Yolk cholesterol and yolk total fat was monitored during the three month period. The supplemented isoflavones in the feed decreased the content of yolk cholesterol during the 3-month feeding trial ($P<0.05$). The supplemented isoflavones has not any influence on the concentration of fat in the egg yolk and egg yolk mass. Therefore, supplementation of the commercial feed with isoflavones could be used as a tool for the reduction of the yolk cholesterol.*

***Key words****: isoflavones, cholesterol, fat, egg yolk.*

## INTRODUCTION

During past decades, the lipids composition (30–40%) of chicken egg has been an area of consumer concern, due to the relationship between specific dietary lipids and the development of coronary heart diseases, atherosclerosis, increasing stroke rate, high proportion of gallstones, enhancing depression rate and as a consequence is deleterious to human health and life expectancy (Imran M. et al., 2015). Vorlova et al., (2001) reported that theaverage content of cholesterol per egg was from 153.45 to 263.90 mg and it varies depending on genotype and, mainly,on the diet (Campo, 1995; Pesti and Bakalli, 1998). Recently, different dietary supplementations as probiotic strains (Mikulski etal., 2012; Abdelqader et al., 2013; Lei et al., 2013), vegetable oils (Faitarone et al., 2013) and fermented feed ingredient (Loh et al., 2009; Zhao et al., 2013) have been used to decreased the egg yolk cholesterol content. Soy isoflavones as functional phytoestrogenic products content in soybean preventing certain types of cancer (Adlercreutz, 1995), reducing the risk of osteoporosis (Adlercreutz, 1995), mineral regulation (Greendale et al., 2002) and also decreasing plasma cholesterol (Ho et al., 2000) in human population.

**The objective of this experiment** was to evaluate the influence of the isoflavones (dietary daidzein and genistein) on the egg cholesterol content and fat concentration in the yolk during late faze of egg production.

## MATERIALS AND METHODS

The experiment was performed with ISA Brown laying hens, 63 weeks old at the beginning of the experiment. The experimental laying hens were randomly assigned to 4 groups, 20 birds per group. The laying hens were housed in laying cages (2 birds per cage)

in a standard poultry house set to a 16L:8D cycle. The laying hens were fed 120 g basal feed per day (control group) and the same amount of the isoflavones supplemented feed per hen of the experimental groups. Water was offered for ad-libitum consumption throughout the experiment. The experiment was conducted under permitted ethical regulations and rules. The experiment was lasting three months.

Laying hens were randomly assigned to receive basal feed (without supplemented isoflavones), and 1000, 2000 and 3000 mg/kg supplemented isoflavones in feed. The experimental feed was supplemented with concentrated product, 408.8g isoflavones per kg product, produced by the North China Pharmaceutical Corporation. The isoflavone composition of the product is presented in Table 1.

Table 1.Composition of the isoflavonic product

| Isoflavone | g/kg |
|---|---|
| 1. Genistin | 73.0 |
| 2. Genistein | 12.6 |
| 3. Daidzin | 221.2 |
| 4. Daidzein | 17.4 |
| 5. Glycitin | 80.1 |
| 6. Glycitein | 4.5 |
| 7. Total | 408.8 |

The composition and nutritive value of the experimental diet is presented in Table 2.

Table 2.Composition and nutritive value of the experimental diet

| Ingredients, g/kg | Basal feed (BF) |
|---|---|
| Maize | 430.1 |
| Soybean meal, 44% protein | 144.3 |
| Sunflower meal, 33% protein | 153.0 |
| Wheat bran | 107.0 |
| Vegetable oil | 43.2 |
| Methionine, 99% | 0.40 |
| Calcium carbonate | 99.4 |
| Mono calcium phosphate | 7.6 |
| $NaHCO_3$ | 3.0 |
| Potassium carbonate | 0.9 |
| Zeolites | 3.0 |
| Salt | 1.9 |
| Vitamin and mineral mixture | 5.0 |
| Isoflavones, 40% | 0.0 |
| Total | 1000 |
| Chemical composition, calculated | |
| Dry matter, g/kg | 903.1 |
| Metabolic energy, Kcal/kg | 2750 |
| Crude protein, g/kg | 165.0 |
| Crude fat, g/kg | 65.2 |
| Calcium, g/kg | 40.0 |
| Phosphorus (available), g/kg | 3.0 |
| Lysine, g/kg | 7.4 |
| DL Methionine, g/kg | 3.6 |
| Methionine + cystine, g/kg | 6.1 |

The control group was blank and fed without SI (basal diet - BF) in the feed and the other 3 experimental groups was fed with SI in the feed in amount of 1000, 2000 and 3000 mg in kg feed.

Concentration of total fat and cholesterol was measured in the yolks produced from the experimental hens. Egg samples of 6 eggs per group, were collected at the beginning and at the end of 1$^{st}$, 2$^{nd}$, and 3$^{rd}$month. The eggs were measured, cracked, the yolks were separated and measured, then mixed, homogenized, stored frozen and analyzed up to 7 days.

The total cholesterol in the egg yolks was measured using the modified method according Washburn and Nix (1974) and Pearson et al. (1953). Briefly, total lipid was extracted by solution of chloroform and methanol 2:1 (v/v). Cholesterol determination wasdone using a commercial test kit for cholesterol analysis (BioSystems S.A., Barcelona, Spain) and analyzed spectrophotometrically at the wavelength of 625 nm.

The results were reported as means ±SEM.

The total fat was analyzed with extraction with diethyl ether according Soxhlet protocol.

Statistical analysis was performed by Statgraph 3 software package.

One-way analysis of variance (ANOVA) was used for the differences between groups. When the F values were significant, the Duncan's Multiple Range Test was performed.

## RESULTS AND DISCUSSIONS

From the obtain data reported in table 3, there can be clear noticed the reduction of cholesterol concentration per gram of yolk, from the 1$^{st}$ till the 3$^{rd}$ month of experiment in the groups fed with supplemented isoflavones in the feed.

The inclusion of 1000, 2000, and 3000 mg isoflavones per kg of feed, at the end of the experiment (3$^{rd}$ month) significantly affect the yolk cholesterol contents expressed as mg/g of yolk or as mg/yolk) ($P<0.05$).

There is reduction of the cholesterol in egg yolk in the experimental groups fed with diet supplemented with isoflavones (1000 mg, 2000 mg and 3000 mg).

Table 3. Content of cholesterol in egg yolk produced from the experimental hens

| | At the begining | 1st month | 2nd month | 3rd month |
|---|---|---|---|---|
| **Basal feed (BF)** | | | | |
| Yolk, g | 16.92±0.61 | 17.85±1.52 | 17.23±0.96 | 16.72±1.00 |
| mg/g yolk | 15.04±0.21 | 14.72±1.31a | 13.73±0.59a | 14.39±1.37a |
| mg/yolk | 254.44±2.63 | 262.75±2.38a | 236.57±3.88 | 240.60±3.68a |
| **BF +1000 mg SI/kg** | | | | |
| Yolk, g | 16.90±0.66 | 17.86±1.09 | 17.95±1.22 | 17.73±0.35 |
| mg/g yolk | 14.43±0.36 | 16.53±1.58a | 12.30±2.38a | 12.74±0.54b |
| mg/yolk | 243.87±2.05 | 295.29±3.11a | 220.85±4.25 | 225.83±3.51b |
| **BF +2000 mg SI/kg** | | | | |
| Yolk, g | 17.46±0.03 | 17.10±1.93 | 17.55±1.07 | 17.13±1.67 |
| mg/g yolk | 15.54±0.50 | 12.85±0.24b | 11.00±2.38b | 12.68±0.46b |
| mg/yolk | 271.40±2.75 | 219.68±4.02b | 193.10±4.17 | 217.26±3.88b |
| **BF +3000 mg SI/kg** | | | | |
| Yolk, g | 16.17±1.18 | 17.67±1.50 | 16.65±1.67 | 17.08±0.69 |
| mg/g yolk | 14.38±1.15 | 12.31±0.66b | 13.28±0.38ab | 11.00±0.23b |
| mg/yolk | 232.52±3.52 | 217.46±2.67b | 221.10±3.38 | 187.93±3.93b |

SI, supplemented isoflavonesValues are means ± S.D
a, b – values in the same column with no common superscript differ significantly (P<0.05).

The content of the total fat in 100 g egg yolk in the control group at the end of the experiment was 25.50 g, and in the experimental groups fed with different amount of supplemented diet were 28.50 g, 28.78 g and 27.95 g in group with 1000 mg, 2000 mg and 3000 mg supplemented isoflavones, respectively. The diet had no significant influence on total fat in the egg yolk (P>0.05). The obtain results are presented in Table 4.

Table 4.Content of total fat in egg yolk produced from experimental hens

| | At the begining | 1st month | 2nd month | 3rd month |
|---|---|---|---|---|
| **Basal feed (BF)** | | | | |
| Yolk, g | 16.92±0.61 | 17.85±1.52 | 17.23±0.96 | 16.72±1.00 |
| g/yolk | 4.95±0.08 | 5.25±0.13 | 4.26±0.07 | 4.26±0.12 |
| g/100g | 29.23±0.48 | 29.42±0.74 | 24.73±0.43 | 25.50±0.70 |
| **BF + 1000 mg SI/kg** | | | | |
| Yolk, g | 16.90±0.66 | 17.86±1.09 | 17.95±1.22 | 17.73±0.35 |
| g/yolk | 5.07±0.21 | 5.10±0.11 | 4.87±0.07 | 5.05±0.01 |
| g/100g | 30.00±1.23 | 28.55±0.59 | 27.16±0.39 | 28.50±0.04 |
| **BF + 2000 mg SI/kg** | | | | |
| Yolk, g | 17.46±0.03 | 17.10±1.93 | 17.55±1.07 | 17.13±1.67 |
| g/yolk | 5.22±0.01 | 5.15±0.06 | 5.09±0.03 | 4.93±0.07 |
| g/100g | 29.90±0.02 | 30.14±0.36 | 29.01±0.18 | 28.78±0.43 |
| **BF + 3000 mg SI/kg** | | | | |
| Yolk, g | 16.17±1.18 | 17.67±1.50 | 16.65±1.67 | 17.08±0.69 |
| g/yolk | 4.67±0.04 | 5.14±0.11 | 4.59±0.04 | 4.77±0.17 |
| g/100g | 28.88±0.28 | 29.11±0.60 | 27.54±0.25 | 27.95±0.97 |

SI, supplemented isoflavones; Data statistically insignificant (P>0.05)

The content of total fat in the egg yolk in the experimental groups has a similar trend as a content of total fat in the control group.

There are a few investigation conducted to investigate the effect of additional isoflavones on egg-yolk cholesterol.

The present results demonstrated that inclusion of isoflavones in different addition levels in layer diet decreased significantly the yolk cholesterol after the 3 months feeding period (P<0.05) (Figure 1).

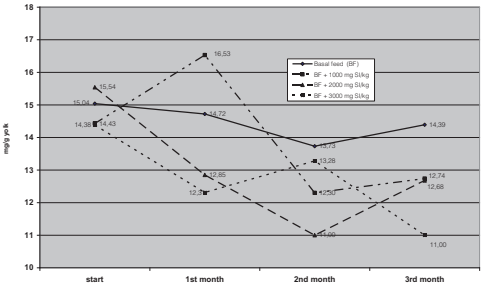


Figure 1. Changes in yolk cholesterol content of layers fed with supplemented isoflavones

However, there was no significant difference in cholesterol contents among the treatments with supplemented 1000, 2000 and 3000 mg $kg^{-1}$ isoflavones in basal feed. Egg cholesterol content (mg/g yolk) decreased by 23.56% in the group fed with the supplementation of 3000 mg/kg isoflavones in the diet. The decreasing of 19% was reported by Yin et al. (2004) in his study when the hens were fed with addition of 40 mg/kg daidzein in the diet. Also Fujiwara et al. (2008) reported the suppression of yolk cholesterol by adding a fermented soybean "Natto" supplement in the layer diet. Our finding of decreasing of yolk cholesterol are in agreement with these mentioned studies. Other study was conducted by Hong H. et al. (2010) with fermented soybean with *A. Oryzae* and with *B. subtilis var. Natto.* The results showed that egg cholesterol content in egg yolk was lower (p<0.05) than those in the control group.There were no significant differences in total fat in egg yolkin mentioned study. Kanpai et al. (2004) reported that administration of White KwaoKrua (*Pueraria mirifica*) in feed which containing potent phytoestrogen not influenced significantly in egg yolk cholesterol content among the experimental groups. Nasra et al. (2010) conducted experiment with fenugreek and licorice which are source of phytoestrogens, and the major findings in this study are that the yolk percent of the egg was decreased significantly by 0.5% fenugreek compared to control group. The significant differences between day 0 and day 3 (P<0.05) in the content of yolk cholesterol are also reported by Saitoh et al. (2001)in the

experiment conducted with diet contained high concentration of soy isoflavones, but no significant differences are noticed among days 0, 1, 6, 12 and 18 ($P>0.05$). Disagreement about the results may be due of different amount and source of isoflavones and different rearing periods of the animals.

## CONCLUSIONS

The results demonstrate that supplementation diet with isoflavones has a beneficial effect. The supplemented isoflavones in the diet reduced the content of yolk cholesterol during the 3-months feeding period ($P<0.05$).

There are no differences between the concentration of the total fat in the yolk of the control group and the fat concentration in the yolk of the experimental groups. The supplemented isoflavones in the diet has not affect the concentration of fat in the egg yolk.

Therefore, our findings suggest that supplemented diet with isoflavones for laying hens in late faze of egg production improve the egg quality to provide low-cholesterol eggs.

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# EFFECTS OF THE USE OF HIGH-PROTEIN RAPESEED FEED ON LAYING PRODUCTIVITY AND EGG QUALITY IN JAPANESE QUAILS

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***Abstract***

*The objective of this scientific paper is to establish the chemical composition and the nutritive value of a high-protein rapeseed feed produced in Bulgaria, and to study the possibilities for replacing soybean meal with this product in the diet of Japanese quails. Chemical composition of the tested product was determined, and the following results regarding its contents were obtained: crude protein – 42.50%, crude fibers – 8.50%, crude fats – 3.50%, lysine – 2.24%, methyonine – 0.85%, tryptophan – 0.51%, threonine – 1.81%, glucosinolates – 11.2 μmol/g. An experiment was performed with a total of 200 female Japanese quails from Pharaoh breed, 8 weeks old, randomly divided in four groups – a control and three experimental groups, 50 quails in each. The poultry from the control group received diet with soybean meal as the basic protein component, while in the experimental groups a part of the soybean meal was substituted with 5%, 10% and 15% of the tested product (for experimental groups I, II and III, respectively). All diets were equal by their nutritional value. During the experiment the feed consumption, laying capacity and health condition were monitored every day. Eggs morphological characteristics and their taste were controlled periodical. In experimental groups I and II, where 5% and 10%, respectively, of high-protein rapeseed product was included in the quail's diets, laying capacity and laying intensity were significantly higher than in the control group ($P<0.05$). The addition of 5%, 10% or 15% of the tested product didn't have negative influence on the egg weight, the eggshell strength, the yolk or the albumen colour, or the boiled eggs' taste and smell. The examined diets with inclusion of 5%, 10% and 15% of high-protein rapeseed product, according to the results obtained in this experiment, are recommendable for use in the practice.*

***Key words:*** *rapeseed product, quail, laying productivity, egg quality.*

## INTRODUCTION

The protein problem on a world scale demands new sources of protein to be looked for. Many countries in Europe and Asia, as well as Canada, increased their production of protein, by extending the areas where various cultures are grown – beans, canola and other, in order to reduce the dependence of their animal husbandry on the import of expensive protein feed, like soybean meal (Naseem et al., 2006; Wickramasuriya et al., 2015). Around 90% of the byproducts obtained in the processing of rapeseed into oil, appertain to the rapeseed extraction meal (REM). The industrial extraction of oil generates rapeseed meal as a byproduct, which can be acquired by producers at a low price (Moraes et al., 2015). In some countries like Germany, Poland, the Czech Republic and others, REM is in the second place as a protein component in compound feeds, only behind the soybean meal. Rapeseed meal is a good source of protein with high contents of amino acids lysine, methyonine, cysteine, tryptophan and threonine (Min et al., 2009). By the contents of sulphur-containing amino acids and threonine, rapeseed meal excels soybean meal. In the inclusion of rapeseed extraction meal in compound feeds, especially those intended for poultry and pigs, its following properties must be taken into account:

– REM has lower content of crude protein (36%) compared to soybean meal (Wickramasuriya et al., 2015);
– The content of crude fibers in REM is two times higher (12%) than in soybean meal (Jia et al., 2012);
– REM contains antinutritive factors (glucosinolates, sinapine, pentosanes, erucic acid, cellulose, chemicellulose), which are the cause for reducing the nutrients' digestibility and the

metabolizable energy level (Bell**,** 1993; Dale, 1996; Khajali and Slominski, 2012).

In the past few decades, the scientific and research work of selectors, technologists and nutrition experts has been oriented towards improvement of the nutritive value of rapeseed and its products (Slominski et al. 2011). Via selection, a reduction of glucosinolate content has been achieved, from 80–120 μmol/g to 5–25 μmol/g (the so-called 00 sorts have been created, with low contents of erucic acid and glucosinolates). The goal of rapeseed's technological processing is to partially or completely remove the husk which contains a large amount of antinutritive factors, and this way to improve the nutritive value of rapeseed feeds.

The purpose of this study was to characterize the chemical composition and nutritional value of the high protein rapeseed feed produced in Bulgaria based on rapeseed meal; to establish the possibilities of replacing soybean meal with this product in compound feed for laying Japanese quails (*Coturnix coturnix japonica*);to investigate the influence of three different levels (5%, 10% and 15%) of the product on laying productivity and egg quality in order to determine its optimal dose for the practice.

## MATERIALS AND METHODS

The subject of the current research is examination of the high-protein rapeseed feed *Rapro,* manufactured according to the technology worked out by the scientific-research and production team of the company Bonmix EOOD, from Lovech, Bulgaria, a product registered in Europe and Canada.

This technology employs deagglomerative processes for decomposition of meal's agglomerates. For this purpose machines are used that prevent the husk's cellulose layer to be torn into small pieces.

This way a mixture of cellulose, protein and cellulose-protein particles is obtained. Then, these particles are separated by achieving granulometric dimensions suitable for rough sorting and their classification according to the parameters absolute weight and mass density.

The following properties of the tested product *Rapro* were determined: total chemical composition (according to the conventional Weende analysis); the contents of Ca and P (according to AOAC, 2007); the contents of amino acids lysine, methyonine, cysteine, threonine, tryptophan (by amino analyzer *Perkin-Elmer*); the content of linoleic acid (by gas chromatograph); the content of glucosinolates (by spectrophotometer).

In Table 1 are shown the chemical compositions of *Rapro*, rapeseed meal and soybean meal.

Table 1 Chemical composition of *Rapro*, Rapeseed meal and soybean meal

| Items | *Rapro* | Rapeseed meal | Soybean meal |
|---|---|---|---|
| Moisture,% | 12 | 11 | 11 |
| Metabolizableenergy, hens, kcal/kg | 1810 | 1794 | 2200 |
| Crude protein, % | 42.5 | 36.0 | 44.0 |
| Crude fiber,% | 8.50 | 12.8 | 6.30 |
| Crude fat,% | 3.50 | 2.50 | 1.50 |
| Lysine, % | 2.24 | 2.12 | 2.75 |
| Methionine,% | 0.85 | 0.72 | 0.62 |
| Methionine +Cysteine,% | 1.88 | 1.58 | 1.25 |
| Threonine,% | 1.81 | 1.55 | 1.73 |
| Tryptophan,% | 0.51 | 0.44 | 0.60 |
| Crude Ash,% | 6.50 | 6.80 | 6.30 |
| Calcium,% | 0.63 | 0.66 | 0.20 |
| Phosphorus, total, % | 1.00 | 0.92 | 0.65 |
| Phosphorus, available,% | 0.17 | 0.27 | 0.24 |
| Linoleic acid,% | 1.60 | 0.80 | 0.70 |
| Nitrogen free extracts,% | 27.0 | 30.9 | 28.5 |

The present study was conducted in the period October–December 2014 in the quail farm of the company Gary–2 Ltd., Etropole, Bulgaria. An experiment was carried out with a total of 200 female Japanese quails (56 days old) from Pharaoh breed. The poultry were randomly divided into four groups – one control and three experimental groups, 50 quails in each group. The quails from all the groups were housed on the second floor of a five-floor cell battery, on a 16 hours lighting schedule, at 21–24°C air temperature and 70–85% relative humidity. Water was supplied via nipple drinkers. The trial lasted 76 days, 16 days - a preparatory period, and 60 days- an experimental period. In Table 2 are presented ingredient and chemical composition of the compound feed for each group. The diet for the control group didn't contain the product *Rapro*, its basic protein component being the soybean meal. In the compound feeds for the experimental groups a part of the soya meal was substituted with 5%, 10% and 15% of Rapro (for I, II and III experimental groups, respectively). The diets for all the groups were equal by their

nutritional composition and adjusted to the specific needs of laying Japanese quails.

Table 2 Ingredient and chemical composition of compound feeds for laying Japanese quails from control and experimental groups

| Groups / Components, % | CG | I EG | II EG | III EG |
|---|---|---|---|---|
| Maize | 30.844 | 44.795 | 43.165 | 42.223 |
| Wheat | 27.831 | 12.282 | 13.000 | 13.000 |
| Soybean meal, 44% | 31.129 | 27.724 | 23.215 | 18.754 |
| Rapro, 42% | - | 5.000 | 10.000 | 15.000 |
| Sunflower oil | 1.000 | 1.000 | 1.489 | 1.956 |
| L- lysine | 0.086 | 0.110 | 0.138 | 0.165 |
| DL-methionine | 0.167 | 0.148 | 0.114 | 0.081 |
| Limestone | 7.570 | 7.483 | 7.432 | 7.380 |
| Monocalcium phosphate | 0.742 | 0.822 | 0.814 | 0.810 |
| Vitamin premix 15C for layers | 0.200 | 0.200 | 0.200 | 0.200 |
| Choline chloride | 0.050 | 0.050 | 0.050 | 0.050 |
| Salt | 0.260 | 0.265 | 0.262 | 0.260 |
| Bonzyme W-P | 0.100 | 0.100 | 0.100 | 0.100 |
| Ronozyme P 5000 | 0.009 | 0.009 | 0.009 | 0.009 |
| Oxigard | 0.012 | 0.012 | 0.012 | 0.012 |
| **Total:** | **100** | **100** | **100** | **100** |
| **Chemical composition** | | | | |
| Metabolizable energy,kcal/kg | 2800 | 2800 | 2800 | 2800 |
| Crude protein, % | 20.30 | 20.30 | 20.30 | 20.30 |
| Lysine, % | 1.150 | 1.150 | 1.150 | 1.150 |
| Methionine + Cysteine, % | 0.780 | 0.780 | 0.780 | 0.780 |
| Tryptophan, % | 0.239 | 0.229 | 0.228 | 0.226 |
| Threonine, % | 0.688 | 0.708 | 0.717 | 0.727 |
| Calcium, % | 3.220 | 3.220 | 3.220 | 3.220 |
| Phosphorus, available, % | 0.410 | 0.410 | 0.410 | 0.410 |

CG –control group
EG – experimental group

During the experiment, on daily basis, the following parameters were controlled: laying capacity (in egg number /group), laying intensity (in percent/group), and the mortality (in quail number).

Also, the feed intake and feed conversion were calculated for each group.

At the beginning, in the middle and at the end of the experiment, 20 eggs from each group were submitted to the following measurements: egg's weight and eggshell's weight (using an electronic scale OHAUS 2000 with the tolerance of 0.01 g); eggshell thickness (in millimeters) without the shell membrane (using a micrometer Amer 25 EE); shape index (calculated according to the formula: Small diameter / Large diameter × 100); yolk colour (according to the 15-grade Roche scale). Albumen colour was determined visually, as well as the presence of blood stains or others not typical inclusions in the yolk and in the albumen.

Twenty boiled eggs from each dietary group laid at the end of the experiment were evaluated organoleptically by a panel of 10 semi-trained judges on an eight point hedonic scale in terms of colour, flavor, taste, smell, odor, texture and overall acceptability. The taste and the smell of the boiled eggs were evaluated while the eggs were still warm.

The results are presented as mean values with their standard errors. Statistical analysis of *Rapro'*s effects on the egg production and eggs' morphological characteristics was performed using Excel 200, Single factor, Anova program.

## RESULTS AND DISCUSSIONS

In Table 1 are shown the results of the chemical analysis of the tested high-protein rapeseed product *Rapro*. The table also contains data on the chemical composition of both rapeseed and soybean meal, thus giving the opportunity for making comparative analysis of all the three nutrients. The metabolizable energy is the highest in the soybean meal (2200 kcal/kg), the lowest in the rapeseed meal (1794 kcal/kg), while *Rapro*'s metabolizable energy is 1,810 kcal/kg. The content of crude protein in *Rapro* is higher (42.50%) than in rapeseed meal (36%), being very close to the level of crude protein in soybean meal (44%). Rapeseed meal has higher content of crude fibers (12.80%) than the soybean meal (6.30%), while as a result of the employed technical processing, the content of crude fibers in *Rapro* is reduced to 8.50%. Rapeseed meal and *Rapro* have lower contents of the amino acid lysine (2.12% and 2.24%, respectively), at the same time having higher contents of the sulphur-containing amino acids (methyonine and cysteine) than the soybean meal which contains 2.75% lysine and 1.25% methyonine + cysteine. *Rapro* has two times higher content of linoleic acid (1.60%) than rapeseed (0.80%) and soybean meal (0.70%). The content of glucosinolates in *Rapro* is 11.20 μmol/g.

In Table 3 are presented the laying capacity (in egg number/week/group), for the period between 11 and 18 weeks' age of the quails; the laying intensity (in percent). The highest amount of eggs (287) was obtained in experimental group II where *Rapro*'s share in

the feed was 10%, and the lowest amount (249) in experimental III group where *Rapro*'s share was 15%, the difference between the latter group and the control group being statistically insignificant (P>0.05).

Table 3 Weekly egg intensity and egg capacity of quail layers from control and experimental groups

| Groups / Age,week | CG | I EG | II EG | III EG |
|---|---|---|---|---|
| **Laying capacity, eggs'number/week** | | | | |
| 11 | 266 | 272 | 281 | 256 |
| 12 | 259 | 260 | 288 | 257 |
| 13 | 256 | 281 | 293 | 259 |
| 14 | 270 | 274 | 302 | 252 |
| 15 | 261 | 279 | 291 | 252 |
| 16 | 255 | 279 | 285 | 235 |
| 17 | 263 | 277 | 282 | 238 |
| 18 | 235 | 286 | 277 | 246 |
| Average | 258 ± 10.59 a. b | 276 ± 7.75 a | 287 ± 7.95 b | 249 ± 8.91 |
| **Laying intensity, %/week** | | | | |
| 11 | 76.0 | 77.7 | 80.3 | 74.6 |
| 12 | 74.0 | 74.3 | 83.9 | 74.9 |
| 13 | 73.1 | 80.3 | 87.2 | 75.5 |
| 14 | 78.7 | 78.3 | 86.3 | 73.5 |
| 15 | 76.1 | 79.7 | 87.5 | 73.5 |
| 16 | 74.3 | 79.7 | 86.6 | 68.5 |
| 17 | 78.3 | 79.1 | 85.7 | 70.8 |
| 18 | 69.9 | 81.7 | 86.0 | 73.2 |
| Average | 75.1 ± 2.88 a. b | 78.9 ± 2.21a | 85.4 ± 2.35 b | 73.1 ± 2.33 |

a, b – values in the same row with no common superscript differ significantly (P<0.05).

Similar results were obtained regarding laying intensity. The poultry receiving diet with 5% and 10% *Rapro* havesignificantly higher laying intensity (P<0.05) than the control group. The laying intensity of the group receiving 15% *Rapro* in the diet is the lowest (73.10%), but the difference between this value and that of the control group is statistically insignificant (P>0.05).

During the whole experimental period, the poultry from all the groups consumed the diets willingly. The results about feed intake, feed conversion and mortality of the quails from control and experimental groups are presented in Table 4. The daily feed intake per quail was 29.60 g, 30.50 g, 30.40 g and 29.20 g (for the control group, I, II and III experimental groups, respectively), which proves that all the diets used in this experiment had equally good taste. The feed conversion was the best in the group receiving 10% of *Rapro* in the diet (experimental group II), where 37.10 g of forage were spent for obtaining one egg. The next best result (38.60 g) was achieved in experimental group I, receiving 5% of *Rapro*. In the control group and the group with 15% content of *Rapro* in the diet (experimental group III) these values are much closer (40.26 and 41.10, respectively). In the whole period of the experiment the poultry were in good health, very lively, and with good looks and feathering. The number of dead poultry during the whole experiment was 2 for control group, 0 for I experimental group, 2 for II experimental group and 2 for III experimental group. The analysis of the results obtained justifies the dosage of 10% of *Rapro* in the diets to be designated as the optimal one. These results are similar with the results obtained by other researchers who made experiments with rapeseed meal in laying hens (Sommers et al., 1988) and in Japanese quails (Elangovan et al., 2001; Hameed et al., 2002).

Table 4. Feed intake and mortality of the quails from control and experimental groups

| Groups / Items | CG | I EG | II EG | III EG |
|---|---|---|---|---|
| Total eggs number | 2203 | 2368 | 2454 | 2131 |
| Feed intake during the whole experimental period, kg | 88,700 | 91,500 | 91,200 | 87,500 |
| Feed intake/quail /day, g | 29.60 | 30.50 | 30.40 | 29.20 |
| Feed intake per one egg, g | 40.26 | 38.6 | 37.1 | 41.1 |
| Mortality, quails' number | 2 | 0 | 2 | 2 |

Data about eggs' morphological characteristics are given in Table 5. Non significant difference was found between the groups regarding eggs' weight at the beginning, in the middle and at the end of the experiment. On the average, in all the groups, it is a higher in the end of the experiment, which is a normal occurrence related with the advance of quails' age. For the eggs' quality of big importance is the eggshell thickness. The percentage of *Rapro* used in the experimental diets had no influence on this parameter which was 0.192 mm at the beginning, 0.197 mm in the middle and 0.201 mm at the end of the experiment, in all the experimental groups). The ratio eggshell mass/egg mass, which is an indicator of the eggshell's strength, was 9.70% at the beginning, 10.25% in the middle, and 10.10% at the end of the experiment, taken on average

for all the groups, and no problems regarding eggshell's strength were noticed.

Table 5 Morphological characteristics and organoleptic properties of the quails' eggs from control and experimental groups (X±SE)

| Items \ Groups | CG | I EG | II EG | III EG |
|---|---|---|---|---|
| **At the beginning of experiment** | | | | |
| Egg weight, g | 11.19±0.27 | 11.30±0.17 | 11.51±0.38 | 11.16±0.19 |
| Eggshell weight, g | 1.12±0.03 | 1.07±0.02 | 1.11±0.03 | 1.10±0.03 |
| Eggshell weight in % of the egg weight | 10.00±0.78 | 9.47±0.69 | 9.64±0.72 | 9.68±0.76 |
| Eggshell thickness, mm | 0.193±0.003 | 0.197±0.001 | 0.195±0.003 | 0.187±0.003 |
| Shape index, % | 76.93±1.02 | 79.28±0.68 | 79.69±1.18 | 78.20±0.83 |
| Yolk colour, Roche's scale | 1.73±0.18 | 1.67±0.16 | 2.13±0.29 | 1.53±0.17 |
| Taste and smell of boiled eggs | Normal Typical | Normal Typical | Normal Typical | Normal Typical |
| **In the middle of experiment** | | | | |
| Egg weight, g | 11.04±0.27 | 11.27±0.25 | 11.10±0.30 | 11.27±0.36 |
| Eggshell weight, g | 1.11±0.03 | 1.14±0.02 | 1.17±0.03 | 1.16±0.04 |
| Eggshell weight in % of the egg weight | 10.10±0.73 | 10.10±0.70 | 10.50±0.74 | 10.30±0.79 |
| Eggshell thickness, mm | 0.197±0.003 | 0.199±0.003 | 0.195±0.003 | 0.196±0.003 |
| Shape index, % | 77.61±0.73 | 78.99±0.88 | 78.77±0.97 | 76.79±0.90 |
| Yolk colour, Roche 's scale | 3.73±0.18 | 3.20±0.30 | 3.80±0.26 | 3.66±0.19 |
| Taste and smell of boiled eggs | Normal Typical | Normal Typical | Normal Typical | Normal Typical |
| **At the end of experiment** | | | | |
| Egg weight, g | 11.60±0.27 | 11.77±0.26 | 11.89±0.28 | 11.31±0.23 |
| Eggshell weight, g | 1.20±0.04 | 1.17±0.03 | 1.17±0.03 | 1.17±0.05 |
| Eggshell weight in % of the egg weight | 10.30±0.74 | 9.94±0.72 | 9.84±0.76 | 10.30±0.79 |
| Eggshell thickness, mm | 0.206±0.003 | 0.199±0.003 | 0.200±0.004 | 0.200±0.004 |
| Shape index, % | 75.71±2.47 | 78.31±0.69 | 77.73±0.65 | 77.54±0.69 |
| Yolk colour, Roche 's scale | 2.87±0.32 | 3.40±0.50 | 3.72±0.32 | 3.47±0.32 |
| Taste and smell of boiled eggs | Normal Typical | Normal Typical | Normal Typical | Normal Typical |

Similar results were reported by Moraes et al. (2015) who investigated the influence of different amounts of rapeseed meal in the diet of laying Japanese quails on morphological characteristics of eggs. The amount of *Rapro* in the feed mixtures had no negative influence on yolk and albumen colour. The eggs from all the groups did not have any blood stains and other not typical inclusions. In the degustation of the boiled eggs no unspecific, unpleasant or side smell or taste were found, in any of the groups.

## CONCLUSIONS

The high-protein product *Rapro*, tested in this study, contains 1,810 kcal/kg metabolizable energy, 42.50% CP, 8.50% crude fibers, 3.50% crude fats, 2.24% lysine, 1.88% methyonine + cysteine, 1.81% threonine, 0.51% tryptophan, 1.60% linoleic acid, 11.20 μmol/g glucosinolates. As a result of the technological processing employed, the content of crude fibers in Rapro was reduced whereas the contents of ME and CP were increased compared with those in the initial rapeseed meal, which way Rapro's digestibility and nutritive value were improved.

In the cases where Rapro's share in the diet given to laying Japanese quails was 5% or 10%, the laying intensity was significantly higher ($P<0.05$) in relation to the control group. In the group receiving 15% of *Rapro* in the diet, the laying intensity was close to that in the control group.

In all the experimental groups (i.e. regardless whether Rapro's share in the compound feeds was 5%, 10% or 15%), feed intake and feed conversion were very close to those in the control group.

The inclusion of Raproin the diets for Japanese quails in the dosage up to 15% has no negative influence on egg weight, eggshell strength, shape index, yolk colour, and boiled eggs' smell and taste. The tested product *Rapro* can partially replace soybean meal in the compound feeds for laying Japanese quails. The optimum dosage of *Rapro* is 10%, and the maximum is 15%. So, the examined diets for Japanese quails with the inclusion of 5%, 10% or 15% of *Rapro*, are recommendable for use in the practice.

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# DETERMINATION OF CHEMICAL CONTENT AND DRY MATTER DIGESTIBILITY OF SOME UNDER- UTILIZED FEEDS IN RUMINANTS FEEDING THROUGH TWO *IN VITRO* METHODS

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***Abstract***

*Chemical analyses, enzymatic and Tilley and Terry digestibility were used to describe the feeding values of thirteen feed samples. Two samples were from common used feeds in ruminant animals feeding: alfalfa hay of two successive cuts and nine other feeds representing under-utilized ones in ruminant feeding like as cereal straw, faba bean straw and pea straw collected at the end of vegetation. All samples were analyzed for their chemical content of dry matter (DM), crude protein (CP), ash, crude fat, neutral detergent fiber (NDF), acid detergent fiber (ADF), acid detergent lignin (ADL), acid detergent insoluble nitrogen (ADIN) and neutral detergent insoluble nitrogen (NDIN) and enzymatic digestibility of dry matter. The under-utilized feed, pea straws and faba pods have similar chemical content for main Weende parameters. They have higher values for CP that cereal straws (31-34%) and lower value of CF (11.6%). The NDF and ADF content of pea straw and faba pod resulted lower that in cereal straw respectively 24% and 14%. The dry matter digestibility (DMD) determined with enzymatic and Tilley and Terry methods resulted to be higher in pea straw and faba pod in comparisons with that of cereal straw respectively 20% and 18%. The dry matter digestibility values determined with Tilley and Terry method for all feeds included in the study resulted higher than DMD determined with enzymatic method. The results of DMD determined by two "in vitro" methods were strongly correlated. According to $R^2$-value (0.99) the DMD determined by Tilley and Terry method could be predicted from enzymatic test as most convenient since it does not need animals.*

***Key words****: under-utilized feeds; leguminous straw; chemical content; in vitro digestibility; ruminant feeding*.

## INTRODUCTION

Among all feed resources that are frequently used in ruminant nutrition some of them are under-utilized like as cereals straw and leguminous straw due to lack of information on their chemical composition and nutritive value. Cereals straws are partly used during the scarcity of feeds and most of their production is burned on fields causing environmental pollution. The leguminous straws are rarely used in ruminant feeding. Leguminous grains like as pea bean and faba bean, are well used in human diets and recently there is an emphasizing of their increasing in animal nutrition as a healthy vegetable protein resources. Under severe shortage of hay, straw can become valuable low-cost forage that can be used effectively, especially in extensive ruminant production systems based on low inputs (López et al., 2005). To provide balanced diets that include straw, it is important to know the nutritive value of this roughage and its variability, as straw sources vary in their nutrient content and digestibility. Cereal straws have been characterized for their chemical content and "*in vivo*" digestibility in other previous studies in Albania like as (Papa et al., 2000). There are data available for nutritive value of cereal straw and recommendations for their uses in ruminants feeding. There are not data about chemical characterization and digestibility of leguminous straw. Component chemical analyses can provide important biochemical information leading to a better understanding of the factors that may limit the animal performances. Chemical characterization methods cannot give a direct estimate of nutritive value, but rather relay on statistical association to measure digestibility and intake (Cherney, 2000). Different methods are used to describe the digestibility of forages and roughages. The attempts to develop enzymatic assays are due to the undesirability cost that has

keeping rumen fistulae animals for experimental intentions. Variations in the cellulase method have been studied, mainly focusing on enzyme concentration and incubation time (Jones and Hayward, 1975, Aufrere, 1982, Aufrère, Baumont et al., 2007, Nousiainen, Rinne et al., 2003). Although the predicted *in vivo* digestibility derived from this methods may be less accurate than that from the Tilley and Terry method (Tilley and Terry, 1963), they are simpler, less time consuming, more convenient and reproducible and don't require fistulated animals.

The aim of the study is to provide scientific information and characterization of locally available feed resources that are often under-utilized due to lack of information on their chemical composition and nutritive value. Providing knowledge on feed resources, including unconventional and lesser known ones, we hope to contribute the development and use of innovative and appropriate feeding options and strategies.

## MATERIALS AND METHODS

### Samples

Thirteen feed samples were used in this study. Two samples were from common used feeds in ruminant animals feeding: alfalfa hay of two successive cuts and nine other feeds representing under-utilized ones in ruminant feeding like as cereal straw, faba bean straw and pea straw collected at the end of vegetation. All these representative samples had an identification number and have been brought from Albania to Animal Science Laboratory of Abel Salazar Biomedical Sciences Institute (ICBAS), University of Porto, Portugal, in vacuum small bags, previously air-dried and ground in a sieve 4 mm. Thereafter, they have been milled again at ICBAS Institute in a sieve 1 mm as rapid as possible, to avoid their exposure to the atmosphere, set in small container labelled with an identification code and type of material and left in the room temperature for their evaluation according to the procedure for its analysis.

### Chemical analysis

All feed samples were analyzed for their chemical parameters according Weende Proximate Analysis (AOAC 2000) for dry matter (DM), ash, crude fiber (CF), crude protein (CP), ether extract (EE). DM was determined after drying at 105°C, and ash after combustion at 550°C (Regulation No. 497/2004, 2004). Crude fat was extracted for 6 h with petroleum ether, whereas the Kjeldahl method was used to determine nitrogen (N) (AOAC, 1990). CP was calculated as N × 6.25. Van Soest detergent system (Van Soest and Robertson, 1985; AOAC, 2000) was used to determine neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL). Hemicellulose was calculated as NDF – ADF and cellulose as ADF – ADL (Rinne et al., 1997a). Acid detergent insoluble nitrogen (ADIN) and neutral detergent insoluble nitrogen (NDIN) were determined according (Van Soest, Robertson et al. 1991).

### Digestibility triels

Two "*in vitro*" methods were used to determine the DMD of selected feeds: enzymatic (celulase and pepsin) and Tilley and Terry method.

### Tilley and Terry method

The two stage Tilley and Terry (1963) procedure modified by Van Soest, Wine et al. (1966) was used to determine the digestibility of feeds. Briefly, rumen fluid obtained from two fistulated non-pregnant and non-lactating Holstein cows after a two weeks adaptation period to the diet with continuous access to fresh drinking water, was diluted anaerobically with four parts of (Marten and Barnes 1980) buffer solution, under $O_2$-free $CO_2$ and dispensed anaerobically into 50 mL conical centrifuge tubes (Corning Inc., New York, NY, USA) containing 250 mg DM of each sample, ground at 1 mm screen and incubated in a water-bath at 39◦C. Incubations were stopped after 48 h. Blanks and samples were incubated in duplicate per inoculum and per incubation, incubations being replicated in two separate runs, resulting in eight replicates for each feedstuff. The calculations for DM digestibility (DMD), were made based on the weight of the dry residue at 103ºC.

### Pepsin-cellulase procedure

The feedstuffs were analyzed for DDM according to the pepsin-cellulase method (Aufrère, Baumont et al., 2007). The enzymatic digestion was carried out by adding fifty millilitres pre-heated solution of 2% pepsin-HCl solution (Pepsin 1:10.000, Biotechnology,

VWR AMRESCO, LLC, Fountain Parkway) in a 50 mL capped conical centrifuge tubes (Corning Inc., New York, NY, USA) with 300 mg DM of each sample (ground to pass a 1 mm screen and incubated in a water bath at 39ºC for 24 h, shaking some time during 24 h. After 24 h incubation, the tubes were transferred into a water bath at 80°C for an acid hydrolysis for 30 min. The residue was then washed with 300 mL hot distilled water at 40°C and vacuum filtered in a Dosi-Fiber equipment (JP Selecta S.A., Spain) to glass crucibles that were then incubated for another 24 h in a water bath at 39°C with 50 mL pre-heated cellulase-buffer from *Trichoderma viride* (Onozuka R-10, Yakult Pharmaceutical, Japan). At the end of second incubation the residue was washed again with 300 mL hot distilled water and vacuum filtrated in the Dosi-Fiber, dried for 48 h in an air-forced ventilated oven at 103°C, cooled to room temperature and weighed. Blanks and samples were incubated in triplicate. The DMD was estimated from the weight of the dry residue.

**Statistical analysis**

The data of dry matter digestibility measured through two in vitro methods and ADF and ADL content were elaborated according to linear regress analyses with least square method. MINITAB software was used to perform the t- test on differences between DMD-TT and DMD-PC and for assessing regression between TT and PC data to develop prediction equations for Tilley and Terry DMD.

## RESULTS AND DISCUSSIONS

Chemical composition of the investigated feedstuffs for different group of feeds is presented in Table 1. The average values of chemical content of analyzed feeds of similar group are presented in the Figure 1. The aim of the study was to evaluate the feeding value of under-utilized feed like as legume straw and cereals straw. Alfalfa hay is included just to make comparisons with under-utilized and not well known feeds. The under-utilized feed, pea straw and faba pod have similar chemical content for main Weende parameters. They have higher values for CP that cereal straw (31-34%) and lower value of CF (11.6%). The NDF and ADF content of pea straw and faba pod resulted lower that in cereal straw respectively 24% and 14%. Makkar et al., 1996 found that chemical composition of chickpea straw differs from typical cereal straw in that it general contains more protein and metabolize energy concentrations and lower neutral detergent fiber (NDF) contents that cereal straw. Higher values of lignin content in both peas straw and faba pods and their differences with other feeds especially with alfalfa hay was expected based on their structure. According to Aufree et al. (1996) legumes are less rich in cell wall material, and composition and structure of their lignin are different and resulting in lower effect on cell wall digestibility. There are not significant differences in insoluble lignin between different varieties of pea straw and faba pod. The higher values were found in Belshi VF (Albania) faba pod with 11.6% followed by pea straw Belshi (Albania) with 10.7%. These values for lignin content are comparable with the one found in official feed tables of Feedipedia.

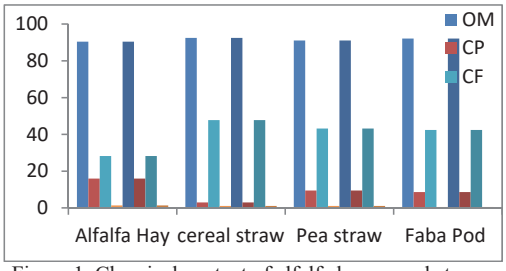


Figure 1. Chemical content of alfalfa hay, cereal straw, pea straw and faba pod (% of DM)

The dry mater digestibility data (DMD) with two "*in vitro*" methods are presented in Table 2. The average values of DMD determined by both in vitro methods of group of feeds showed that pea straw and faba pod had higher DMD than cereal straw, but lower than alfalfa hay. There were differences between DMD determined with two in vitro methods ($p \leq 0.05$). For pea straw and faba pod there were small differences in digestibility prediction by the two methods used, indicating good correlation between the linear function and the experimental values. The pepsin-cellulas method produced consistently lower DMD contents than Tilley and Terry method as more similar to in vivo digestibility. Similar results have been noticed by (Papa et al., 2011) where

pepsin-cellulas method produced lower values of DMD for straws than gas-test method. Within-species variability was noticed also in pea straws and faba pods. The pea straw of Belshi (Albania) varieties showed the highest digested DM than other varieties in both in vitro methods (50.8 and 65.65%). The faba straw of Aguadulce (Italy) varieties showed also the highest DMD (43.4 and 70.32%). The DMD of Aguadulce faba pod was close to DMD of alfalfa hay of second cut (70.46%). Similar results have been reported by (Haddad et al., 2001) where the nutritive value of lentil straw was close to nutritive value of alfalfa hay. The regress linear model used to describe the relations between two in vitro methods is shown in Figure 2.

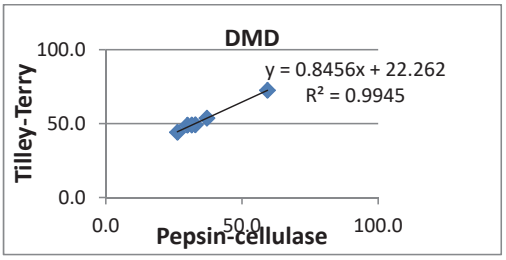


Figure 2. Linear regression between pepsin-celulas and Tilley and Terry method

According to $R^2$-value (0.99) for p≤0.001 the DMD determined by Tilley and Terry method could be predicted from enzymatic test as most convenient since it does not need animals. $R^2$=0.99 (p≤0.001).

Chemical components ADF and ADL are very good predictors of Tilley and Terry dry matter digestibility.

The multiply regression equation gives a reliable prediction judging from $R^2$-value = 0.97 and residual mean square error = 2.006.

**Y=96.045-1.241ADF+2.395ADL**

In accordance with the present study, Nousiainen et al., 2003; Huhtanen et al., 2006b, found ADL as the best single predictor of *in vivo* organic matter digestibility for grass silages ($R^2$ = 0.62) and for a set of forage samples (grasses, legumes and whole crops) ($R^2$ = 0.43), respectively.

Table 1. Chemical content of feeds

| Feeds | Varieties | DM | Ash | OM | CP | CF | EE | NDF | ADF | ADL | NDIN | ADIN |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Alfalfa Hay | Second cut | **91.8** | **10.2** | **89.8** | **12.2** | **29.6** | **1.2** | **47.5** | **33.4** | **6.4** | **0.58** | **0.21** |
| Alfalfa Hay | Third cut | **91.2** | **8.9** | **91.1** | **19.7** | **26.8** | **1.5** | **38** | **31.4** | **7.6** | **0.40** | **0.23** |
| Oat straw | | **92.4** | **7.9** | **92.1** | **2.7** | **46.5** | **1.5** | **78.9** | **53.8** | **8.7** | **0.12** | **0.14** |
| Wheat straw | | **92.6** | **6.9** | **93.1** | **2.6** | **48.5** | **0.8** | **83.4** | **56.3** | **8** | **0.15** | **0.16** |
| Ryegrass straw | | **91.9** | **7.8** | **92.2** | **3.6** | **48.3** | **0.9** | **78** | **54.3** | **7.7** | **0.20** | **0.18** |
| Pea straw | Belshi (Albania) | **91.7** | **8.4** | **91.6** | **7.3** | **45.9** | **1.5** | **64.1** | **50.4** | **10.7** | **0.37** | 0.26 |
| Pea straw | Belshi (Albania) | **91.8** | **9.8** | **90.2** | **9.3** | **36.3** | **1.2** | **53.3** | **42.3** | **8.1** | **0.37** | 0.19 |
| Pea straw | Vitra* (Ltonia) | **91.8** | **9.8** | **90.2** | **11.1** | **44.1** | **1.9** | **61** | **48.5** | **9.9** | **0.36** | 0.25 |
| Pea straw | Alderman* (Italy ) | **91.5** | **7.6** | **92.4** | **10.1** | **46.3** | **1** | **65.4** | **48.5** | **9.9** | **0.34** | 0.24 |
| Fava pod | Belshi VF (Albania) | **91.4** | **6.9** | **93.1** | **7.5** | **47.9** | **0.5** | **64.8** | **54.8** | **11.6** | **0.27** | 0.25 |
| Fava pod | Aguadulce* (Italy) | **90.9** | **8.5** | **91.5** | **9.5** | **37** | **0.5** | **55.8** | **41.6** | **7.6** | **0.36** | 0.25 |
| Fava pod | Jogeva* (Letonia) | **91.7** | **8.1** | **91.9** | **7.4** | **45.4** | **0.6** | **63.8** | **49.2** | **10.4** | **0.34** | 0.23 |
| Fava pod | Skrapari* (Albania) | **91.2** | **8** | **92** | **10.0** | **39.3** | **0.6** | **56** | **49.6** | **9.8** | **0.32** | 0.28 |

Table 2. Dry matter digestibility with enzymatic and Tilley and Terry methods

| | Enzymatic digestibility | | | Tilley and Terry | | |
|---|---|---|---|---|---|---|
| Feeds | Mean ±SD | Minimum | Maximum | Mean ±SD | Minimum | Maximum |
| Alflalfa hay | 59.3±6.1 | 55.0 | 63.7 | 72.48±2.9 | 70.46 | 74.50 |
| Cereal straw | 29.7±3.3 | 26.3 | 32.9 | 47.4±2.9 | 44.06 | 49.15 |
| Pea straw | 42.7±6.1 | 36.0 | 50.8 | 58.76±5.0 | 54.17 | 65.65 |
| Faba pod | 38.6±3.9 | 35.5 | 43.4 | 61.01±7.2 | 53.84 | 70.32 |

## CONCLUSIONS

The data of the study confirm that the under-utilized feeds pea straw and faba pod have, have similar chemical content for main Weende parameters. They have higher values for CP that cereal straws, higher protein content and and lower value of CF. The NDF and ADF content of peas straw and faba pod resulted lower that in cereal straw. There are not significant differences in insoluble lignin between different varieties of pea straw and faba pod. The average values of DMD determined by both in vitro methods of group of feeds showed that pea straw and faba pod had higher DMD than cereal straw but lower than alfalfa hay. There were differences between DMD determined with two in vitro methods. The pepsin-cellulase method produced consistently lower DMD contents than Tilley and Terry method. Pea straw and faba pods could be good feed resources for ruminant animals and must not be under valuated.

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# EFFECTS OF HERBAL MIXTURE AND DON OR T-2 TOXIN EXPOSURE ON SOME GLUTATHIONE REDOX AND LIPID PEROXIDATION PARAMETER OF BLOOD AND LIVER IN BROILER CHICKENS

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***Abstract***

*The purpose of present study was to investigate the short-term effect of DON and T-2 toxin exposure on some blood and liver lipid peroxide and glutathione redox parameters in broiler chickens. A total of 120 three-week old Cobb 540 broiler chickens were randomly assigned into five experimental groups of 24 chickens in each. The short-term trial lasted for 48 hours, after 12 hours of feed deprivation. The experimentally mycotoxin-contaminated diets contained (1 kg) 3.74 mg T-2 or 16.12 mg DON, respectively. Herbal mixture (Herbamix Basic Premix™, Herbamix Trade Ltd., Budapest) was added to the complete feed at the dose of 600 mg/kg. Six birds of each group were slaughtered at 12th, 24th, 36th and 48th hours of the experiment. Parameters of the lipid peroxidation (malondialdehyde) and the glutathione redox system (reduced glutathione content and glutathione peroxidase activity) were measured in blood plasma and liver homogenate. Malondialdehyde content did not change in blood plasma but it was significantly lower in liver homogenate in both mycotoxin loaded groups fed with herbal mixture supplemented feed at 24 hour as compared to the control. Reduced glutathione content did not change significantly in blood plasma, but in liver homogenate, at 24 hour sampling, T-2 toxin alone or in combination with herbal mixture showed significantly higher values as compared to the control. In conclusion, the investigated trichothecene mycotoxins at the dose applied, activated the glutathione redox system in liver of broiler chicken, while addition of herbal mixture has moderate effect against the mild oxidative stress as caused by DON or T-2 toxin.*

***Key words:*** *T-2 toxin, DON, lipid peroxidation, glutathione redox system, medicinal herb.*

## INTRODUCTION

Moulds produce different mycotoxins that have importance in farm animal nutrition because of their widespread occurrence and diversity (Leeson et al. 1995). Among various trichothecene mycotoxins, those produced by *Fusarium* moulds, such as a 'type B' trichothecene deoxynivalenol (DON) is often found in feed ingredients even at high concentrations in different parts of the world under different environmental conditions (Jelinek et al. 1989).T-2 toxin, which is a 'type A' trichothecene is also important to the poultry industry because of their toxicity and co-occurrence in feeds (Devegowda and Murthy, 2005).

The maximum recommended concentration of T-2 toxin and its metabolite, the HT-2 toxin in feeds for broilers is 0.25 mg/kg complete feed (2013/165/EU) and in case of DON 5.0 mg/kg complete feed (2006/576/EC).

This relative high tolerance of poultry in case of DON is possibly due to the de-epoxidation in the gut before absorption (Awad et al., 2008).

Application of some herbal extracts of plant origin like turmeric (*Curcuma longa*), garlic (*Allium sativum*) and asafetida (*Ferula asafetida*) have shown to counteract mycotoxicosis in poultry through their antioxidant activity.

Several herbal products contain antioxidant substances capable of scavenging free radicals

and enhancing antioxidant enzymes (Nyandieka et al., 1990).
The purpose of present study was to investigate the short-term effect of T-2 or DON on lipid peroxidation processes and on the glutathione redox system in broiler chicken, in connection with the dietary addition of a medicinal herb mixture.

## MATERIALS AND METHODS

A total of 120 three-week old Cobb 540 broiler chickens (body weight: 749.60±90.98 g) was randomly assigned into five experimental groups of 24 chickens in each. The short-term trial lasted for 48 hours, after 12 hours of feed deprivation. The basal diet was a commercial broiler feed (13.4 MJ/kg AME, 20% crude protein, 10% crude fat, 3.5% crude fibre, 35mg/kg vitamin E and 0.25mg/kg selenium). The nutrient content of the diet met the requirements for broiler chickens (Hungarian Feed Code, 2004). Measured mycotoxin concentrations of the commercial diet (1 kg) were: T-2: <0.10 mg; DON: 0.25 mg. The experimentally contaminated diets contained (1 kg) 3.74 mg T-2 and 1.26 mg HT-2 or 16.12 mg DON, respectively. Herbal mixture (Herbamix Basic Premix™, Herbamix Trade Ltd., Budapest) was added to the complete feed in powder form at the dose of 600 mg/kg. The main components of the applied herbal mixture are rosemary (*Rosmarinus officinalis*), oregano (*Origanum vulgare*) thyme oil (*Thymus vulgaris*) and Mary thistle (*Silybum marianum*).
DON was produced by *Fusarium graminearum* (NRRL 5883) and T-2 by *Fusarium sporotrichioides* (NRRL 3299) strains on corn substrate according to Fodor et al. (2006).DON content of feed was determined according to Pussemier et al. (2006), and T-2 and HT-2 concentration were measured based on the method of Trebstein et al. (2008) with HPLC after immunoaffinity cleanup.
Six birds were exterminated from each group at 12th, 24th, 36th and 48th hours of the experiment.
After cervical dislocation, blood samples were collected into EDTA-$Na_2$ containing tubes. The whole blood was separated by centrifugation (2,500×g, 20 min) and the blood plasma was stored at −70°C until analysis.
*Post mortem* liver samples were taken for biochemical analyses and stored at -70°C until analysis. Before the biochemical analysis liver homogenates were made with 9-fold cold (4°C) physiological saline (0.65% w/v NaCl).
Initial phase products of lipid peroxidation, conjugated dienes (CD) and trienes (CT) were measured by spectrometry at 232 nm (dienes) and 268 nm (trienes) according to AOAC (1984) in the liver. Determination of malondialdehde (MDA) concentration was carried out in the native liver homogenates, while the other parameters were determined in the 10,000×g supernatant fraction of the homogenate. MDA content of blood plasma was determined using the 2-thiobarbituric acid method according to Placer et al. (1966), in liver homogenates according to Botsoglou et al. (1994). The concentration of MDA was calculated using standard curves of increasing 1,1,3,3 tetraethoxypropane (Fluka, Buchs). Reduced glutathione (GSH) content of blood plasma, and a 10,000×g supernatant fraction of liver homogenates was measured as described by Sedlak and Lindsay (1968). Glutathione peroxidase (GPx) activity was determined according to Lawrence and Burk (1976). GSH content and GPx activity were expressed in protein content, which was determined in blood plasma by the biuret method (Weichselbaum, 1948) or with Folin-phenol reagent in a 10,000×g supernatant fraction of liver homogenates (Lowry et al., 1951).
Statistical analyses Statistical analysis of data (calculation of means and standard deviations, one-way analysis of variance with Tukey's post-hoc test) was performed with GraphPad Prism 5.04 for Windows (GraphPad Software, San Diego, CA, USA).

## RESULTS AND DISCUSSIONS

There was no mortality during the trial, and no clinical signs of toxicity were observed. Calculated mycotoxin intake, which was calculated from feed intake and measured mycotoxin content of the particular complete feed, was almost the same between the groups fed with mycotoxin contaminated and mycotoxin contaminated and herbal mixture supplemented groups (Table 1).

Table 1. Calculated mycotoxin intake of broiler chickens

| Group | Calculated mycotoxin intake (mg/bird) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 0-12 h | | 12-24 h | | 24-36 h | | 36-48 h | |
| | DON | T-2 | DON | T-2 | DON | T-2 | DON | T-2 |
| **Control** | 0.017 | | 0.011 | | 0.019 | | 0.022 | |
| **T-2 toxin** | | 0.245 | | 0.174 | | 0.179 | | 0.390 |
| **DON** | 1.139 | | 0.811 | | 0.960 | | 2.163 | |
| **Herbamix™** | 0.017 | | 0.013 | | 0.013 | | 0.031 | |
| **T-2 toxin + Herbamix™** | | 0.256 | | 0.178 | | 0.178 | | 0.430 |
| **DON + Herbamix™** | 1.102 | | 0.833 | | 0.907 | | 1.934 | |

Early markers of lipid peroxidation, the level of CD and CT did not change significantly as effect of T-2 toxin or DON, and it was not modified by the supplementation of herbal mixture in liver (data not shown).

End product of lipid peroxidation, the MDA concentration did not change in blood plasma (data not shown), but it was significantly lower in the liver homogenate in both mycotoxin loaded groups fed with herbal mixture supplemented feed at 24 hour sampling as compared to the control (Table 3).

GSH concentration did not change significantly in blood plasma (data not shown), but in liver homogenate showed significant differences at 24 hour sampling, when T-2 toxin alone ($p<0.05$) or in combination with herbal mixture resulted in higher values as compared to the control, while significantly ($p<0.05$) higher GSH concentration was found in case of DON only when it was combined with herbal mixture supplementation (Table 3).

GPx activity in blood plasma showed moderate changes. At 12th hour it was lower in DON and herbal mixture treated group as compared to T-2 toxin and herbal mixture group, and at 48th hour herbal mixture alone caused significantly lower enzyme activity in blood plasma than the control (Table 2).

In liver homogenate GPx activity changed significantly at 24 hour sampling, when higher values were found as effect of T-2 toxin, also in combination with herbal mixture, and in DON + herbal mixture group, as compared to control group and to the group fed with DON contaminated diet alone (Table 3).

Reduced glutathione (GSH) content in liver homogenate showed higher values when T-2 toxin contaminated feed was fed, alone or in combination with herbal mixture.

It means that the moderate oxidative stress in the liver as effect of T-2 toxin activates the glutathione synthesis, as part of the antioxidant response (Zimniak et al., 1997).

The results revealed that trichothecene mycotoxins, DON or T-2 toxin, have an effect on oxygen free radical formation, and it activates the glutathione redox system in liver of broiler chicken.

Table 2. Individual and combined effect of T-2 toxin, DON and herbal mixture on glutathione peroxidase activity in blood plasma (mean±SD; n=6)

| Time | Control | T-2 toxin | DON | Herbamix | T-2 toxin + Herbamix | DON + Herbamix |
|---|---|---|---|---|---|---|
| **GPx (U/g protein content)** | | | | | | |
| **12th hour** | 10.0$^{ab}$± 1.42 | 9.88$^{ab}$± 0.74 | 8.16$^{ab}$± 1.94 | 8.45$^{ab}$± 1.13 | 10.27$^{b}$± 1.88 | 8.06$^{a}$± 1.85 |
| **24th hour** | 9.80± 0.83 | 11.45± 2.72 | 9.52± 2.94 | 11.92± 2.74 | 10.53± 0.55 | 10.82± 0.64 |
| **36th hour** | 7.41± 1.47 | 9.61± 2.60 | 6.96± 1.33 | 7.56± 2.06 | 8.53± 1.60 | 8.39± 1.83 |
| **48th hour** | 11.00$^{b}$± 2.86 | 10.37$^{ab}$± 2.81 | 8.35$^{ab}$± 1.64 | 7.88$^{a}$± 0.98 | 8.38$^{ab}$± 0.56 | 8.30$^{ab}$± 1.40 |

$^{a,b}$ Means designated with different letters within the same rows mean significant difference ($p<0.05$)

Table 3. Individual and combined effect of T-2 toxin, DON and herbal mixture on lipid peroxidation and glutathione redox system of liver homogenates (mean±SD; n=6)

| | Control | T-2 toxin | DON | Herbamix™ | T-2 toxin + Herbamix™ | DON + Herbamix™ |
|---|---|---|---|---|---|---|
| | MDA (µmol/) | | | | | |
| **12th hour** | 10.99± 2.15 | 10.12± 3.02 | 9.36± 0.54 | 9.74± 2.10 | 9.15± 2.40 | 9.88± 4.47 |
| **24th hour** | 17.95$^{b}$± 3.69 | 12.62$^{ab}$± 1.88 | 15.00$^{ab}$± 4.94 | 14.42$^{ab}$± 3.51 | 11.99$^{a}$± 2.60 | 11.72$^{a}$± 1.61 |
| **36th hour** | 10.05± 1.37 | 11.37± 2.31 | 10.04± 2.12 | 10.15± 2.33 | 10.69± 0.90 | 9.16± 2.49 |
| **48th hour** | 12.57± 3.47 | 15.51± 2.68 | 12.03± 3.24 | 11.81± 3.64 | 12.46± 2.31 | 14.54± 6.23 |
| | GSH (µmol/g protein content) | | | | | |
| **12th hour** | 3.03± 0.75 | 4.03± 1.12 | 3.55± 0.89 | 3.15± 0.27 | 3.22± 0.51 | 3.19± 0.77 |
| **24th hour** | 2.84$^{a}$± 1.07 | 4.56$^{bc}$± 0.74 | 3.00$^{ab}$± 1.17 | 3.55$^{abc}$± 0.70 | 4.17$^{abc}$ ± 0.86 | 4.51$^{b}$± 0.50 |
| **36th hour** | 3.46± 0.83 | 3.60± 0.58 | 3.08± 0.60 | 2.93± 0.39 | 3.42± 0.32 | 3.19± 0.48 |
| **48th hour** | 2.62± 0.40 | 2.92± 0.51 | 3.15± 0.58 | 3.03± 0.66 | 2.82± 0.30 | 2.74± 0.29 |
| | GPx (U/g protein content) | | | | | |
| **12th hour** | 3.01± 0.66 | 3.99± 0.91 | 3.48± 0.68 | 3.14± 0.21 | 3.21± 0.53 | 3.07± 1.35 |
| **24th hour** | 3.10$^{a}$± 1.24 | 4.78$^{b}$± 0.67 | 2.89$^{a}$± 0.96 | 3.51$^{ab}$± 0.94 | 4.69$^{b}$± 0.74 | 4.70$^{b}$± 0.44 |
| **36th hour** | 3.23± 0.88 | 3.44± 0.61 | 3.16± 0.73 | 3.06± 0.63 | 3.40± 0.44 | 3.23± 0.62 |
| **48th hour** | 1.99± 0.37 | 2.54± 0.48 | 2.84± 0.49 | 2.64± 0.58 | 2.72± 0.61 | 2.31± 0.37 |

$^{a,b}$ Means designated with different letters within the same rows mean significant difference ($p<0.05$)

Addition of herbal mixture has moderate effect against the mild oxidative stress as caused by DON or T-2 toxin at the dose applied. No clinical signs of toxicity and mortality was observed at the dose level applied, which supported by the relatively high tolerance of broiler chicken to DON (Dänicke et al., 2001) or T-2 toxin (Eriksen and Pettersson, 2004). The results revealed that addition of herbal mixture did not modify the TBARS values, probably because of the lack of marked oxidative stress in liver as effect of the mycotoxin doses used in this trial. Glutathione peroxidase activity in liver homogenate changed significantly at 24 hour sampling, when higher values were found as effect of T-2 toxin, also in combination with herbal mixture and DON in combination with herbal mixture, but also when DON was used alone. In liver homogenate significant changes were found at 48 hour sampling, where T-2 toxin load resulted higher activity when it was used together with herbal mixture, as compared to herbal mixture supplemented group. T-2 toxin induced glutathione peroxidase activity, without additional effect of herbal mixture. Lack of effect of DON probably explained the high tolerance of broiler chicken which is probably caused by the higher rate of metabolism in the liver (Awad et al., 2014). It means that both trichothecene mycotoxins activate the enzymatic antioxidant defence, in this case glutathione peroxidase activity, but herbal mixture has no additional effect, and when it used alone did not has effect. the results revealed that trichothecene mycotoxins, DON or T-2 toxin, have effect on oxygen free radical formation, and it is activate the glutathione redox system in liver of broiler chicken. Addition of herbal mixture has moderate effect against the mild oxidative stress as caused by DON or T-2 toxin at the dose applied, in antioxidant but also in xenobiotic transformation.

## CONCLUSIONS

The results of this study showed that the applied trichothecene mycotoxins, DON or T-2 toxin, activated the glutathione redox system in liver of broiler chicken, while addition of herbal mixture had moderate effect against the mild oxidative stress as caused by DON or T-2 toxin at the dose applied. In conclusion the results revealed that trichothecene mycotoxins, DON or T-2 toxin, have effect on oxygen free radical formation, and it is activate the glutathione redox system in liver of broiler chicken. Addition of herbal mixture has moderate effect against the mild oxidative stress as caused by DON or T-2 toxin at the dose applied, which would important not only in antioxidant but also in xenobiotic transformation.

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# EFFECT OF SUPPLEMENTED STARTER CULTURE ON TOFU DREG SILAGE QUALITY

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***Abstract***

*Research used experimental method with split-plot design. Supplemented starterculture (without starter, molasses and Lactobacillus plantarum) were whole plot and time of observed (1, 2, 3, and 4 weeks) were subplot. Every time of observed for each treatment was replicated four times.The experiment showed that bacteria population among treatments for every time observed wasn't significant (P>0.05). Lactic acid bacteria population from first making silage until fourth weeks for all treatments showed increased (P<0.05). First week of observed among treatments showed significant for silage pH.pH of silage that supplemented molasses and Lactobacillus plantarum were lower significant (P<0.05) than pH of silage without starter culture. Nevertheless, furthermore time of observed among treatment wasn't significant (P>0.05). The first week observed N-Ammonia of silage wasn't significant (P>0.05) among treatments. Significance N-ammonia of silage among treatments was began at second week. N-Ammonia Silage that supplemented Lactobacillus plantarum was lowest (P<0.05) significant than others.*

***Key words****: starter culture, tofu dreg silage, molasses, Lactobacillus plantarum, lactic acid bacteria, pH, N-ammonia.*

## INTRODUCTION

Tofu dreg is waste product a processing of soybeans into tofu. Protein contentof tofu dreg in dry matter basicsis 29.17% (Lopez, 1963). That can be used as an alternative source of feed protein because it is relatively cheap and abundant enough availability. This is evidenced by the increase in tofu dreg as much as 32,832 to 36,937 thousand tons per year by 81 companies produce tofu in 1994 and in 1999 to 116 companies in Indonesia (the Central Bureau of Statistics, 1999). Tofu often found in West Java in the area around Lembang, Sumedang, Sukabumi, Pangalengan, and Bogor.

However, the weakness of tofu dreg is to have a high water content and so can not be stored longer and easily damaged, so at normal temperature can only survive about 24 hours. Preservation efforts can be done by drying in the sun, but drying it got into trouble because of the drying process takes more than 24 hours. Due to this tofu dreg to be rotten before drying. Therefore, it is necessary preservation which is easily done without harm by way of silage as feed material processing are stored in a fresh state with anaerobic atmosphere. The goal is to maintain nutrition, color, palatability and durable (Susetyo et al., 1977).

Silage preservation method in principle is to increase the growth of lactic acid bacteria that produce lactic acid which can provide acidic conditions, which is expected to inhibit the growth of spoilage bacteria.

For the growth of lactic acid bacteria needed water soluble carbohydrate is added to the material preserved in anaerobic atmosphere. It can also be added directly lactic acid or lactic acid bacteria.

## MATERIALS AND METHODS

### Materials

The materials used for making of silage in this study is tofu dreg. Tofu dreg obtained from the factory in Cileunyiand Bandung area.

Tofu dreg 5kg packed in plastic with anaerobic conditions by starter culture (molasses, *Lactobacillus plantarum*) or without a starter. The amount of packaging is 36 packs.

Addition Molasses

Tofu dreg that is gave molasses as much as twelve packs. Each package is added 3% molasses or 150 grams per pack. Molasses obtained from KUD Tanjung Sari.

Addition inoculant *Lactobacillus plantarum*

Tofu dreg by *Lactobacillus plantarum* inoculants twelve pack. *Lactobacillus plantarum* was is obtained from the Laboratory of Microbiology and Bioprocess Department of Technical Chemistry Bandung Institute of Technology.

*Lactobacillus plantarum* grown on agar (Agar, beef extract, and skim milk) and then is incubated for 48 hours in a test tube.

Take a sample of the reaction tube randomly that have was planted and incubated, for the calculated of population with Method Total Plate Count (TPC).

Subsequently tubes are another plus physiological NaCl solution, and inoculated into tofu dreg. Provision of *Lactobacillus plantarum* inoculant as much as 5 x $10^5$ CFU/g fresh Tofu dreg.

**Treatment and experimental design**

The treatment will be attempted is a wide variety of starter culture. The design used was split plot design treatment with the addition of starter culture (without the starter, molasses, and *Lactobacillus plantarum*) as the main plots and observation time (1, 2, 3, and 4 weeks) as the subplot treatment.

Each treatment was repeated three times. The treatment was :

1. Tofu dreg without starter.
2. Tofu dreg + molasses (3% of the weight of Tofu dreg).
3. Tofu dreg + *Lactobacillus plantarum* (population 5 x $10^5$ CFU bacteria/g Tofu dreg).

**Variables Measured**

Every week for each treatment taken as many as three packs for further analysis:

1. pH with a pH meter brands of "Hanna"
2. The content of N-ammonia was determined by the method of "Micro Diffusion Conway" (AOAC, 1980)
3. The population of lactic acid bacteria, with "Method Total Plate Count (TPC)" according Fardiaz (1992).

Observations were made up to four weeks analysis method. Data were analyzed by analysis of variance followed by Duncan's multiple range test (Gomez and Gomez, 1995).

## RESULTS AND DISCUSSIONS

**The Effect of adding Starter Culture on Lactic Acid Bacteria Population**

Observations (Figure 1) shows that the population of lactic acid bacteria from the beginning of making silage until the fourth week of the overall treatment showed increased ($P<0.05$).

Results of analysis of variance influence between administration starter every week on the population of lactic acid bacteria showed no significant differences ($P>0.05$).

Nevertheless, the observation every week tofu dreg that inoculated bacteria *Lactobacillus plantarum* decreased from the first week to the second week and the next week showed an increase in population.

The decline in the population of lactic acid bacteria compared to the beginning of making silage was reported also by Yatno (1999).

The decline in the population of lactic acid in the tofu dreg out inoculated with *Lactobacillus plantarum* possibility that the competition between the lactic acid bacteria existing in the tofu dreg out with *Lactobacillus plantarum* were inoculated into tofu dreg.

Yatno research results (1999) in the tofu dreg out existing lactic acid bacterial strain *Lactobacillus sp*. Inoculation *Lactobacillus plantarum* suppress the growth of lactic acid bacteria present in tofu dreg, so that the total population of lactic acid bacteria is reduced.

McDonald (1991) states that *Lactobacillus plantarum* is very dominant in the ensilage process, highly competitive and rapidly produce more acid.

After *Lactobacillus plantarum* achieve dominance in the second week, the next week is growing rapidly.

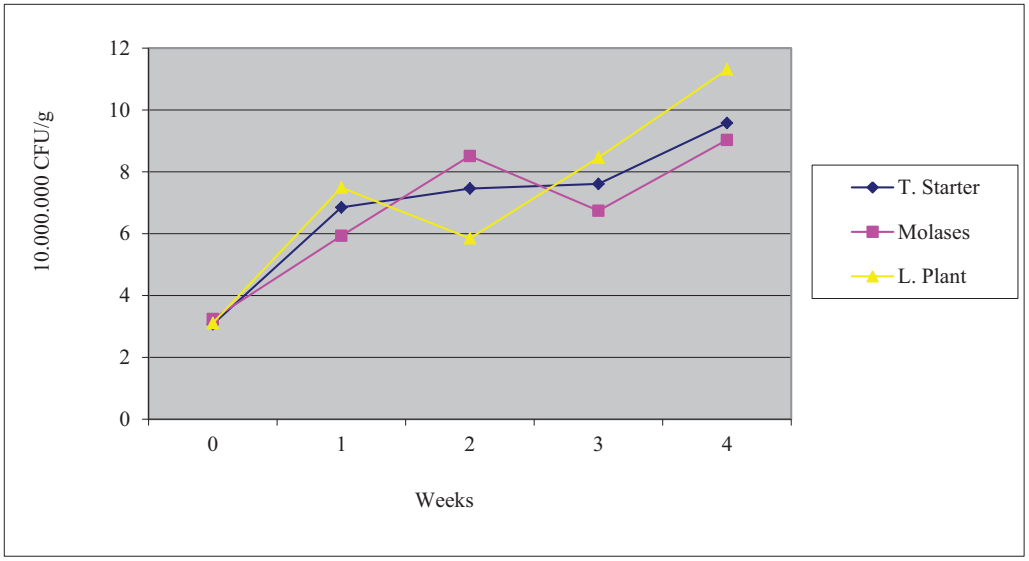


Figure 1. Population Changes Lactic Acid Bacteria ($10^7$ CFU/ g Silage Fresh Tofu dreg) Every Weeks

Table 1. Effect of the Starter Addition on Lactic Acid Bacteria Population ($10^7$ CFU/ g Silage Fresh Tofu Dregs) (Every Sunday)

| Time | Without Starter (X $10^7$ CFU/g) | Molases (X $10^7$ CFU/g) | *L plantarum* (X $10^7$ CFU/g) |
|---|---|---|---|
| 1st week | 6.85 a | 5.93 a | 7.49 a |
| 2nd week | 7.46 a | 8.51 a | 7.49 a |
| 3rd week | 7.61 a | 6.74 a | 8.46 a |
| 4rt week | 9.58 a | 9.03 a | 11.31 a |

Description: The same alphabet to the rowsshowed no significant different (P<0.05)

**Effect of adding starter culture on pH Tofu dreg**

The level of acidity (pH) of silage that has been measured by using a pH-meter indicates there has been a decrease in pH in the first week compared with the beginning of making silage. pH silage real differences among treatments occurred in the first week (P<0.050).

The pH of silage that was inoculated *L. plantarum* and that was added molasses was significantly lower (P<0.05) than without starter. But in the next few weeks between treatments showed no significant differences (P>0.05)

Decrease in pH than at the start of making silage, because it produces acid compounds during ensilage by lactic acid bacteria.

The acid formed during the process include lactic acid, acetic acid and butyric acid as well as several other compounds such as ethanol, carbon dioxide, methane, carbon monoxide nitrite (NO) and heat (Cullison, 1978).

In the first week seemed the pH of silage that was added molasses and that was inoculated *Lactobacillus plantarum* significantly (P<0.05) lower than pH of silage without starter. Molasses is a material containing a water-soluble carbohydrates.

The molasses can be used as a stimulant of microorganisms forming lactic acid.

Woolford (1998) suggested that the ensilage process requires rapid formation of lactic acid.

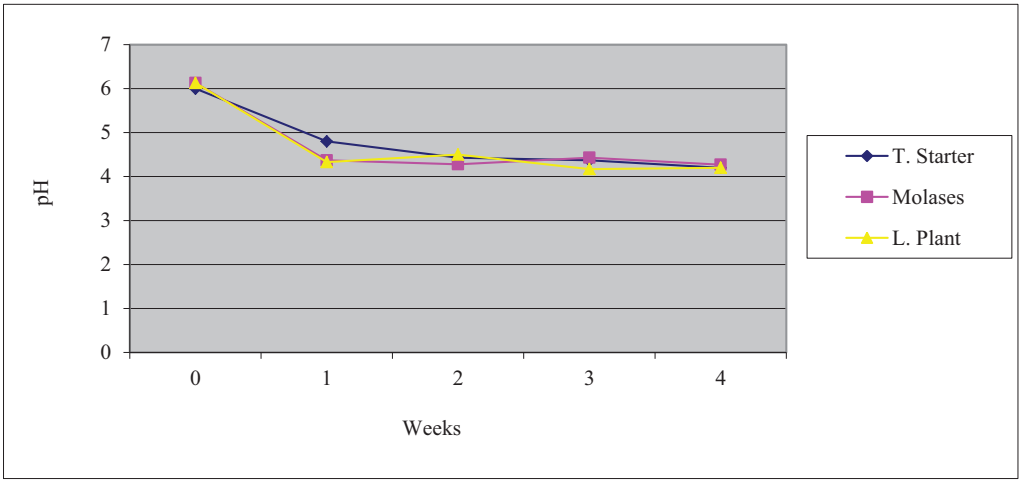


Figure 2. Changes in pH Silage Tofu Dregs Every Weeks

Table 2. Effect of the Addition Starter on Silage pH Tofu Dregs Every Week

| Time | Without Starter pH | Molases pH | *L plantarum* pH |
|---|---|---|---|
| 1st week | 4.80 a | 4.37 b | 4.33 b |
| 2nd week | 4.43 a | 4.28 a | 4.50 a |
| 3rd week | 4.37 a | 4.43 a | 4.17 a |
| 4rt week | 4.20 a | 4.27 a | 4.20 a |

Description: The not same alphabet to the rows showed significant different(P <0.05)

Carbohydrates are readily soluble in water/WSC is a source for starting and maintaining the process of fermentation, lactic acid bacteria can multiply rapidly under conditions where available feeds rich in carbohydrates (Cullison, 1978).

To accelerate the formation of lactic acid in ensilage process can be done by direct stimulant that with the addition of lactic acid bacteria.

*Lactobacillus plantarum* is group of bacteria that have the ability to convert carbohydrates such as lactose and glucose is fermented into lactic acid in large quantities.

*Lactobacillus plantarum* is a group of bacteria homofermentatif, which will produce 2 moles of lactic acid for every mole of glucose and fructose.

According Rahayu et al. (1992) homofermentatif acid bacteria can change 95% glucose and other hexoses to lactic acid and carbon dioxide with a small amount of volatile acids (butyric acid).

The implications of the addition molasaes or *Lactobacillus plantarum* on ensilage process is able to accelerate a decrease in pH.

**Effect of Adding Starter Culture on N-Ammonia Silage Tofu Dreg**

The content of N-Ammonia is one of the criteria considered to determine the success of the ensilage process (Wilkins, 1988). Results of analysis of variance showed that the adding of starter culturegave significant effect (P<0.05) on N-ammonia in silage.

Duncan's multiple range test showedthat the first week N ammonia content showed nonsignificant.

The significant difference was occured in the second week of observation, it appears that the content of the N-Ammonia lowest caused by the addition of an inoculant treatments *Lactobacillus plantarum.*

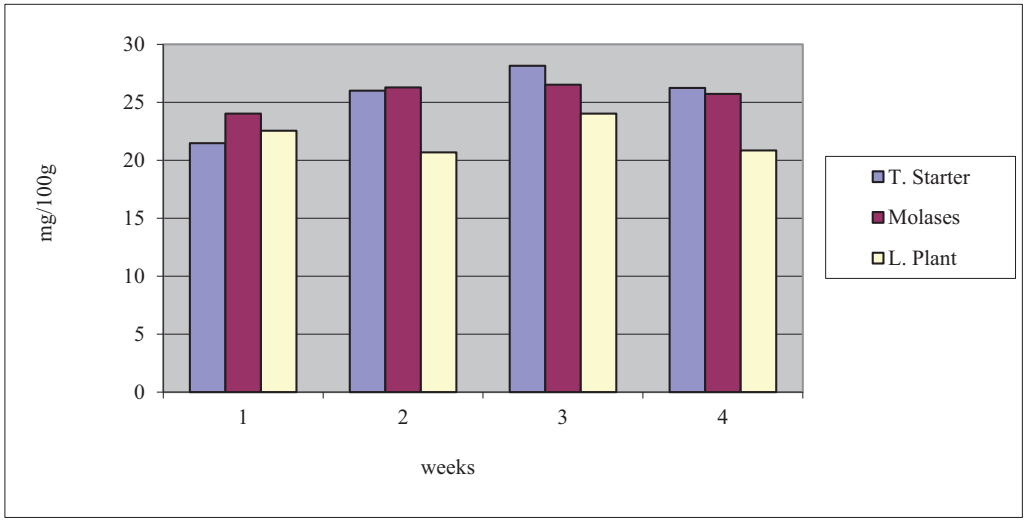


Figure 3. Production of N-Ammonia (mg/100g Silage Fresh Tofu Dregs)

Table 3. Effect of the Addition of Starter on N-Ammonia Production (mg/ 100 g Silage Fresh Tofu Dregs) every Weeks

| Time | Without Starter (mg/ 100 g Silage Fresh Tofu Dregs) | Molases (mg/ 100g Silage Fresh Tofu Dregs) | *L plantarum* (mg/ 100 g Silage Fresh Tofu Dregs) |
|---|---|---|---|
| 1st week | 21.48 a | 24.03 a | 22.55 a |
| 2nd week | 26.01 a | 26.29 a | 20.68 b |
| 3rd week | 28.16 a | 26.52 ab | 24.03 b |
| 4rt week | 26.24 a | 25.73 a | 20.85 b |

Description: The not same Alphabet to the rows showed significant different($P < 0.05$)

Their N-ammonia in silage is presumably because the process of deamination by bacteria other than lactic acid bacteria that are proteolytic so will outline glutamic acid and lysine into acetic acid, pyruvic acid and N compounds in the form of $NH_3$.

According to McDonald et al (1991) that as many as 60 types of Clostridium and 7 species are microorganisms that are often involved in the process of fermentation of the silage.

Further explained that Clostridia are saccharolytic as *Clostridium butiricum* capable of fermenting organic acids and sugar and little ability to ferment proteins and amino acids, but it also is a proteolytic like *Clostridium sporogenes* which is able to ferment the amino acid glutamic acid, lysine, arginine, histidine, alanine and glycine).

Meanwhile, according Bolsen and Sapienza (1993), there are three genera, namely *Escherichia, Klebsiella* and *Erwinia.*

These bacteria are divided into two groups: the fermenting sugars and organic acids and the ferment of free amino acids. This bacteria is not undesirable in the ensilage process.

These bacteria are sensitive to low pH and require wet conditions for its development. These bacteria can be suppressed as low as possible by accelerating the fermentation produced by lactic acid-producing bacteria, this is due to the optimal pH for growth of bacteria is 6-7.

The implications of N-ammonia content is that the higher N-ammonia produced from ensilage proses is mean an lot of amino acids degradated by proteolytic bacteria.

The smaller the content of N-ammonia in silage is the better, because the proteolityc process is a little occurs. From the research it appears that administration of *Lactobacillus plantarum* inoculum provide the best results because it can prevent proteolytic process, which in turn contains N-ammonia in silage is lower.

## CONCLUSIONS

Conclusion

From the observation of silage tofu dreg for four weeks showed that the addition of *Lactobacillus plantarum* give better effect than with the addition of molasses or without a starter that is characterized by low content of N-Ammonia silage.

Suggestion

1. To maintain the quality of the tofu dreg by way of silage suggested the addition of lactic acid bacteria *Lactobacillus plantarum*.
2. Do further research on comparing the quality of the tofu dreg with the addition of various types of lactic acid bacteria.

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# MONITORING LIPID PEROXIDATION IN EGGS ENRICHED IN OMEGA 3 POLYUNSATURATED FATTY ACIDS (Ω – 3 PUFA)

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***Abstract***

*The 6-week experiment used 168 Tetra SL (26 weeks) layers assigned to 4 groups. Compared to the conventional diet formulation used for the control group (C), in order to enrich the eggs in ω – 3 PUFA, the diet formulations used for the experimental groups (E1, E2, E3) included 7% flaxseed meal. Diet E1 used vitamin E (100 mg/kg feed) as antioxidant, while the other diets used 3% (E2) and 1.5% (E3) grape seeds powder. Egg samples were collected throughout the experimental period: initially, in the beginning of the experiment, in weeks II, IV and VI and when the experiment ended. The collected eggs were used to form egg yolk samples, which were assayed for fatty acids, peroxide value (PV), concentration of conjugated dienes (CD) and concentration of conjugated trienes (CT) and total antioxidant capacity. The yolks of the eggs from the experimental groups had a significantly (P≤0.05) higher concentration of ω – 3 PUFA compared to those from the control group. The lowest PV was determined in the yolk from E2 groups (3% grape seeds powder). The lowest CD concentrations were traced in the eggs collected on week II from groups E1 (6.278±1.931 µmol/g) and E2 (6.254±0.196 µmol/g). The yolks from groups E2 and E3 collected on weeks II and VI had the lowest CT absorbance. The antioxidant capacity was higher in the yolk from groups E1 (100 mg vitamin E/kg CF) and E2 (3 % grape seeds powder). The yolks from E3 (1.5% grape seeds powder) eggs collected in the end of the experiment had a significantly (P≤0.05) lower antioxidant capacity than E1 and E2.*

***Key words*****:** *antioxidant capacity, dienes, eggs, peroxidation, PUFA ω – 3, trienes.*

## INTRODUCTION

The interest for foods high in polyunsaturated fatty acids (ω – 3 PUFA ) increased a lot over the past two decades (Siro et al., 2008). However, the susceptibility of autoxidation, major reaction of lipid degradation, is a major concern both for the food industry and for the consumers. The oxidation of the fatty acids from feeds and animal foods increases with their level of unsaturation and affects drastically the nutrients, the flavour and safety of the product, its storage and the economic efficiency (Labuza, 1971; Frankel, 1980). Once started, oxidation progresses throughout a chain mechanism with free radicals (Labuza, 1971). Within this context, lipid peroxidation must be assessed by tracing the primary lipid peroxidation products, the hydroperoxides (Nouroozzadeh J. et al., 1994), the conjugated dienes (Iversen SA et al., 1985), or some secondary products such as malondialdehyde (Draper et al., 1993), alkanes (Burk and Ludden, 1989).

The content of hydroperoxides can be determined by several methods such as iodometric titration (Gray, 1978); spectrometry (Griffiths et al., 2000; Bou R. et al., 2008); chemiluminiscence (Miyazawa et al., 1987; Shahidi et al., 2002); chromatography (Dobarganes and Velasco., 2002). Monitoring the amount of hydroperoxides function of time shows whether the lipids are in the stage of increase or decrease of concentration. This information can be used to consider the acceptability of a food in terms of the extent of deterioration.

The lipids which contain methyl dienes or polyenes undergo a change in the position of the double bond during oxidation due to the isomerization and conjugation (Logani and Davies, 1980). The resulting conjugated dienes and trienes are quantified by spectrophotometry

(Papuc et al., 2012). Lipid oxidation in foods and other biological systems is often determined by spectrophotometry with 2-thiobarbituric acid (TBA) and reading at 530-532 nm wavelengths (Tarladgis et al., 1964). Shahidi et al. (1986) developed an alternative spectrophotometric method to monitor lipid oxidation, measuring the total volatile carbonyl compounds that were formed through hydroperoxides degradation, based on the absorbance of the quinoidal ion, derivative of aldehydes and ketones.

The production of eggs enriched in PUFA ω-3, food with functional properties, can be achieved by using flax in layer diets under different forms: seeds (Bean and Leeson, 2003; Criste et al., 2009); oil (Milinsk et al., 2003; Souza et al., 2008); flaxseed meal (Aziza et al., 2013; Panaite et al., 2016). The presence of a material rich in PUFA (flaxseed meal, for instance) in layer diets requires the presence of an antioxidant. This is any substance which delays significantly or inhibits the oxidation of a substrate (Semb, 2012). The dietary antioxidants can minimize lipid oxidation, therefore preserving the quality of the eggs enriched in ω – 3 PUFA (Qi and Sim, 1998; Galobart et al., 2001). Despite the efficiency, constant quality and the rather low cost of the synthetic antioxidants, there is a recent worldwide trend to use natural antioxidants (Frankel, 2005, Pokorný, 1991). Many scientists reported that the winery by-products are rich in polyphenols (Katalinić et al., 2010) and flavonoids (Yilmaz and Toledo, 2004). Antioxidant capacity can be assessed by determining their antioxidant capacity. Burits and Bucar (2000) evaluated the antioxidant capacity by the capacity to annihilate radical 1,1-diphenyl-2-picrylhydrazyl (DPPH), as shown by the discoloration of the DPPH solution. Another method to determine the antioxidant capacity is the ABTS spectro-photometric assay (Paulova et al., 2004).

The purpose of this experiment was to monitor lipid peroxidation in the egg enriched in ω – 3 PUFA during an experiment in which the layers were fed diets high in ω – 3 PUFA. The following determinations were performed on the eggs collected at different moments during the feeding trial: peroxide value (PV), concentration of conjugated dienes (CD) and concentration of conjugated trienes (CT), total antioxidant capacity.

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## MATERIALS AND METHODS

A 6-week feeding trial was conducted to obtain eggs enriched in ω – 3 PUFA. The experiment used 168 TETRA SL layers (aged 26 weeks), assigned to 4 groups of 42 layers each. The birds were housed in an experimental hall under controlled environmental conditions (average temperature 21.94±1.96°C; air humidity 56.83±6.38 %), in agreement with TETRA SL management guide. The light regimen (16 h light/24 h), in agreement with TETRA SL management guide, was provided by light bulbs. The layers had free access to the feed and water.

The compound feeds for the 4 groups had the same basic formulation (Table 1). Compared to the conventional formulation, given to the control group (C), the diets of the experimental groups (E1, E2, E3) included 7% flax seeds meal, to enrich the eggs in ω – 3 PUFA. Diet E1 used vitamin E (100 mg/kg feed) as antioxidant, while the other diets used 3% (E2) and 1.5% (E3) grape seeds powder, mechanically degreased.

Samples were collected from each batch of compound feed and assayed for the basic chemical composition and fatty acids concentration. The methods from Regulation (CE) 152/2009 (Methods of sampling and analysis for the official control of feed) have been used: the gravimetric method for dry matter (DM) and ash; the Kjeldahl method for crude protein (CP); extraction in organic solvents for ether extractives (EE); successive hydrolysis in alkali and acid environment for crude fibre (CF). The fatty acids concentration has been determined by gas chromatography according to standards SR CEN ISO/TS 17764-1/ 2008 (Feeds. Determination of the fatty acids content. 1. Preparation of the fatty acids methyl esters) and SR CEN ISO/TS 17764-2/ 2008 (Feeds. Determination of the fatty acids content. 2. Method of gas chromatography) (Panaite et. al., 2016).

Table 1. Diet formulations

| Ingredients | Control group (C) | Experimental group 1 (E1) | Experimental group 2 (E2) | Experimental group 3 (E3) |
|---|---|---|---|---|
| Corn, % | 20 | 20 | 20 | 20 |
| Wheat, % | 28.25 | 26.6 | 24.18 | 24.47 |
| Rice bran, % | 10 | 10 | 10 | 10 |
| Soybean meal, % | 18.72 | 13.28 | 15.32 | 13.36 |
| Rapeseeds meal, % | 8 | 8 | 4.68 | 8 |
| Oil, % | 3.53 | 3.29 | 3.9 | 3.84 |
| *Flaxseeds meal*, % | - | *7* | *7* | *7* |
| *Grape seems powder*, % | - | - | *3* | *1.5* |
| Methionine, % | 0.08 | 0.16 | 0.18 | 0.16 |
| Lysine, % | - | 0.17 | 0.16 | 0.16 |
| Carbonate, % | 8.72 | 8.74 | 8.78 | 8.74 |
| Monocalcium phosphate, % | 1.27 | 1.32 | 1.36 | 1.33 |
| Salt, % | 0.38 | 0.39 | 0.39 | 0.39 |
| Choline, % | 0.05 | 0.05 | 0.05 | 0.05 |
| Premix A6 (IBNA) *, % | 1 | - | 1 | 1 |
| A6 (100 mg vit.E/kg CF) , % | - | 1 | - | - |
| Total | 100 | 100 | 100 | 100 |

*1kg premix IBNA (A6) 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;

To determine the fatty acids concentration in the egg yolks, 18 eggs per group were collected in the beginning of the trial, and on weeks 2, 4 and 6. Six samples per group (3 egg yolks/sample) were formed and dried in a drying cabinet at $65^0$C, after which the fatty acids profile was determined by gas chromatography. For the preparation of the fatty acids methyl esters in agreement with the standard ISO 5508:2002, we weighed a sample of about 1 g of fat extracted from the dried yolk ($65^o$C). The analysis of the methyl esters was done according to standard SR EN ISO 5509:2002 (Panaite et al., 2016).

To monitor lipid peroxidation in the eggs enriched in omega 3 polyunsaturated fatty acids (ω – 3 PUFA), we collected eggs in the first experimental weeks, on weeks 2 and 4, and in the final week of the experiment (week 6). In the initial sampling we collected 30 eggs, used to form 10 egg yolk samples. To evaluate lipid degradation, in the experimental weeks 2, 4 and 6, we collected a total of 72 eggs (18 eggs/group), and six samples per group (3 egg yolks/sample) were formed. The yolk samples were stored in 15 mL plastic tubes and frozen. Before analysis, the yolk samples were thawed, and assayed for the peroxide value, concentration of conjugated dienes and trienes and for the total antioxidant capacity.

To determine the peroxidation products of the lipids, the lipids from the yolk samples were extracted according to the method described by Folch et al. (1957). We homogenized 2 g yolk sample with 10 mL methanol and 20 mL de chloroform, and stirred for one hour. The resulting solution was filtered through a separation funnel, to which 7.5 mL 0.88% KCl solution was added, and the phases were thus separated. The lower layer was collected in a 100 mL Berzelius beaker and left to evaporate at room temperature until the next day. The beakers with the sample were weighed and the amount of fat extracted from the sample was determined by the difference from the initial beaker weight.

Peroxide value determination relied on peroxide capacity to oxidize the ferrous ion at

low pH, using the ferrous oxidation-xylenol orange (FOX) assay. A blue-violet compound is formed with the ferrous ion, whose concentration is determined spectrophotometrically (at 560 nm). Between 0.01- 0.05 g of the fat extracted from the yolk samples were put into a glass tube, to which 9.9 mL chloroform-methanol mixture (7:3, v/v) was added and stirred. After the addition of 50 µL xylenol orange solution, 10 mM, and of 50 µL $FeCl_2$ (1000 mg/kg) solution, the mixture was homogenized for five minutes and the absorbance was read at 560 nm, using a JASCO V-530 spectrophotometer. The standard curve was obtained using a $FeCl_3$ (10 mg/kg) solution. The peroxide value was expressed in mEq $O_2$/ kg fat.

The conjugated dienes and trienes were determined by molecular absorption spectrometry in UV, as described by Papuc et al. (2012). The aliquots from the fat collected in Berzelius beakers after lipid extraction, as described above, was mixed with 10 mL isooctane to be dissolved. If the fat didn't dissolve, more isooctane was gradually added until a maximum volume of 50 mL. For the limpid solutions, we read the absorbance at 233 nm (conjugated diene) and at 268 nm (conjugated trienes), using a JASCO V-530 spectrophotometer. The concentration of conjugated dienes was expressed in µmol/g fat, while the concentration of conjugated trienes was expressed in absorbance units ($A_{268}$ nm).

The total antioxidant capacity was determined using the spectrophotometric method, as described by Prieto et al. (1999). The method relies on the reduction of Mo (VI) to Mo (V), by the sample analytes and the subsequent formation of a green phosphate/ Mo (V) complex, at acid pH. We weighed 1 g egg yolk, in 30-50 mL centrifuge tubes. We added 10 mL 80% methanol solution, homogenized and stirred for one hour in darkness. We centrifuged at 10,000 rpm, for 15 minutes, then collected the supernatant. In 15 mL tubes we pipetted 0.2 mL sample solution, added 4 mL ammonium phosphomolybdate reagent and incubated for 90 minutes at 95°C. The samples were left to cool down and the absorbance was read at 695 nm, compared to the blank, using a JASCO V-530 spectrophotometer. The results were expressed in mM ascorbic acid equivalent.

*Statistical analysis*

The analytic data were compared by variance analysis (ANOVA) using Stat View for WINDOWS (SAS, version 6.0).

The differrences of the means were considered significant for $P \leq 0.05$. The results were expressed as mean± SD for all measurements.

## RESULTS AND DISCUSSIONS

The analysis of the 4 compound feeds (Table 2) has showed that the crude fat percentage increased in the feeds for the experimental groups, compared to the control, due to the dietary flaxseeds meal.

The same ingredient also increased significantly ($P \leq 0.05$) the α-linolenic acid (ω – 3 PUFA acid) concentration n the feeds for the experimental groups, compared to the control group (Table 2).

Table 2.Chemical characterization of the dietary compound feeds for layers

| Item | Control group (C) | Experimental group 1 (E1) | Experimental group 2 (E2) | Experimental group 3 (E3) |
|---|---|---|---|---|
| Dry matter, % | 89.85±0.081 | 90.27±0.208 | 90.27±0.129 | 90.38 ± 0.067 |
| Crude protein, % | 19.147±0.765 | 19.29±0.856 | 19.33±0.637 | 19.02±0.667 |
| Fat, % | $7.037 \pm 0.117^{cd}$ | $8.03 \pm 0.07^{cd}$ | $8.133 \pm 0.188^{ab}$ | $8.05 \pm 0.243^{ab}$ |
| Crude fibre, % | $5.283 \pm 0.27^{bcd}$ | $6.05 \pm 0.218^{a}$ | $6.567 \pm 0.257^{a}$ | $6.547 \pm 0.598^{a}$ |
| Ash, % | 13.72±0.805 | 13.51±1.245 | 14.583±0.211 | 14.133±0.775 |
| Linoleic acid C 18:2n6 | $48.485 \pm 0.403^{bd}$ | $45.315 \pm 0.912^{a}$ | 47.32±0.17 | $46.425 \pm 1.336^{a}$ |
| α-linolenic acid C 18:3n3 | $1.195 \pm 0.177^{bcd}$ | $6.865 \pm 0.163^{a}$ | $6.42 \pm 0.297^{a}$ | $6.43 \pm 0.523^{a}$ |

*Where: a,b,c,d, significant differences ($P \leq 0.05$) compared to C, E1, E2, E3;

The concentration of linolenic acid was, in average, 5.5 times higher than in the control feeds. The literature shows that the flaxseeds meal is rich in polyunsaturated fatty acids,

which account for 73% of the total fatty acids; it has a moderate content of monounsaturated fats (18%) and a low amount of saturated fats (9%) (Cunnane et al., 1993; Dubois et al., 2007).

Because the concentration of polyunsaturated fatty acids was rather similar in the eggs collected on weeks 2, 4 and 6, table 3 shows the mean of the determinations conducted during the experiment.

The supplemental flaxseed meal given to the experimental groups increased significantly ($P \leq 0.05$) ω – 3 PUFA in the egg yolk, compared to the control group (Table 3). Regarding ω – 6 PUFA, its concentration was higher ($P \leq 0.05$) in the yolk of the eggs from group C, compared to the experimental groups (Table 3). ω – 6/ ω – 3 ratio was significantly higher ($P \leq 0.05$), by about 3 times, in the yolk of the eggs collected from the control group, compared to those from the experimental groups (Table 3). Our data confirm the literature findings, which show that the use of flax, under different forms, in layer diets, increased PUFA concentration in the eggs (Leeson et al., 1998; Aziza et al., 2013).

Table 3. Fatty acids concentration (g% total fatty acids) in egg yolk, depending on their level of saturation

| Item | Initial | Mean of the determinations performed during the experiment | | | |
|---|---|---|---|---|---|
| | | Control group (C) | Experimental group 1 (E1) | Experimental group 2 (E2) | Experimental group 3 (E3) |
| ΣSFA | 34.60±1.112$^{b}$ | 34.43±0.814$^{a,e}$ | 33.91±1.025 | 33.61±0.923 | 32.85±0.916$^{b}$ |
| ΣUFA | 64.62±0.850cde | 65.45±0.838$^{e}$ | 65.93±1.041$^{a}$ | 66.22±0.8$^{a}$ | 66.99±0.906$^{ab}$ |
| ΣMUFA | 56.62±0.668$^{bcde}$ | 33.96±0.944$^{a}$ | 33.94±1.156$^{a}$ | 33.29±0.903$^{ae}$ | 34.44±0.914$^{ad}$ |
| ΣPUFA Of which: | 7.99±0.338$^{bcde}$ | 31.49±0.303 $^{acde}$ | 31.99±0.300$^{abde}$ | 32.92 ±0.255$^{abc}$ | 32.55±0.512$^{abc}$ |
| -ΣPUFA ω - 3 | 1.71±0.07$^{cde}$ | 1.56±0.19$^{cde}$ | 4.58±0.21$^{abe}$ | 4.39±0.24$^{abe}$ | 4.13±0.20$^{abcd}$ |
| -ΣPUFA ω - 3 | 28.62±0.45 $^{bc}$ | 29.93±0.3 $^{acde}$ | 27.41±0.18$^{abde}$ | 28.54±0.30$^{bc}$ | 28.42±0.49$^{bc}$ |
| -PUFA ω – 6/ ω - 3 | 16.75±0.55 $^{bcde}$ | 19.23±2.39$^{acde}$ | 5.99±0.27$^{ab}$ | 6.50±0.41$^{ab}$ | 6.88±0.35$^{ab}$ |

Where: SFA = saturated fatty acids; UFA = unsaturated fatty acids; MUFA = monounsaturated fatty acids; PUFA – polyunsaturated fatty acids; a,b,c,d,e= significant differences ($P \leq 0.05$) compared to initial, C, E1, E2, E3.

The evaluation of lipid degradation level in the eggs collected during the experiment started with the determination of the peroxide value (PV).

Frankel (2005), showed that if no antioxidant is used, the higher is the proportion of unsaturated fatty acids in the fat, the higher is the peroxide value. PV decreased (Figure 1) in the yolk of the eggs collected from groups E1 and E2, unlike the control group and E3 (increasing trend).

Throughout the experimental period, irrespective of the sampling week, the yolk from E2 eggs had the lowest PV (Figure 1), with the lowest one being in the eggs collected in the end of the experiment (week 6).

This shows a higher oxidative stability of the yolk from E2 eggs (3% grape seeds powder), compared to groups C, E1 (100 mg vitamin E/kg CF) and E3 (1.5% grape seeds powder).

Compared to the initial sampling, yolk PV decreased in all experimental groups (Figure 1).

Significant ($P \leq 0.05$) differences were noticed, in the concentration of conjugated dienes (CD) andtrienes (CT), secondary products of lipid oxidation, between the experimental groups (E1, E2, E3) and the control group, C, for each week of sampling and between these intervals.

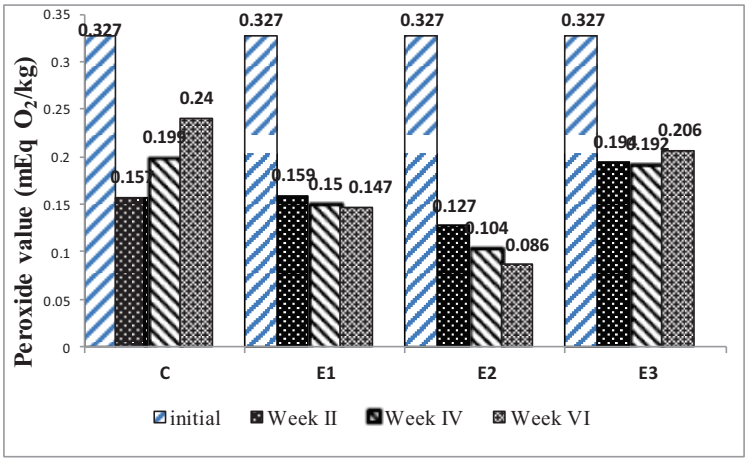


Figure 1.Evolution of yolk PV

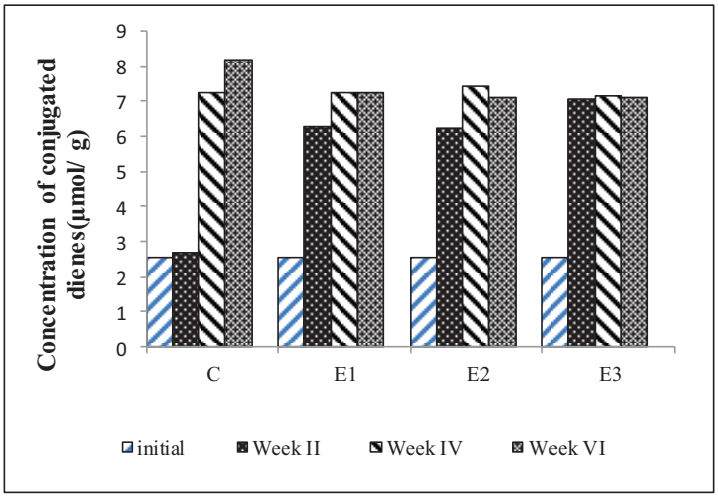


Figure 2. Evolution of yolk concentration of conjugated dienes (CD)

Compared to the initial sampling, CD concentration increased in the yolk from all groups (C, E1, E2, E3), throughout the experiment (Figure 2).

Comparable concentrations were noticed, during the last week of experiment, in the yolk of the eggs from groups E2 and E3, which shows the beneficial effect of the supplemental grape seeds powder (diet formulations E2 and E3) on the oxidative stability of the lipids in the yolks enriched in ω – 3 PUFA.

Figure 3 shows the results of CT ($A_{268}$ nm) absorbance from the yolk samples, and the chemical changes of the lipids determined during the successive sampling periods (weeks 2, 4 and 6), compared to the initial sampling.

Conjugated CT absorbance displays a decreasing trend in all groups (C, E1, E2, E3). The yolk of the eggs collected from groups E2 and E3 were rather similar, and were the lowest in weeks 2 and 6.

Corroborating Figures 2 and 3, one may observe that the yolk of the eggs collected from groups E2 and E3 displayed the highest stability against lipid peroxidation.

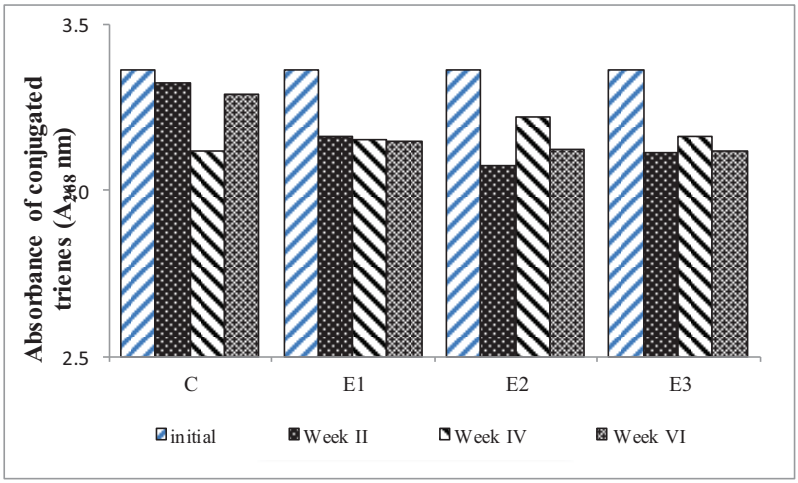


Figure 3. Evolution of conjugated trienes ($A_{CT268}$ nm) formation in the yolk

Throughout the experimental period, the yolk from groups E1 and E2 displayed a higher antioxidant capacity (Table 4). The yolk of E3 eggs collected in week 6, had a significantly ($P \leq 0.05$) lower antioxidant capacity than the other experimental groups (Table 4).

This result, corroborated with the peroxide values (Figure 1), shows that the supplemental 1.5% grape seeds powder was too low to inhibit yolk lipids peroxidation. The yolk of the eggs collected in week 6 from group E2 had a higher antioxidant capacity (8.672 mM ascorbic acid equivalent) compared to the other periods of sampling. This is due to the antioxidant activity of the grape seeds powder (E2).

Papuc et al., (2008) have shown that the oxidation of the unsaturated fatty acids from the foods can be slowed down by the addition of plants rich in compounds with antioxidant activity.

Table 4. Antioxidant capacity of the yolk depending on the period of sample collection

| Item | Initial | Week II | Week IV | Week VI |
|---|---|---|---|---|
| | mM ascorbic acid equivalent | mM ascorbic acid equivalent | mM ascorbic acid equivalent | mM ascorbic acid equivalent |
| Initial | $4.78 \pm 1.644^{abcde}$ | - | - | - |
| Group C | - | $5.037 \pm 1.67^{cd}$ | $5.557 \pm 0.68$ | $5.795 \pm 1.78^{ae}$ |
| Group E1 | - | $6.543 \pm 1.79^{abde}$ | $5.866 \pm 3.57$ | $7.866 \pm 0.71^{ae}$ |
| Group E2 | - | $7.806 \pm 1.17^{abce}$ | $7.079 \pm 1.89^{a}$ | $8.672 \pm 3.67^{ae}$ |
| Group E3 | - | $5.199 \pm 0.67^{cd}$ | $6.465 \pm 0.75^{a}$ | $6.103 \pm 0.36^{abcd}$ |

* Where: a,b,c,d,e, significant differences ($P \leq 0.05$) compared to initial, C, E1, E2, E3.

## CONCLUSIONS

The lowest PV of the yolk lipids was determined in group E2. This shows the beneficial action of the 3% dietary grape seeds powder to delay the onset of lipid peroxidation in the yolks, compared to the lower concentration (1.5%) of dietary grape seeds powder, as noticed in group E3. The concentration of conjugated dienes, secondary product of lipid degradation, was lower in the yolk from the experimental groups, compared to the control group, irrespective of the sampling period. The highest antioxidant capacity was determined in the yolk of the eggs from the experimental groups E1 (100 mg vitamin E/kg CF) and E2 (3 % grape seeds powder).

The experimental results show the opportunity of using natural antioxidants in the compound feeds rich in omega 3 polyunsaturated fatty acids. If they are added in a proper

concentration, they have a constant effect of preventing lipid peroxidation.

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# EFFECTS OF DIETARY SYMBIOTICS AND ORGANIC ZINC ON TRACE MINERALS COMPOSITION OF PORK

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***Abstract***

*An experiment was conducted to evaluate the effects of symbiotics and organic zinc on pork quality (five anatomical parts and three types of organs) of growing pigs. The 28 days study was conducted on 8 castrated Topigs growing pigs, males, with an initial bodyweight of 18.25 ± 0.43 kg. The pigs were assigned to 2 groups (C, E), housed in individual metabolic cages and fed on conventional diets with 18.54% CP and 3129.6 kcal/kg ME. The diets of E group contained organic Zn (E.C.O. Trace® Trace minerals, Biochem Zusatzstoffe Handels- und ProduktionsgesellschaftmbH Küstermeyerstr, Germany) and it was supplemented with 10 g/kg symbiotics (BiominR IMBO Pro/prebiotic, BIOMIN, GmbH Austria). At the end of experiment, all pigs were slaughtered and meat (tenderloin; loin; ham; shoulder; belly) and organ (liver, kidney and spleen) samples were collected. The mineral quality of the collected samples was evaluated. For the samples of loin, tenderloin and belly, the iron concentrations were significantly (P≤0.5) increased for E group, compared to C group (loin: 7.3 ± 0.4 ppm for C; 10.05 ± 0.8 ppm for E; tenderloin: 14.51 ± 2.3 ppm for C; 18.82 ± 0.9 ppm for E and belly: 14.31 ± 1.14 ppm for C; 19.89 ± 1.5 ppm for E). Similar results were obtained for iron concentrations in organs, but, the differences recorded were only numeric. No significant differences were noticed between groups for Zn, Cu and Mn concentrations in the collected samples. The conclusion of the study was that symbiotics and organic zinc had positive effects on mineral metabolism and these results confirm the synergistic interrelation of Zn and Fe.*

***Key words:*** *symbiotics, organic zinc, pigs, mineral quality.*

## INTRODUCTION

The dramatic changes in the international market require high standards of quality assurance in terms of diversity of food and environmental issues, ethics and animal welfare in meat production. Both carcass composition and quality of products from pork depend on many factors: genotype, terms of growth (the feeding, housing and environmental production system), conditions of slaughter, handling and processing of meat and carcases (Sellier, 1998; Monin, 2003), storage time (Granit et al., 2001) and minimizing the growth of pathogens in the gastrointestinal tract (Engber et al., 2002). Also, food is the basic tool to manipulate the growth of animal also influencing the sensory characteristics of meat by increasing the intramuscular fat (DeVol et al., 1988).

Knowledge of the structure, chemical composition, biochemical transformations occurring in meat and factors responsible for changing the main characteristics of the meat, is an important milestone for establishing the nutritional quality of meat. Various biochemical transformations occur in meat and a number of other processes positively affect the organoleptic characteristics.

On the other hand, improper storage conditions lead to degradation through an inevitable deterioration of the quality. Probiotics are viable, defined microorganisms in sufficient numbers, which alter microflora in a compartment of the host and, by that, exert beneficial health effects in this host. Prebiotics are nondigestible food ingredients that beneficially affect the host. A symbiotic is a product containing prebiotics and probiotics and in which the prebiotic compound selectively favours the probiotic compound (Scholz-Arenz et al., 2007).

The purpose of this study was to evaluate the effects of symbiotics and organic zinc on pork quality (five anatomical parts and three types of organs) of growing pigs.

## MATERIALS AND METHODS

The experiment was performed in compliance with Directive 2010/63/EU on the protection of animals used for scientific purposes and all procedures described and it was approved by Ethical Commission of National Research and Development Institute for Biology and Animal Nutrition, Balotesti, Romania.

The 28 days experiment was conducted on 8 growing male, castrated hybrid TOPIGS (Large White × Hybrid (Large White × Pietrain) female × Talent (mainly Duroc), aged 47±3 days, under conditions of experimental balance. Throughout the experimental period, the piglets were randomly assigned to 2 groups (4 animals per group), kept in individual metabolic cages (Agrico, Rybarska, Czech Republic) with an area of 0.87 $m^2$, placed in an experimental hall under controlled environmental conditions (temperature of $24^0$C, humidity 50-60%). The piglets received their diets in one daily meal, at 8.00 a.m., *ad libitum*.

The amount of feed given to each pig was weighed daily, as well as the leftovers (collected each morning). Water was supplied *ad libitum* via drinking nipples. They received a commercial diet designed for this category of animals differing between groups by the level of symbiotic supplement (Tables 1 and 2).

The diets of E group contained organic Zn (E.C.O. Trace® Trace minerals, Biochem Zusatzstoffe Handels- und Produktions gesellschaft mbH Küstermeyerstr, Germany) and 10 g/kg symbiotics (Biomin$^R$ IMBO Pro/prebiotic, BIOMIN, GmbH Austria).

The productive parameters (average daily gain, feed conversion) were calculated from the records of the body weights and feed intake.

At the end of experiment, after blood samples collection, all pigs were slaughtered in an experimental abattoir. Five cuts (tenderloin, shoulder, loin, ham and belly) and organs (liver, spleen and kidney) were dissected, deboned, external fat removed, frozen at -$80^0$C and kept until chemical analysis. Trace mineral concentrations were determined in meat and organ samples applying flame atomic absorption spectrometry (FAAS) as described by Untea et al. (2012) after microwave digestion. The used equipment was as follows: Atomic absorption spectrometer Thermo Electron – SOLAAR M6 Dual Zeeman Comfort (Cambridge, UK), with deuterium lamp for background correction and air-acetylene flame and microwave digestion system with remote temperature measurement, BERGHOF, Speedwave MWS-2 Comfort (Eningen, Germany). Stock solutions of Cu, Fe, Mn, Zn, 1000 ppm traceable to SRM from NIST, were used to standardize the flame atomic absorption spectrometer. Class A glassware was used for transvasation, dilution and storage.

Each pig was considered an experimental unit. All data are expressed as mean value ± standard error of the mean (SEM). The analytical data were compared performing analysis of variance (ANOVA), using STATVIEW for Windows (SAS, version 6.0). The differences between mean values in the groups were considered significant at $P<0.05$.

Table 1. Formulation and chemical composition of feed concentrates used for hybrid Topigs piglets

| Item | C (%) | E (%) |
|---|---|---|
| Soybean meal, 46.0% | 11.80 | 11.80 |
| Lysine | 7.20 | 7.20 |
| Dl - methionine | 1.00 | 1.00 |
| Calcium carbonate 37% | 36.00 | 36.00 |
| Mono calcium phosphate | 18.00 | 18.00 |
| Salt | 4.00 | 4.00 |
| Premix 1%* | 20.00 | 20.00 |
| Choline 60% | 2.00 | 2.00 |
| **Characteristics of the concentrate feeds – Chemical analysis (theoretical calculation)** | | |
| Dry basis, % | 94.35 | 94.35 |
| ME, Kcal/Kg | 785.02 | 785.02 |
| Crude protein, % | 13.14 | 13.14 |
| Crude fat, % | 0.21 | 0.21 |
| Crude fibre, % | 0.71 | 0.71 |
| Calcium, % | 21.57 | 21.59 |
| Phosphorus, % | 4.13 | 4.13 |
| Phosphorus available, % | 3.98 | 3.98 |
| Sodium, % | 1.59 | 1.59 |
| Chloride, % | 3.81 | 3.81 |
| Lysine, % | 6.02 | 6.02 |
| Methionine, % | 1.07 | 1.07 |
| Met + cis, % | 1.16 | 1.16 |
| Threonine, % | 0.22 | 0.22 |
| Tryptophan, % | 0.07 | 0.07 |
| Linoleic acid, % | 0.11 | 0.11 |

*the premix of E group contained organic Zn

Table 2. Formulation and chemical composition of compound feeds used for hybrid Topigs piglets

| Item | C (%) | E (%) |
|---|---|---|
| Maize | 35.70 | 35.69 |
| Wheat | 26.00 | 26.00 |
| Rice bran | 9.00 | 9.00 |
| Corn gluten | 3.00 | 3.00 |
| Soybean meal | 17.30 | 17.30 |
| Sunflower meal | 4.00 | 4.00 |
| **Feed concentrate** | **5.00** | **5.00** |
| Symbiotics | - | 0.01 |
| **Chemical composition** | | |
| Dry basis. % | 86.89 | |
| ME, Kcal/Kg | 3129.60 | |
| Crude protein. % | 18.54 | |
| Crude fat, % | 1.65 | |
| Crude fibre, % | 4.12 | |
| Calcium, % | 1.20 | |
| Phosphorus, % | 0.65 | |
| Phosphorus available, % | 0.33 | |
| Sodium, % | 0.11 | |
| Chloride, % | 0.07 | |
| Lysine, % | 1.11 | |
| Methionine, % | 0.36 | |
| Met + cis, % | 0.69 | |
| Threonine, % | 0.66 | |
| Tryptophan, % | 0.21 | |
| Linoleic acid, (c18:2) (%) | 1.06 | |

## RESULTS AND DISCUSSIONS

Pig performance values showed no significant differences between groups. During the experimental period, all productive parameters, final weight, average daily gain (ADG), average daily feed intake (ADFI) and feed conversion efficiency were slightly increased (P > 0.05), for E group (Table 3). There was no effect on overall growth performance of pigs fed supplemental organic Zn and symbiotics.

Table 3. Production parameters

| Parameter | C | E |
|---|---|---|
| Initial weight, (kg/head) | 18.25 ± 0.433 | 18.375 ± 0.315 |
| Final weight , (kg/head) | 35.83 ± 2.553 | 37.00 ± 2.041 |
| ADG, (g/head/day) | 0.628 | 0.665 |
| ADFI, (g/head/day) | 1199.673 | 1422.054 |
| Feed efficiency, (g/kg) | 2.08±0.324 | 2.13±0.343 |

The copper, iron, manganese and zinc concentrations in piglet organs and meat were chosen to obtain an indicator for measurement of the trace elements bioavailability (Richards et al., 2010) from the diet supplemented with symbiotics. The deposits of Cu, Fe, Mn and Zn in the main organs (liver, spleen and kidney) were evaluated and the results are presented in table 4. No significant differences (P>0.05) were noticed between groups for mineral concentrations determined in the selected organs. A slight increase of Fe concentrations was observed, but due to a large range of values, the differences were inconsistent (P>0.05). Similar ranges of values of mineral organs concentrations in pigs were reported in the scientific literature (Luo and Dove, 1996; Jondreville et al., 2005; Apgar et al., 1995).

Table 4. Mineral composition of selected organs (liver, kidney, spleen)

| Organs | Cu (mg/kg) | Fe (mg/kg) | Mn (mg/kg) | Zn (mg/kg) |
|---|---|---|---|---|
| *Liver* | | | | |
| Control | 4.36±0.49 | 102.23±20.48 | 3.98±0.16 | 39.10±4.76 |
| Experimental | 4.23±0.18 | 118.39±28.08 | 3.73±0.73 | 38.12±3.19 |
| *Kidney* | | | | |
| Control | 5.84±2.55 | 40.20±9.36 | 1.13±0.30 | 20.54±2.47 |
| Experimental | 5.71±1.33 | 52.76±5.59 | 1.36±0.21 | 21.24±2.40 |
| *Spleen* | | | | |
| Control | 0.64±0.07 | 101.10±18.63 | 0.26±0.06 | 21.58±1.24 |
| Experimental | 0.68±0.03 | 129.61±22.91 | 0.24±0.05 | 21.55±0.81 |

Results are expressed as a mean ± SD.

Table 5. Mineral composition of anatomical parts (tenderloin; loin; ham; shoulder, belly)

| Anatomical parts | Cu (mg/kg) | Fe (mg/kg) | Mn (mg/kg) | Zn (mg/kg) |
|---|---|---|---|---|
| *Ham* | | | | |
| Control | 0.43±0.18 | 9.82±0.67 | 0.04±0.02 | 14.31±0.91 |
| Experimental | 0.41±0.11 | 10.66±1.32 | 0.04±0.05 | 15.75±2.29 |
| *Belly* | | | | |
| Control | 0.67±0.05 | 14.31±1.14 [b] | 0.35±0.10 | 21.22±2.22 |
| Experimental | 0.75±0.26 | 19.89±1.53 [a] | 0.31±0.13 | 21.74±2.56 |
| *Shoulder* | | | | |
| Control | 0.78±0.01 | 14.19±2.12 | 0.03±0.03 | 22.32±2.85 |
| Experimental | 0.69±0.08 | 14.62±2.37 | 0.02±0.02 | 22.19±2.62 |
| *Loin* | | | | |
| Control | 0.35±0.09 | 7.30±0.40 [b] | 0.20±0.09 | 12.21±0.51 |
| Experimental | 0.45±0.10 | 10.05±0.85 [a] | 0.28±0.06 | 11.91±0.88 |
| *Tenderloin* | | | | |
| Control | 0.59±0.10 | 14.51±2.29 [b] | 0.18±0.14 | 13.63±0.21 |
| Experimental | 0.69±0.09 | 18.82±0.96 [a] | 0.23±0.05 | 13.48±0.87 |

In the same column,different superscripts mean significantly different (P<0.05) from C (a) respective E (b).
Results are expressed as a mean ± SD.

Five anatomical parts were considered for the evaluation of symbiotics and organic zinc effect on carcass mineral properties: tenderloin; loin; ham; shoulder, belly.

The recorded trace mineral concentrations are presented in table 5. In the case of iron, significant differences (P<0.05) were noticed between groups for loin, tenderloin and belly. These results sustain the previous observation (Table 4) about the positive effect noticed on iron concentrations in organs.

Prebiotics are non-digestible food ingredients that beneficially affect the host by selectively stimulating the growth and / or activity of one or a limited number of bacteria (probiotics) in the gastrointestinal tract, and thus exert a health promoter effect (Roberfroid, 2002).

It was shown that there is an increase in intestinal absorption of minerals due to indigestible carbohydrates (Coudray et al., 2006).

Vanhoof and DeSchrijver (1996) tested the effect of inulin on Fe and Zn absorption in rats and pigs and noticed that Zn absorption was significantly higher for experimental group. Yalçınkaya et al. (2012) performed a study on broilers and showed that prebiotics mannan-oligosaccharide facilitates absorption of Fe and Zn and stimulate Cu retention.

## CONCLUSIONS

The results of the study indicate that symbiotics and organic acids supplements improve mineral parameters of pork quality. The concentrations of Fe were improved in meat and organ samples, indicating the symbiotic and organic zinc potential in developing functional foods.

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# FEEDING QUALITY OF THE MEAT FROM BROILERS FED WITH DIETARY FOOD INDUSTRY BY-PRODUCTS (FLAXSEED, RAPESEEDS AND BUCKTHORN MEAL, GRAPE POMACE)

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***Abstract***

*A feeding trial was conducted on 75, ROSS308 (0-42days) broilers, to evaluate the quality of the meat from broilers which received food industry by-products in their diets: rapeseeds meal and grape pomace, or flaxseeds meal and buckthorn meal. The broiler chicks were assigned to 3 groups (C, E1 and E2), and were housed in an experimental hall with controlled environmental conditions: average air temperature 27.07±2.75°C, humidity 64.80±9.57%. For 10 days, during the starter phase, all chicks received a conventional compound feed. In the other two stages (growing, finishing), compared to the conventional diet given to the C group, the diet formulations of the experimental groups included different proportions, depending on the phase of development, rapeseeds meal and grape pomace (E1), or flaxseeds and buckthorn meals (E2). The highest polyphenols concentration was determined in the finishing diet formulation for group E1 (with 8% rapeseeds meal and 4% grape pomace). The dietary concentration of ω 3 polyunsaturated fatty acids (ω 3 PUFA) in the diet formulations for group E2 increased with the level of dietary flaxseeds meal (2.5% during the grower phase and 8% in the finishing phase). Six broilers from each group were slaughtered in the end of the trial and meat (breast and thigh) and liver samples were collected and assayed for dry matter, protein, fat, ash, fatty acids and cholesterol. The highest concentration of ω 3 PUFA, which are essential for human health, were determined in the breast and thigh of E2 broilers (flaxseeds meal and buckthorn meal). The cholesterol level in the breast meat and thigh samples was not significantly different between groups; however, it was lowerin the experimental groups than in the control group. The fat level in the liver samples collected from C group broilers was significantly (P≤0.05) higher than in the experimental groups.*

***Key words:*** *broilers, by-products, feeding quality, breast meat, thigh, liver.*

## INTRODUCTION

The increasingly large amounts of vegetable by-products resulting from the food industry causes economic losses and bears an adverse impact on the environment. At the same time, the increasing cost of animal feeding increase the total production costs, in which the cost of feeding represents 60-70%. Most people are familiar with the three "R" concept (reduce, reuse, recycle). A fourth R, responsibility, might be the key to a sustainable society. The most efficient disposal of the by-products is to use them as animal feeds ingredient, but it is sometimes limited by the legislation and by the nature of the particular by-product. Researchers have been increasingly concerned, lately, with finding new feeding solutions for poultry, which would allow achieving high performance at low costs (Lup, 2010).

The food industry by-products are rich in valuable nutrients: vitamins, minerals, polyphenols, polyunsaturated fatty acids, pigments, etc. Much of the food industry by-products, the meals, come from the oil extraction industry, being the wastes that remain after oil extraction from the oil seeds. Rapeseed meal is among the "classical" meals used in farm animal feeding. Rapeseed is

mainly an oil-source 40–45% DM, but the rapeseed meal obtained after extraction of oil is an interesting protein-source with protein content varying between 32% and 45% DM. While the advantage of rapeseed meal is the quality of its protein (its amino acid profile is more interesting than that of soybean), it contains a high proportion of fibre besides other anti-nutritional factors ANF such as tannins, sinapin and phytic acid (Burel et al., 2000). Several, authors warn against using a high level of rapeseeds meal in broiler diets (Karunajeewa, 1999; McNeill et al., 2004). On the other hand, Wetscherek et al. (1990) showed that up to 20% rapeseed oil meal can be included in broiler diets without affecting performance. Another oil industry by-product, the flaxseed meal, is increasingly used in poultry production due to its large content of fatty acids: 12.50% saturated FA, 24.21% monounsaturated FA, 43.23% ω:3 FA and 20.06% ω:6 FA (Aziza et al., 2013). Mridula et al. (2011) noticed that the alpha-linolenic acid, omega 3 acid, content in both breast and thigh meat was higher with an increasing level of flaxseed meal in the diets without affecting the sensory acceptability of meat. The same authors consider that ca up to 10% of flaxseed meal may be used in broiler diet to enhance the alpha-linolenic acid content in the broiler meat.

As the industry of natural food supplements developed, new by-products appeared, such as the buckthorn meal. Buckthorn is a rich source of natural antioxidants such as ascorbic acid, tocopherols, carotenoids, flavonoids, while they contain proteins, vitamins (especially vitamin C), minerals, lipids (mainly unsaturated fatty acids), sugars, organic acids and phytosterols (Christaki, 2012). Kaushal and Sharma (2011) have shown that the buckthorn fruits are adequate for animal feeding too. In a study on the effect of flavones of sea Buckthorn on carcass characteristics and meat quality of Arbor Acres broilers, Li et al. (2008) have noticed that at the dose of 0.2% flavones of sea buckthorn, ether extract of thigh muscle and serum triglyceride were significantly decreased ($P \leq 0.05$).

Grape (*Vitis vinifera*) is one of the world's largest fruit crops (FAO-STAT, 2007). Recent investigations have stressed the importance of by-products from wine processing as plant materials that are particularly rich in polyphenols and have a wide range of biological activities. Grape pomace is the residue left after juice extraction by pressing grapes in the wine industry. This by-product (constituted by seeds, skin, and stem) is used every year either as animal feed (with low nutritional value) or for ethanol production by fermentation and distillation (Viveros et al., 2011). Grapes contain a large amount of polyphenols which include the phenolic acids, anthocyanins and proanthocyanidins (Lu and Foo, 1998). Goni et al. (2007) found out that the dietary grape pomace can delay lipid oxidation in breast and thigh chicken meats and reduce the potential risk induced by lipid oxidation products.

Within this context, we conducted a study to evaluate the quality of broiler chicken treated with pairs of food industry by-products: rapeseed meal and grape pomace, or flaxseed meal and buckthorn meal.

## MATERIALS AND METHODS

A feeding trial was conducted on 75, ROSS 308 broilers, from 0 to 42 days of age. The day-old chicks were weighed individually and assigned to three homogenous groups: 42.39 ± 0.18 g (C); 42.16 ± 0.24 g (E1); 42.748 ± 0.21 g (E2). The broiler chicks were housed in an experimental hall with controlled environmental conditions, according to the management guide: average air temperature 27.07 ± 2.75°C, humidity 64.80 ± 9.57%, ventilation/broiler 0.50 ± 0.24%; $CO_2$ level, 686.39 ± 104.38 (ppm). The broilers had free access to the feed and water.

Diet formulation was calculated using the results of the chemical analysis of the feed ingredients in agreement with the feeding requirements (NRC, 1994) and using a mathematical model for poultry diets formulation (Burlacu et al., 1999). For 10 days, during the starter phase, all chicks received a conventional compound feed, with the purpose of developing a good appetite and reaching the standard body weight at 7 days. In the other two stages (growing, finishing), compared to the conventional diet given to the control broilers, the diet formulations of the experimental groups included different proportions, depending on the phase of development (Table 1).

Table 1. Diet formulations

| Ingredient | Phase II – growth (11 – 28 days) | | | Phase III – finishing (29 - 42 days) | | |
|---|---|---|---|---|---|---|
| | C | E1 | E2 | C | E1 | E2 |
| | % | | | | | |
| Corn | 51.32 | 46.1 | 50.22 | 60.23 | 51.98 | 59.61 |
| Soybean meal | 38.32 | 32.6 | 35.54 | 30.04 | 24.29 | 23.26 |
| *Rapeseed meal* | - | *8.00* | - | - | *8.00* | - |
| *Grape pomace* | - | *2.00* | - | - | *4.00* | - |
| *Buckthorn meal* | - | - | *2.00* | - | - | *2.00* |
| *Flaxseed meal* | - | - | *2.50* | - | - | *8.00* |
| Plant oil | 5.73 | 6.90 | 5.04 | 5.16 | 7.45 | 2.41 |
| Lysine | 0.02 | 0.05 | 0.13 | 0.11 | 0.14 | 0.36 |
| Methionine | 0.25 | 0.21 | 0.29 | 0.23 | 0.20 | 0.32 |
| Choline | 0.05 | 0.05 | 0.05 | 0.05 | 0.05 | 0.05 |
| Calcium carbonate | 1.67 | 1.46 | 1.58 | 1.63 | 1.35 | 1.45 |
| Monocalcium phosphate | 1.23 | 1.22 | 1.24 | 1.17 | 1.15 | 1.15 |
| Salt | 0.41 | 0.41 | 0.41 | 0.38 | 0.39 | 0.39 |
| Premix | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 |
| **Total** | **100** | **100** | **100** | **100** | **100** | **100** |

*1kg IBNA premix (A1)contains: = 1100000 IU/kg vit. A; 200000 IU/kg vit. D3; 2700 IU/kg vit. E; 300 mg/kg vit. K; 200 mg/kg vit. B1; 400 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; 2000 mg/kg vit. C; 8000 mg/kg manganese; 8000 mg/kg iron; 500 mg/kg copper; 6000 mg/kg zinc; 37 mg/kg cobalt; 152 mg/kg iodine; 18 mg/kg selenium; 6000 mg/kg antioxidant.

Samples of the studied by-products (rapeseed, flaxseed and buckthorn meals, grape pomace) and of the compound feeds were collected and assayed for the basic chemical composition: dry matter (DM), crude protein (CP), ether extractives (EE), crude fibre (CF) and ash (Ash), using the chemical methods from Regulation (CE) no. 152/2009 (Methods of sampling and analysis for the official inspection of feeds). The fatty acids were determined by gas chromatography, according to SR CEN ISO/TS 17764 -2:2008. The content of total phenols in the methanol feeds extracts, egg white and yolk extracts was determined according to the method described by Mihailovic et al. (2013), while the flavonoids content of the methanol feeds extracts was determined according to the method described by Zhishen et al. (1999).

In the end of the feeding trial, according to the working protocol approved by the ethic commission of IBNA Balotesti (decision nr. 52/30.07.2014), 6 broilers/group were slaughtered and meat (breast and thigh) and liver samples were collected and assayed for their feeding quality (dry matter, protein, fat, ash, fatty acids and cholesterol).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 $550^0$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 method used to determine the cholesterol was in agreement with AOAC International standard, 2002 (Cholesterol in multicomponent foods – Gas Chromatographic method. Assoc. of Anal. Chem. Arlington, VA). The working principle is the saponification of the sample followed by extraction is petrol ether, concentration and addition of chloroform. The sample is split in the GC, it is separated in the chromatographic column, and the results are compared with the standard chromatograms by measuring the peak area. It was used a Perkin Elmer-Clarus 500 chromatograph fitted with flame ionization

detector (FID) and capillary separation column HP-5, 30 in length, and 0.320 mm inner diameter, 0.10 µm thick film.
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

The basic chemical analysis of the dietary by-products (Table 2) revealed a high protein level in the rapeseed meal and flaxseed meal. The high fibre level from all used by-products show that they must be included in moderate level sin poultry diets. The values from Table 2 are in agreement with the literature data, although the by-products have a lower level of chemical composition stability.
The rapeseed meal had 43.49% dry matter, 37,98% crude protein (Bell,1990) and 12.8% crude fibre (Jensen, 1994). The grape pomace had 88.44% dry matter, 10.64% crude protein and 40.66% crude fibre (Olteanu et al., 2014). Panaite (2016) found that the flaxseed meal had 20.91% crude protein and 7.18 % crude fibre.

Table 2. Chemical composition of the studied by-products*

| Item | Rapeseed meal | Flaxseed meal | Grape pomace | Buckthorn meal |
|---|---|---|---|---|
| DM% | 90.03 | 90.46 | 89.92 | 88.94 |
| CP% | 31.15 | 29.97 | 12.33 | 11.66 |
| EE% | 1.02 | 15.69 | 5.95 | 12.46 |
| CF% | 12.40 | 11.16 | 35.17 | 15.10 |
| Ash% | 5.71 | 3.87 | 2.83 | 2.69 |
| Σ SFA | 14.88 | 13.67 | 30.06 | 10.13 |
| ΣMUFA | 41.53 | 19.51 | 42.63 | 19.55 |
| Σ PUFA, (g/100g fatty acids) of which | 43.19 | 70.33 | 66.60 | 27.33 |
| - ω:3 | 5.68 | 43.42 | 1.12 | 4.88 |
| - ω:6 | 37.51 | 26.91 | 65.48 | 22.45 |
| - ω:6/ Ω: ω3 | 6.60 | 0.62 | 58.40 | 4.60 |

where:DM=dry matter; CP=crude protein; EE= ether extractives; CF=crude fibre; Ash=ash; Σ= sum; SFA =saturated fatty acids; MUFA=monounsaturated fatty acids; PUFA= polyunsaturated fatty acids.
*Chemical composition on dry matter (DM) basis.

The fatty acids profile (Table 2) showed that the flaxseed meal is particularly rich in omega 3 polyunsaturated fatty acids (omega 3 PUFA), as also shown by other authors (Aziza et al., 2013; Panaite et al., 2016).
The data shown in Table 3 reveal that the level of omega 3 polyunsaturated fatty acids (ω 3 PUFA) was higher in the diet formulation with flaxseed meal (E2), in both phases of development, than in groups C and E1. The level of ω 3 PUFA was higher in the finishing formulation for E2, with a higher flaxseed meal level, than in the grower formulation for the same group. In terms of the oxidative status of the compound feeds (Table 3), the highest concentration of polyphenols (2.60 mg gallic acid equivalents/g) was determined in E1 formulation for the finishing phase (8% rapeseed meal and 4% grape pomace).
The high antioxidant activity of the grape pomace has also been reported by other authors who used it in broiler diets (Goñi et al., 2007; Brenes et al., 2008). The concentration of polyphenols in E2 diet formulation for both phases, which contained 2% buckthorn meal, was also higher than in the control group (Table 3).

Table 3. Chemical composition of the compound feeds *

| Item | Growth phase (11 – 28 days) | | | Finishing phase (29 - 42 days) | | |
|---|---|---|---|---|---|---|
| | **C** | **E1** | **E2** | **C** | **E1** | **E2** |
| DM % | 89.17 | 89.03 | 88.81 | 89.10 | 89.40 | 88.92 |
| CP % | 22.62 | 20.79 | 22.20 | 18.80 | 19.53 | 18.81 |
| EE % | 7.51 | 8.41 | 6.95 | 7.22 | 9.56 | 6.06 |
| CF % | 4.06 | 5.88 | 4.03 | 3.87 | 5.66 | 4.62 |
| Ash % | 5.60 | 5.74 | 6.03 | 6.02 | 5.41 | 5.29 |
| g /100g total fatty acids: | | | | | | |
| Σ SFA | 12.15 | 11.66 | 12.94 | 13.17 | 12.81 | 13.91 |
| Σ MUFA | 28.24 | 28.73 | 28.25 | 29.32 | 29.25 | 28.24 |
| Σ PUFA, of which: | 59.09 | 59.20 | 58.37 | 57.07 | 57.62 | 57.54 |
| - ω:3 | 1.03 | 0.92 | 4.13 | 1.48 | 1.02 | 9.71 |
| - ω:6 | 58.06 | 58.27 | 54.24 | 55.59 | 56.60 | 47.83 |
| - ω:6/ ω:3 | 56.36 | 63.31 | 13.13 | 37.69 | 55.25 | 4.92 |
| Polyphenols (mg galic acid equivalents/g | 1.160 | 1.79 | 1.59 | 1.161 | 2.60 | 1.68 |

where:DM=dry matter; CP=crude protein; EE= ether extractives; CF=crude fibre; Ash=ash; Σ= sum; SFA =saturated fatty acids; MUFA=monounsaturated fatty acids; PUFA= polyunsaturated fatty acids.
*Chemical composition on dry matter (DM) basis.

The total gains of the broilers, recorded in the end of the experiment, were comparable between groups: 2.39 kg (C); 2.32 kg (E1); 2.18 kg (E2). Therefore, the quality of broiler meat can be compared between the three groups. Table 4 data show that the best results were obtained for the breast meat samples from the broilers which received E2 formulations, rich in ω 3 PUFA. A significant (P≤0.05) decrease of the fat percentage was noticed in this group, compared to the broilers fed on the conventional formulation (group C). The concentration of ω 3 PUFA in the breast meat samples was higher (P≤ 0.05) in the experimental groups than in the control group. Betti et al., (2009) reported 300 mg ω 3 PUFA per 100 g breast meat, in 26.2 days, with 10% flaxseed meal. Kamboh and Zhu (2013) also reported a low proportion of saturated fatty acids and an increasing proportion of polyunsaturated fatty acids in the broiler meat, by supplementation with bioflavonoids, such as groups E1 (grape pomace) and E2 (buckthorn) from this experiment.

The protein level didn't vary in the thigh samples collected from the 3 groups. However, unlike the breast meat samples, the proportion of fat was significantly (P≤0.05) higher in thigh from group E1 than in groups C and E2 (Table 5). The highest value (P≤0.05) of ω 3 PUFA concentration in the broiler thigh (Table 5) was noticed, same as for the breast meat samples, in group E2. However, an ω 6/ ω 3 PUFA ratio closer to the ideal value of 1 (Simopoulos, 2002) was determined in the thigh meat samples from group E2, the value of this ratio being 49.83% lower than in group C and 53.53% lower than in group E1 (Table 5).

Table 4. Chemical composition of the breast muscle

| Item | C | E1 | E2 |
|---|---|---|---|
| Dry matter, % | 24.07±1.23 | 24.74±0.58 | 23.34±2.16 |
| Protein, % | 22.09±1.72 | 22.17±0.51 | 21.71±2.82 |
| Fat, % DM | 1.12±0.07 $^{c,}$ | 1.25±0.13 $^{c}$ | 0.83±0.18 $^{a,b,}$ |
| Ash, % DM | 1.19±0.10 $^{c}$ | 1.17±0.05 | 1.09±0.09 $^{a}$ |
| Σ PUFA, (g/100g fatty acids) of which: | 31.73±0.29 $^{b}$ | 33.94±1.48 $^{ac}$ | 32.24±1.93 $^{b}$ |
| - ω:3 | 1.865±0.116$^{bc}$ | 2.74±1.495$^{a}$ | 3.212±0.329$^{a}$ |
| - ω:6 | 29.68±0.306 | 30.1±0.897 | 28.745±1.601 |
| - ω:6 / ω:3 | 15.97±1.069b$^{c}$ | 10.737±5.819 $^{a}$ | 8.99±0.419 $^{a}$ |

where: a,b,c, significant differences (P≤0.05) compared to C, E1, E2;
Σ= sum; PUFA= polyunsaturated fatty acids

Table 5. Chemical composition of the thigh samples

| Item | C | E1 | E2 |
|---|---|---|---|
| Dry matter, % | 25.51±1.06 | 25.72±1.32 | 26.49±0.89 |
| Protein, % | 18.96±0.62 | 19.21±1.21 | 19.22±0.70 |
| Fat, % DM | 4.08±0.37 $^{b,}$ | 5.01±0.34 $^{a,c}$ | 4.28±0.33 $^{b}$ |
| Ash, % DM | 0.99±0.06 | 0.91±0.07 | 0.88±0.04 |
| Σ PUFA, (g/100g fatty acids) of which: | 33.11±0.57$^{b,c}$ | 39.53±0.68$^{a,c}$ | 41.40±0.19$^{a,b}$ |
| - ω:3 | 1.75±0.08$^{c}$ | 1.95±0.15$^{c}$ | 4.18±0.10$^{a,b}$ |
| - ω:6 | 31.00±0.57$^{b,c}$ | 37.16±0.55$^{a}$ | 37.07±0.22$^{a}$ |
| - ω:6 / ω:3 | 17.70±0.92$^{b,c}$ | 19.11±1.55$^{a,c}$ | 8.88±0.22$^{a,b}$ |

where: a,b,c, significant differences (P≤0.05) compared to C, E1, E2;
Σ= sum; PUFA= polyunsaturated fatty acids

The diet formulations enriched in ω 3 PUFA determine a higher content of these fatty acids in the meat and eggs, resulting thus foods that are a natural source of these essential nutrients for the consumers (Leskanich and Noble, 1997).

Figure 1 shows the concentrations of ω 3 PUFA, which are essential acids for human health (Simopoulos et al., 2000), in the breast and thigh meat samples.

The concentration of α –linolenic acid (C 18:3n3) was 68.80% and 68.52 % lower in the breast from group C broilers, than in the breast samples from groups E1 and E2, respectively.

The concentration of docosapentaenoic acid (C 22:5n3) too, was 43.3 % and 76.09% lower in the breast samples from group C broilers, than in the breast samples from groups E1 and E2, respectively.

The docosahexaenoic acid (C 22:6n3) was 13.79% (E1) and 44.44% (E2) higher in the breast samples of these groups, than in group C.

The concentration of α-linolenic acid in the thigh samples from group E2 was 7 times higher than in the thigh samples from group C, while the concentration of docosahexaenoic acid was over 4 times higher.

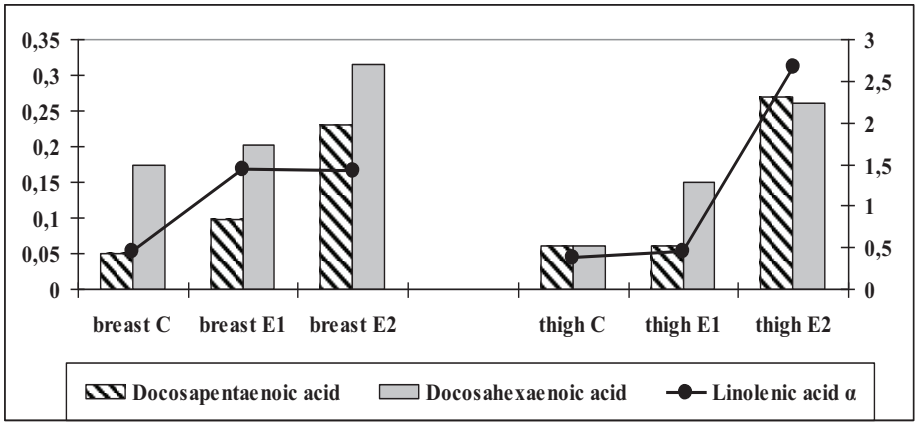


Figure 1. Concentration of omega 3 polyunsaturated fatty acids (g/ 100g total acids) in the breast and thigh meat sample

Figure 2 shows that the cholesterol concentration in the thigh samples from groups C and E2 was higher than in the breast meat but the difference was not statistically significant. The lowest cholesterol concentration in the thigh samples was recorded in group E1 (Figure 2). The breast samples from group C had the highest concentration of cholesterol, but also the difference was not statistically significant.

The chemical analysis of the liver samples (Table 6) collected in the end of the experiment showed that the fat level of these samples was significantly (P≤0.05) higher in group C than in the experimental groups (E1, E2).

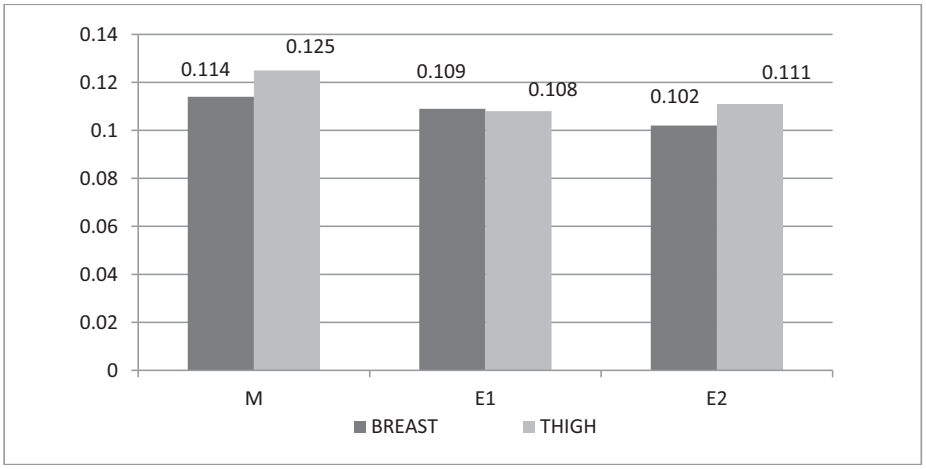


Figure 2. Cholesterol (g %) concentration in the breast and thigh meat samples

Table 6. Chemical composition of the liver samples

| Item | C | E1 | E2 |
|---|---|---|---|
| Dry matter, % | 26.73±2.57 | 25.96±0.82 | 25.71±0.74 |
| Protein, % | 18.11±1.10 | 17.85±0.95 | 18.12±0.79 |
| Fat, % DM | 3.92±1.15 [b,c] | 2.95±0.34 [a] | 2.60±0.10 [a] |
| Ash, % DM | 1.21±0.15 | 1.30±0.08 | 1.30±0.08 |
| Σ PUFA, (g/100g fatty acids) of which: | 36.85±3.92 [b.c] | 43.68±1.89 [a] | 42.32±1.07 [a] |
| ω:3 | 1.90±0.31 [c] | 1.60±0.15 [c.] | 5.46±0.60 [a .b] |
| ω:6 | 34.84±3.73 [b.] | 41.89±1.79 [a.c] | 36.71±1.10 [b] |
| ω:6 / ω:3 | 18.58±2.48 [b.c] | 26.31±2.20 [a.c] | 6.80±0.75 [a.b] |

where: a,b,c, significant differences (P≤0.05) compared to C, E1, E2; Σ= sum; PUFA= polyunsaturated fatty acids.

The concentration of omega 3 fatty acids determined in the liver samples from group E2 was significantly (P≤0.05) higher than in the samples from groups C and E1 (Table 6). All the omega 3 acids essential to human health, α-linolenic, docosapentaenoic and docosahexaenoic, were significantly (P≤0.05) higher in the liver samples from group E2 (flaxseed meal) than in the other groups. Also in this group, ω 6/ ω 3 PUFA ratio was significantly (P≤0.05) lower than in the other groups.

## CONCLUSIONS

The high fibre level from all the used by-products shows that they have to be included in moderate levels in the diet formulations for poultry. The highest polyphenols concentration was determined in the diet formulation for group E1, the finishing phase (8% rapeseed meal and 4% grape pomace), which is due to the high antioxidant activity of the grape pomace. The dietary grape pomace also increased the level of ω 3 PUFA in this group. As the dietary level of flaxseed meal increased in the formulations for group E2 (2.5% in the growing phase and 8% in the finishing phase), the dietary concentration of ω 3 PUFA also increased.

The feeding trial has shown that the highest concentration of ω 3 polyunsaturated fatty acids, essential to human health, was determined in the breast and thigh meat samples from E2 broilers (flaxseed and buckthorn meals). The cholesterol concentration in the breast meat samples was not significantly different between groups, although they were lower in the experimental groups than in the control group. In group C, the fat level from the liver samples was significantly (P≤0.05)

higher than in the liver samples from the experimental groups.

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# THE USE OF TURMERIC (*Curcuma domestica* Val) MEAL IN THE RATIONAS FEED ADDITIVE ON HEN-DAY PRODUCTION AND EGG QUALITY OF SENTUL CHICKEN

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***Abstract***

*Sentul chicken is recognized as a dual purpose local chicken breed and it is very potential because it growth rapidly and it has high eggs productivity. They are able to adapt to the environment and it remains productive even thoughthe diets given are low of quality, and they resist to diseases and the husbandry of the does not require a special skill. One of the alternative to improve eggs quality is by giving the ration added with a Turmeric (Curcuma domestica Val) meal. Turmeric is one of the medicinal plants used as an herbal medicine containing atsiri oil (volatile oil) and curcuminoid. The aim of this research was to evaluate the hen-day production and thequality of Sentul's egg added turmeric meal in the ration. Sixty Sentul Chickens at 42 weeks of age were used. The data were analyzed using a Completely Randomized Design consisted of four treatments, which were 0, 0.1, 0.2, and 0.3 percent of turmeric meal, and each treatment was repeated five times. The statistical analysis indicated that the effect of the addition of turmeric meal (Curcuma domestica, Val) in ration was significant (P<0.05) on hen-day production, egg weight, and egg yolk colorscore, but it was not significant (P>0.05) on yolk index, Haugh unit value and shell thickness. All and all the use of turmeric meal in the ration of Sentul chicken up to 0.2 percent support the high quality egg formation, and theaddition of 0.3 percent turmeric into Sentul chicken's ration have positive effect on egg yolk color.*

***Key words****: Sentul chicken, turmeric (Curcuma domestica Val) meal, hen-day production,egg quality.*

## INTRODUCTION

Sentul Chicken is a local chicken from Ciamis region, West Java, Indonesia, and it is a dual purpose type which can bepurposed for eggs and meat production.

They are able to adapt with the environment and it remains productive even though their diets are low of quality.

This chicken resiston diseases and their breeding does not require a special technique (Widjastuti, 1996).

The egg of Sentul chicken has high nutritional value which can be accepted by all consumers. The yolk color of it has its own charm, thus from the observations,the yolk color which is paler thanthat of local chicken eggs is often rejected by the cake manufacturing industry. Meanwhile, there is a growing assumption aboutthe egg yolk color which is often defined as the quality of nutrient that the more yellow of yolk is the higher content of nutrition. So in order to face this market assumption, some treatments have to be conducted.

Recently, the demand of the eggs has to be free from the residue of any drugs. The presence of pathogenic bacterial which is causing infectionis often causing diseases in chickens, so it makesthe livestock productivity are decreasing. In order to avoid the bacterial infection, usually, it is given the antibiotics (Agustina, 2006; Khusman et al., 2008). However, the utilization of antibiotics as feed additives is prohibitedsince it endangers the health of both humans and livestock, because, the residue is left behind as well as the resistance of the bacteria. Therefore, in order to face that problem, the alternative which has a same purpose but not harmful to the health of livestock is needed, and the answer is phytobiotic.

The solution, the alternative food ingredients which has good quality,is expected to be able to reduce the production cost. Moreover, several studies have been conducted by using natural ingredients in rations containing curcumoid such as *Curcuma xanthorriza* Roxb, garlic or *Curcuma zedoaria* Rosc meal, which was frequently used as the ingredients of

traditional medicine or herbs (Maheswari, 2002).

Turmeric (*Curcuma domestica* Val) is one of phytobiotic use as herbal medicine component production.

Turmeric are very nutritious for healing the stomachache, reinforcing the digestion and appetite, stimulating intestine movements and eliminating the indigestion (Mangisah, 2005; Widjastuti, 2010).

Turmeric is one of the herbs which can be used as feed additives and it has good enough quality when it is added to ration for poultry (Pratikno, 2010). Turmeric can be used as growth promoters and immunomodulatory or antibacterial in poultry.

Turmeric contains 6.3% crude protein, 5.1% crude fat, 69.4% carbohydrates, 13.1% moisture (Chattopadhyay et al., 2004), 2.4 to 4% essential fatty acids and 4.7 to 8.2% crude ash (Kermanshahi and Riasi, 2006).

The curcuminoid content in turmeric is 3-5% curcumine and its derivatives, called demethoxycurcumine and bisdemethoxy-curcumine and it is also containing atsiri oil (volatile oil) approximately 2.5 to 6%.

Curcumine can improve the performance of the digestive tract, the immune system of poultry, thus it can produce the good quality carcass. In addition to prevent diseases, turmeric can also provide color on the carcass and egg yolk (Somaatmadja, 1981).

Atsiri oil can help the digestion by stimulating the nervous system secretion, produced digestive enzymes that contain pepsin, trypsin, lipase, amylase and secreted into stomach and intestines whichincreas nutrients metabolism (Widjastuti, 2010).

Furthermore, turmeric contains many flavonoid compounds acting as phytoestrogen which have estrogen-like activity, enhancing vitellogenin (an egg yolk protein precursor) synthesis during the egg laying period via it'srespond to estrogen (Saraswati et al., 2013; Rahardja et al., 2015).

Based on those problems, this study was conducted to evaluate the effects of the addition of four different dietary levels (0, 0.1, 0.2, and 0.3%) of turmeric *(Curcuma domestica Val*) meal on Hen-day production and to evaluate egg quality of Sentul chicken hens from 42 to 50 weeks of age.

Figure 1. The Sentul Hen

## MATERIALS AND METHODS

The research used sixty Sentul Chickens at 42 weeks of age with 1.36% coefficient variation. The hens kept in litter system, as much as 20 flock, and each unit was consisted of 3 chickens.

The ration which was consisted of yellow corn meal, fish meal, rice bran meal, soy-bean meal, turmeric meal, $CaCO_3$ and bone meal, resulted 15 percent protein and 2750 Kcal/Kg of metabolism energy.

The formula rations were:

$R_0$ Based ration without turmeric meal
$R_1$ Based ration + 0.1% turmeric meal
$R_2$ Based ration + 0.2% turmeric meal
$R_3$ Based ration + 0.3% turmeric meal

The composition of based ration is in Table 1, while the nutrient and metabolism energy content are in Table 2.

Table1. Composition of based ration (%)

| No | Ingredients | Based Ration |
|---|---|---|
| 1 | Yellow corn | 58.00 |
| 2 | Soy-bean meal | 4.75 |
| 3 | Rice bran meal | 28.00 |
| 4 | Fish meal | 8.00 |
| 5 | CaCo3 | 0.50 |
| 6 | Bone meal | 0.75 |

Table 2. The nutrients and metabolism energy content in rations

| No | Nutrition Component | Based Ration |
|---|---|---|
| 1 | Crude Protein (%) | 15.00 |
| 2 | Crude Fat (%) | 6.66 |
| 3 | Crude Fiber | 4.89 |
| 4 | Calcium (%) | 1.05 |
| 5 | Phosphorus (%) | 0.58 |
| 6 | Lysine (%) | 0.97 |
| 7 | Methionine (%) | 0.35 |
| 8 | Metabolic Energy (kcal/kg) | 2755 |

The Completely Randomized Design (CRD) was used by 4 treatments, and each treatment was replicated 5 times. The data was analyzed by using analysis of variance and the difference among treatments which were tested by using Duncan'S Multiple Range Test. Variable analyses were egg weight, yolk index, Haugh unit value, shell thickness and yolk color score.

## RESULTS AND DISCUSSIONS

The effect of turmeric treatment on theegg weight, yolk index, Haugh unit value, shell thickness and yolk color score of Sentul chicken egg is shown in Table 3.

Table 3. The average of the egg weight, yolk index, Haugh unit value, shell thickness and color yolk score

| Variable | R0 | R1 | R2 | R3 |
|---|---|---|---|---|
| Hen Day (%) | 44.80$^{(b)}$ | 46.56$^{(a)}$ | 47.19$^{(a)}$ | 45.20$^{(b)}$ |
| Egg Weight (g) | 41.35$^{(b)}$ | 43.76$^{(a)}$ | 44.38$^{(a)}$ | 40.87$^{(b)}$ |
| Yolk Index | 0.36$^{(a)}$ | 0,38$^{(a)}$ | 0.40$^{(a)}$ | 0.36$^{(a)}$ |
| Haugh Unit | 95.85$^{(a)}$ | 96.56$^{(a)}$ | 97.19 (a) | 95.36$^{(a)}$ |
| Shell Thickness | 0.33$^{(a)}$ | 0.33$^{(a)}$ | 0.34$^{(a)}$) | 0.33$^{(a)}$ |
| Color Yolk score | 8.01$^{(a)}$ | 8.67$^{(b)}$ | 8.93$^{(b)}$ | 9.01$^{(b)}$ |

### Hen-day Production and Egg Weigh

The average hen-day production and egg weight were various. The R3, the giving of 0.3 percent turmeric meal in the ration, is the lowest while the giving of 0.2 percent turmeric meal was the highest (Table 3). The analysis of variance showed that treatment addedby turmeric meal had significant effect on the Hen-day production and egg weight. The treatment of R1 and R2 on hen-day produce and egg weight were significantly higher than R0 and R3. The difference was due to the consumption of ration containing turmeric meal which was better than that onbased ration without turmeric meal. The decreasing of hen-day and egg weight in based ration containing 0.3% turmeric meal (R3) was reducingthe feed consumption. High turmeric content in the ration will reduce feed consumption, because turmeric can affect the aroma and appetite. This is due to the bitter taste and pungent smell, so the palatability of the ration was decreasedresulting the decreased of feed intake. It means that the turmeric meal from 0.1-0.2% in the ration did not affect the aroma, the palatability of the diets and appetite, but it hada limit on hen-day production achievement. The addition until 0.2% turmeric meal in the ration Sentul chickens had advantages, because the bioactive substance such as curcumin and atsiri oil can help digestion by stimulating the nervous system secretion, producing digestive enzymes which contains pepsin, trypsin, lipase, amylase and secreted into stomach and intestines that increased nutrients metabolism. The high dose ofturmeric used in the ration can be poisonous, so the right utilization of turmeric meal can improve the hen-day and Sentul chicken egg weight.

In addition, the results were similar to those found by Lagana et al. (2011), who found that the addition of 0.2% turmeric into layinghen diets did not affect the egg production, feed consumption and egg specific gravity. Moreover, Riasi et al (2012) suggested that the addition of 0.2% turmeric powder into laying (Hy-Line W-36) hen diets from 100-104 weeks of age significantly increased the egg mass and it improved the feed conversion ratio.

### Yolk Index and Haugh Unit

Table 3 shows that yolk index and Haugh unit tends to increase proportional because of level of turmeric meal increased in the ration. Analysis of variance showed that by addition of turmeric meal as feed additive in ration Sentul chicken has no significant effect on yolk index and Haugh unit. It is meaning that the turmeric meal from 0.1-0.3% in the ration gave the best results on yolk index and Haugh unit. The eggs were tested had relatively similar levels of freshness, because the retrieval of eggs and egg yolk index measurement carried out in the same time. Mountney (1976) states that a fresh egg has a variety of egg yolk index values are relatively small. As the average value of yellow index normal egg range 0.30 to 0.50.

The Haugh unit value of Sentul chicken egg was still included in grade AA, because it has

the value above 75. The quantity of Haugh unit value wasaffected by genetics, the age of chicken, season and storage conditions. According to Sherif (2016), the variations in the effects of the addition of turmeric powderinto laying hen diets among the different studies might be attributed to the differences in the concentrationlevels and periods of turmeric supplemented, age and strain of laying hens, turmeric sources, stability of active compounds, drying method, turmeric products, experimental methods used.

**Shell Thickness**

The average range shell thickness is 0.33 – 0.34. The range is in the range chicken egg shell thickness in general (Nataamijaya et al., 2003). The analysis of variance showed that by giving turmeric meal as feed additive until 0.3% in the ration did not significantly influence (P>0.05) the shell thickness. It means that the bioactive turmeric which was curcumin could improve the performance of the digestive tract, so the absorption calciumand phosphorus were increasing consequently resulting thick eggshell thickness.

**Egg Yolk Color Score**

Table 3 shows that egg yolk color score tends to increase proportional, because of the increasing of turmeric mea in the ration. The results of variance analysisshowed that theaddition of turmeric until 0.3% in ration provided significant effect (P<0.05) on egg yolk color score. Turmeric contains xanthophyll compounds, thus the addition of high doses of turmeric in the ration can produce more natural xanthophyll, so the egg yolk color score is going to increase. According to Gilbert (1971), there was a linear relationship between the level of pigment with the egg yolk color. The molecular structure xanthophyll in turmeric meal decisive to theegg yolk color, because most of xanthophyll from the food is used first for pigment egg yolk color production and only a few are used for the pigment of skin tissue. In accordance with the opinion of Edjeng et al. (2002) which states, if the egg-laying chickens quickly largely xanthophyll of ration, it can be used to production of egg yolk color pigment and just a little to the skin tissue, hence the chickens after the long period of the egg, the network becomes pale or bluish white.

## CONCLUSIONS

It can be concluded that the utilization of the Turmeric (*Curcuma domestica* Val) until 0.2% level in ration was still able to support a good result on hen-day production and egg weight, furthermore the addition of 0.3% turmeric into Sentul chicken ration have positive effect on egg yolk color.

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# IMPROVING MILK AND SOYBEAN FERMENTED WITH PROBIOTIC BACTERIA ON HDL AND LDL BROILER BLOOD

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***Abstract***

*Probiotics can play an important role in immunological, digestive and respiratory functions and could have a significant effect in alleviating lipid. Therefore, a study was conducted to evaluate the effect of Milk and Soybean Fermented with Probiotic on cholesterol status i.e. High Density Lipoprotein (HDL) and Low Density Lipoprotein (LDL) indices of broilers. Materials and Methods: A total of 120, 5 weeks old broilers were used in this study in a Complete Randomized Design (CRD). The birds were randomly assigned into six treatment groups of P0, P1, P2, P3, P4 and P5 with 24 birds treatment $G^1$ replicated 4 times of 5 birds each. The broiler in first group (P0) basal feed, (P1) basal feed with cow's milk, (P2) basal feed with milk fermented, (P3)basal feed with soy milk fermented, (P4) basal feed with combination milk fermented and soy milk fermented, (P5) basal feed with combination milk fermented and soy milk fermented with different bacteria. Results: There were non-significant ($p>0.05$) increasing High Density Lipoprotein (HDL) level of broiler due to probiotic supplementation. Increasing blood HDL levels is (69.73 mg/dL) in group fed P4 (combination milk fermented and soy milk fermented) compared to control (45,16 mg/dL). A statistically significant ($p<0.05$) decrease in total number of Low Density Lipoprotein (LDL) level. Lowest LDL level (33.36 mg/dL) was found in group fed (P4) combination milk fermented and soy milk fermented. In conclusion, addition of probiotic milk fermented with soy milk fermented had beneficial effect increasing HDL levels and decreasing LDL levels broiler blood.*

***Key words:*** *probiotic, fermented milk, fermented soy milk, broiler, HDL, LDL.*

## INTRODUCTION

*Cholesterol in the blood circulation in lipoprotein particles*. In lipoprotein, the most influence on cholesterol is High Density Lipoprotein (HDL) and Low Density Lipoprotein (LDL). The level of HDL and LDL cholesterol is needed to determine which is the total amount of LDL, HDL and 1/5 triglycerides in each deciliter of blood. Usually, the total and HDL cholesterol levels can describe the general conditions of cholesterol levels.

The goal of this study is expected to add science and knowledge, especially regarding meat quality of broilers. In addition, fermented milk, soy milk and the combinations with probiotics bacteria can increase levels of high density lipoprotein (HDL) and decrease levels of low density lipoprotein (LDL) blood broiler

HDL removes cholesterol from tissues and transports it to the liver. HDL is created mostly from components from other degraded lipoproteins. HDL converts cholesterol to cholesteryl esters by LCAT, an enzyme activated by apoA-I in HDL. HDL appears to get cholesterol to the liver 1) by transfer the cholesteryl ester to VLDL which after degradation IDL and LDL is taken to the liver and 2) by direct interactions between HDL and the liver via a specific HDL receptor. The liver disposes of cholesterol as bile acids. HDL is also called "good cholesterol" because it is associated with lowering cholesterol levels.

VLDLs are synthesized by the liver, like chylomicrons, are degraded by lipoprotein lipase. VLDL, IDL, and LDL are interrelated. IDL and LDL appear in the circulation as VLDL remnants. VLDL is converted to LDL by removal of all proteins except apo B-100 and esterification of most of the cholesterol by lecithin-cholesterol acyl transferase (LCAT) associated with HDLs. The esterification occurs by transfer of a fatty acid from lecithin to cholesterol (forming lysolecithin).

HDL can be classified into larger, less dense $HDL_2$ or smaller, denser $HDL_3$ which falls within the density ranges 1.063–1.125 and 1.125–1.210 g/mL, respectively. Although the major proportion of HDL is normally present in $HDL_3$, individual variability in HDL levels in human populations usually reflects different amounts of $HDL_2$.

Giving probiotics is one of the efforts to decrease LDL and increase HDL blood broiler. Probiotics are living organisms used as feed supplements when consumed can improve animal health by balancing the microflora in the digestive tract. One of the food products that contain probiotics are fermented milk and fermented soy milk are known to lower LDL and raise HDL blood. Based on previous studies it is known that fermented soy milk contains flavonoids. Flavonoids are one of the components which can reduce cholesterol by inhibiting the action of the enzyme system 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) reductase. Based on this, the authors are interested in doing a study entitled "Improving Milk and Soybean Fermented with Probiotik Bacteria on HDL and LDL Broiler Blood".

The existence of the cholesterol in the body is very important because it has a function as a component of the cell membrane, so that the body can not function without cholesterol (Laurencio, 2002). Blood cholesterol is influenced by the levels of HDL and LDL cholesterol is a major component of total blood cholesterol (Hendromartono, 2004). Low density lipoprotein (LDL) is a vehicle to bring a lot to the tissues. Normal levels of LDL in the blood of broilers is <130 mg/dL and HDL>22 mg/dL (Basmacioglu and Ergul, 2005).

Milk fermented containing lactic acid, bacteria that can lower total cholesterol, LDL cholesterol, and triglycerides and to increase HDL cholesterol. The processing of the soybean will hydrolyze isoflavone compounds into free aglycone isoflavones higher activity. Lactic acid bacteria in fermented soy milk has a very important role in improving the digestibility of soy isoflavones. This is due to the activity of β-glucosidase enzyme in the bacteria that can hydrolyze aglycon isoflavones into a compound that is easily absorbed (Larkin et al., 2009).

This research is very important for knowing combination milk and soy milk with probiotic bacteria that have result to increase HDL and decrease LDL.

## MATERIALS AND METHODS

Methodology: 120 broilers were adapted period of 1 week was given to the birds, following which a trial was conducted that lasted 5 weeks. The broilers were offered the maintenance ration throughout the study. A total of 120, 5 weeks old were used in this study in a Complete Randomized Design (CRD). The birds were randomly assigned into six treatment groups of P0, P1, P2, P3, P4 and P5 with 24 broilers treatment $G^1$ group replicated 4 times of 5 broilers replicate $G^1$. The birds in the first group (P0) were given basal feed without milk, while as other groups were supplemented with milk (P1), milk fermented (P2), soy milk fermented (P3), combination milk and soy milk fermented (P4), and combination milk and soy fermented with different bacteria (P5). The feed and potable water were supplied *ad libitum* throughout the experimental period of 5 weeks, with strict adherence to all the conventional management practices.

Parameters recorded: Blood samples were randomly collected from 24 broilers per replicate at the end of study. The samples were analyzed at the Laboratory of Physiology and Biochemistry, Faculty of Animal Husbandry, University of Padjadjaran. The parameters recorded were HDL and LDL levels. The HDL and LDL was estimated using a HDL and LDL Direct method using Biolabo KIT.

Statistical analysis: Data collected were subjected to analysis of variance (ANOVA) as per Steel and Torrie[18] and where ever means difference existed, they were compared using Honestly Significant Difference (Tukey test) with 5% significant level.

The treatment consists of :

$P_0$ = Basal feed (Control)
$P_1$ = Basal feed with cow's milk (M)
$P_2$ = Basal feed with milk fermented (MF)
$P_3$ = Basal feed with soy milk fermented (SMF)
$P_4$ = Basal ration with milk fermented + soy milk fermented (MF+SMF)
$P_5$ =Basal ration with milk fermented + soy milk fermented, different bacteria (MF+SMF with different bacteria)

The measured variables were:

1. High Density Lipoprotein (HDL)
2. Low Density Lipoprotein (LDL)

## RESULTS AND DISCUSSIONS

Based on data from Table 1. it can be seen that the average highest to the lowest HDL levels, respectivelly is P4 (MF+SMF)= 69.73 mg/dL,

P3 (SMF) = 64.19 mg/dL, P5 (MF+SMF with different bacteria) = 62.95 mg/dL, P2 (MF) = 60.51 mg/dL, P0 (Control) = 45.16 mg/dL and P1 (M) = 45.11 mg/dL.

Table1. Average High Density Lipoprotein and Low Density Lipoprotein in Broilers Blood

| PARAMETER | TREATMENT | | | | | |
|---|---|---|---|---|---|---|
| | P0 | P1 | P2 | P3 | P4 | P5 |
| | ……..mg/dL…….. | | | | | |
| HDL | 45.16[a] ± 14.53 | 45.11[a] ± 12.38 | 60.51[a] ± 25.48 | 64.19[a] ± 8.74 | 69.73[a] ± 14.76 | 62.95[a] ± 6.22 |
| LDL | 52.96[a] ± 19.47 | 102.83[b] ± 27.14 | 43.73[a] ± 24.99 | 55.7[a] ± 6.88 | 33.36[a] ± 22.46 | 35.66[a] ± 29.31 |

For more details can be seen in the graph in figure 1.

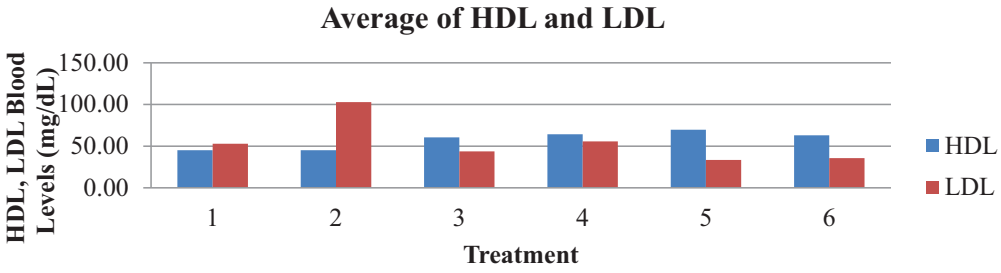


Figure 1. Effect of fermented milk, fermented soy milk and their combination on high density lipoprotein (HDL) and low density lipoprotein (LDL) broiler blood

According Basmacioglu and Ergul (2005), normal HDL levels in the blood of broilers is more than 22 mg/dL. All the treatments in the normal range, based on Table 1. and Figure 1., showed that P2 (MF), P5 (MF+SMF with different bacteria), P3 (SMF) and P4 (MF+SMF) is able to increase cholesterol levels, while levels of HDL P1 (M) lower than other treatments but there is in the normal range. Result of statistical analysis using analysis of variance showed that adding of milk fermented, soy milk fermented, and the combinations was not significantly different (P> 0.05) increase blood HDL levels. Although was not significant difference but the level tend increases. HDL is the smallest lipoprotein particles produced in the liver and small intestine, has the highest density because it contains more protein than cholesterol. The content is the most direct apolipoprotein Apo A-I and Apo A-II. The liver synthesizes lipoproteins as complexes of apolipoproteins and phospholipids, which form particles of cholesterol-free, the complex is capable of taking cholesterol carried internally of cells through the interaction with the ATP-binding cassette transporter AI (ABCA1), an enzyme plasma called lecithin-cholesterol acyl-transferase (LCAT) converts free cholesterol into cholesteryl ester (a more hydrophobic form of cholesterol), which is sequestered into the core of the lipoprotein particles, eventually causing the newly synthesized HDL. HDL particles increases because circulate through the bloodstream and incorporate more cholesterol and phospholipid molecules from cells and other lipoproteins, for example, by interaction with the transporter ABCG1 and Phospholipids Transport Protein (PLTP) (Murray, 2009).

The HDL binds cholesterol and phospholipids in the blood and transfer to other lipoproteins to be released into the bloodstream and flows throughout the body. The results of Moses et al. (2006) study showed that HDL can maintain a balance and not to accumulate inside the cell, the balance is managed by the removal of sterols from the membrane at a rate equal to the amount of cholesterol is synthesized to the liver (Diestchy, 2003).

Increased blood levels of HDL also due to the present of isoflavones from soy milk fermented. The fermentation process will hydrolyze soy isoflavones into compounds called free aglycone isoflavones. This is due to the activity of β-glucosidase enzyme in the bacteria that can hydrolyze isoflavone aglycone into a compound that easily absorbed (Larkin et al., 2009). Isoflavones activate the Peroxisome Proliferator activated receptor α (PPAR α), such as increasing the synthesis of lipoprotein lipase which can increase the catabolism of triglyceride-rich VLDL. HDL levels vary with plasma triacylglycerol and directly with the activity of lipoprotein lipase. This process is caused by byproducts that are release from hydrolysis chylomicrons and VLDL cholesterol, phospholipids, and Apo A-I to form a pre β-HDL. PPARα also can increase the expression of Apo A-I and Apo A-II directly from the cycle and establish praβ HDL-cholesterol after binding with phospholipids and cholesterol. The final result from isoflavones activated will increase HDL cholesterol (Medjakovic et al., 2010).

Based on data from Table 1, the average highest to the lowest levels of LDL, respectively is in P1 (M) = 102.83 mg/dL, P3 (SMF) = 55.70 mg/dL, P0 (Control) = 52.96 mg/dL, P2 (MF) = 43.73 mg/dL, P5 (MF+SMF with different bacteria) = 35.66 mg/dL and P4 (MF+SMF) = 33.36 mg/dL.

According Basmacioglu and Ergul (2005) that normal LDL levels in the blood is <130 mg/dL. Blood levels of LDL in each treatment are in the normal range. Based on Table 1 and Figure 1 showed that P2 (MF) and P4 (MF+SMF) can decrease blood LDL levels, while LDL levels P1 (M) and P3 (SMF) increased compared to the other treatments, but the number is still in the normal range. Results indicate that adding of milk fermented, soy milk fermented and the combinations have significant effect ($P<0.05$) decrease LDL levels.

Decreased levels of blood LDL broiler chicken in milk fermentation occurs because of the activity of lactic acid bacteria that produce enzymes that hydrolyze bile salt hydrolase or sever the bond of C-24, N-acyl amides formed between bile acids and amino acids in the conjugated bile salts. Activities LAB produces the enzyme bile salt hydrolase (BSH) deconyugated bile salts by separating the glycine or taurine of steroids to produce bile salt-free or cause to form cholic acid-free which is poorly absorbed by the small intestine (Surono, 2004; Lengkey and Lovita, 2013) ,

A decrease in blood LDL with combination fermented cow's milk and soy milk is the best result, due to the presence source of food for lactic acid bacteria. Cow's milk contents lactose, sources of food for Lactic acid bacteria, while the type of carbohydrate in soy milk fermented can not be used as a food source.

The nutritional content of cow's milk and soy milk are high in carbohydrates and fat causes an increase in the amount of cholesterol that is hydrolyzed by the help of bile salts in the intestine. Carbohydrates in soy milk oligosaccharides consist of classes that can not be used as an energy source and a carbon source by lactic acid bacteria. In addition, the soy milk is not lactose which is a food source for lactic acid bacteria, so that the fermentation process is not perfect. This causes the population of BAL in the digestive tract of broilers was not optimum, so it is not capable of inhibiting the absorption of cholesterol that cause increase LDL in blood. Thus, dietary inclusion of milk, soy milk and the combination fermented is recommended for the welfare of broilers.

Flavonoids contain in probiotic may release one hydrogen atom from one cluster reducing associated with one free radical synthesis of forming 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) to be blocked that serves as a precursor forming cholesterol and subsequent oxidation of LDL cholesterol is inhibited (Reynertson, 2007).

High LDL levels will cause more cholesterol attached to the walls blood vessels at the time of transport carried out and slowly it will to form stacks which precipitate, such as plaque (Graha, 2010 )

**Comparison of HDL and LDL levels**

HDL levels of broiler chickens in this study did not experience a significant increase and LDL levels did not decrease significantly, but can be seen changes in the balance between the levels of HDL/LDL each treatment, shown in Table 2.

Table 2. Comparison of Blood levels of HDL and LDL Broiler

| Treatment | HDL (mg/dL) | LDL (mg/dL) | Comparation |
|---|---|---|---|
| P0 | 45.16 | 52.96 | 0.85 : 1 |
| P1 | 45.11 | 102.83 | 0.44 : 1 |
| P2 | 60.51 | 43.73 | 1.38 : 1 |
| P3 | 64.19 | 55.70 | 1.15 : 1 |
| P4 | 69.73 | 33.36 | 2.09 : 1 |
| P5 | 62.95 | 35.66 | 1.76 : 1 |

The best ratio of HDL and LDL are 2.09:1 (P4). Levels of HDL have an inverse relationship to each other with various illnesses, so that the ratio of HDL/LDL is an important predictive parameter (Murray et al., 2009).

Low levels of blood LDL are good for health, where the risk of blood vessel will be low, because cholesterol is transported throughout the body slightly.

This research can improve the ratio of HDL/LDL in the blood of 2.09:1 in P4. According Laihad study (2000) that rasio HDL/LDL blood of broiler chickens was 1.6:1.

## CONCLUSIONS

Based on the results of this study concluded that:

Fermented cow's milk, soy milk fermentation and the combination of fermented cow's milk with soy milk fermented able to increase blood HDL levels despite statistical improvement was no significant difference in the numbers HDL levels, but using fermented milk and soy milk cause the number of HDL tend increase, while cow's milk causes a decrease in blood HDL levels.

The best treatment for decreasing blood LDL levels is combination fermented cow's milk and soy milk in (P4) = 33.36 mg/dL.

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# CHANGES OF COMFORT PARAMETERS AND TEST DAY MILK YIELD IN HOLSTEIN COWS

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***Abstract***

*The objective of this investigation was to reveal the changes of comfort parameters and test day milk (TDMY) according to environmental factors in Holstein cows. In total, 99 clinically healthy cows reared at a private farm in the Black Sea region of Turkey were examined. Flank hygiene (FH), rear legs hygiene (RLH), body condition score (BCS) and rumen fill (RF) were selected as comfort parameters and tested in two seasons (spring and summer), parity (first and second) and stage of lactation (<200 d and ≥200 d) groups by t-test. While season affected all comfort parameters and TDMY (P<0.001), stage of lactation only affected BCS (P<0.001) and RF (P<0.05). The cows with second parity had higher milk production (P<0.05). Moderate correlation coefficients were determined between FH and RLH (r=0.557; P<0.01) or between BCS and RF (r=0.525; P<0.01).*

***Key words****: comfort, cow, hygiene, milk production.*

## INTRODUCTION

One of the main goals of the world's dairy sector is achieving high quality and quantity raw milk from milking animals. As well known, genotype and environment are two principal factors affecting success for more milk production. In other words, in addition to high genetic merit, optimum environmental conditions should be ensured for dairy animals in the farms. To measure comfort conditions, some indirect parameters may be used. For instance, hygienic status of animals is highly related to raw milk amount and quality. Besides, rumen fill score (RFS) may be used to determine whether a dairy animal intake sufficient feed or not. At this point, fat-energy storing ability of animal is kept in many farms via body condition score (BCS) observations.

Many studies have been conducted to reveal the changes of comfort parameters in dairy cattle (Busato et al., 2000; Haskell et al., 2006; Atasever and Erdem, 2009). However, studies on the associations of these parameters with milk yield are still limited. Determination of this relation may be seen as a leader approach for dairy owners to boost productivity in their herds.

The aims of the present study were to reveal the changes of comfort parameters and test day milk yield (TDMY) according to environmental factors and to estimate correlations among investigated traits.

## MATERIALS AND METHODS

This study was carried out at a private dairy farm which is located in Samsun province in the Black Sea region of Turkey. In total, 194 Holstein cows were used to be experiment material.

To assess hygienic status of cows, flank hygiene scores (FHS) and rear legs hygiene scores (RHS) were recorded. For both evaluation, a 1 to 4 scale (1: too clean and 4: too dirty) was used. To collect RFS, a similar scale (1: empty and 4: very full) was applied. In BCS evaluation, scores between 1 and 5 (1: emaciated and 5: obese) were performed (Wildman et al., 1982). All cows were clinically healthy and were kept similar feeding or barning conditions during the study period. Test day milk yield (TDMY) data were obtained from computer records of the farm.

In statistical work, independent t-test was applied to determine the effects of factors on the traits in two seasons (spring and summer), parity (parity 1 and 2) and stage of lactation (SL; up to 220 d and higher than 220 d) groups. Correlation coefficients among the traits were

estimated according to Kendall's Tau. All statistical works were performed using SPSS 17 for windows at 0.05 significance level.

## RESULTS AND DISCUSSIONS

Means (±SD) of the investigated features according to season are given in Table 1. As seen, all items were significantly (P<0.001) affected by season factor. FHS and RHS of the examined cows were higher in summer. It seems that feeding regime of the investigated farm had better conditions in the summer due to relatively higher BCS and RFS values. As related to this finding, TDMY achieved to higher level in the summer.

Comfort parameters were also evaluated by parity in this work (Table 1). As seen, parity was not an effective factor on the parameters. However, primiparous cows had lower (P<0.05) milk production when compared to the cows with second lactation rank. This case could be assessed to be an expected result (Erdem et al., 2010).

In addition to season and parity, all traits were evaluated by stage of lactation (SL) in two different groups (Table 1). As seen, BCS and RFS affected by SL, significantly (P<0.001 and P<0.05). This case may be commented with eradication of adverse effect of negative energy balance (NEB) in cows up to 200d.

The general means for FHS (2.34) and RHS (2.67) were found as relatively high. Also, BCS, RFS and TDMY of experimental cows can be assumed to be moderate. At this point, revising managemental conditions of the farm may suggest as urgent process (Nalubwama et al., 2016).

Table 2. Correlation coefficients of the traits

| Parameters | RHS | BCS | RFS | TDMY |
|---|---|---|---|---|
| FHS | 0.557** | -0.048 | 0.044 | 0.174** |
| RHS | | -0.034 | 0.021 | 0.107* |
| BCS | | | 0.525** | 0.111* |
| RFS | | | | 0.101 |

FHS: flank hygiene score, RHS: rear legs hygiene score, RFS: rumen filling score, TDMY: test day milk yield

Correlation coefficients between investigated traits according to Kendall's Tau are given in Table 2.

As expected, positive and moderate (P<0.01) correlations were calculated between FHS and RHS, or BCS and RFS. This result clearly points out that keeping dairy cows in comfort conditions is an interrelated topic.

Table 1. Means (±SD) of the parameters by environmental factors

| Factors | n | FHS | RHS | BCS | RFS | TDMY |
|---|---|---|---|---|---|---|
| **Season** | | | | | | |
| *Spring* | 99 | 2.16±0.86 | 2.48±0.74 | 2.73±0.27 | 2.22±0.58 | 22.02±3.30 |
| *Summer* | 95 | 2.53±0.66 | 2.86±0.70 | 3.04±0.32 | 2.68±0.36 | 24.57±1.88 |
| | | *** | *** | *** | *** | *** |
| **Parity** | | | | | | |
| *1* | 97 | 2.28±0.83 | 2.67±0.73 | 2.89±0.35 | 2.47±0.59 | 22.78±3.10 |
| *2* | 97 | 2.41±0.74 | 2.67±0.76 | 2.87±0.32 | 2.42±0.47 | 23.75±2.80 |
| | | ns | ns | ns | ns | * |
| **Stage of lactation** | | | | | | |
| *1 (<220d)* | 104 | 2.28±0.83 | 2.67±0.73 | 2.81±0.33 | 2.38±0.57 | 22.96±3.44 |
| *2 (≥220d)* | 90 | 2.41±0.74 | 2.67±0.76 | 2.96±0.32 | 2.52±0.47 | 23.61±2.31 |
| | | ns | ns | *** | * | ns |
| **Overall** | 194 | 2.34±0.79 | 2.67±0.74 | 2.88±0.33 | 2.44±0.53 | 23.27±2.98 |

ns: non-significant, *: P<0.05, ***: P<0.001

FHS: flank hygiene score, RHS: rear legs hygiene score, RFS: rumen filling score, TDMY: test day milk yield

## CONCLUSIONS

This investigation revealed that multiple non-genetic factors play an important role on both comfort traits and milk production level.

That's why, keeping dairy cows clean and feeding with balanced rations throughout the lactation period should be regarded as gold steps. However, further studies including all seasons are needed to confirm obtained findings here.

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# THE EFFECT OF CHITOSAN ON LEAD (Pb) CONTENT IN LIVER AND BONE OF QUAIL EXPOSED TO Pb-ACETATE IN DRINKING WATER

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***Abstract***

*The purpose of this study was to determine the effect of chitosan on the Pb content in the liver and bone of quail exposed to Pb-acetate in drinking water. Quail was kept in experimental cage of Poultry House, meanwhile, slaughtering and sampling were conducted in the Laboratory of Animal Physiology and Biochemistry, Faculty of Animal Husbandry, Padjadjaran University; furthermore, samples were analyzed in the Laboratory of Dairy Cattle, Bogor Agricultural Institute. The Experiment based on an experimental design with five treatments and five replications. Chitosan was given in ration as follows $P_0$ (0 ppm), $P_1$ (50 ppm), $P_2$ (100 ppm), $P_3$ (150 ppm) and $P_4$ (200 ppm). Orthogonal polynomial test was used to see the trend of increased or decreased as a result of treatment response. The result of this study showed decreased levels Pb in liver and bone of quail along with increasing doses of chitosan given. The best result obtained at $P_4$ (200 ppm) with the lowest Pb content in liver and bone of quail.*

***Key words***: *lead, liver, bone, quail, Chitosan.*

## INTRODUCTION

Animal products such as meat, egg or milk should be healthy and free from residues of various digestive mainly heavy metals because if the animal products consumed by humans constantly and in large quantities, it will cause health problems and even death for consumers.
Heavy metals such as lead (Pb) are one of the high level of environmental pollutants.
One of the main factors due to the increasing number of industries that use heavy metals pollution that ultimately leads either directly or indirectly to livestock, especially quail.
Direct are effects in the form of pollution through the air, soil and water, whereas the effects indirectly through the form of food and drinking water.
According to Darmono (1995), based on the need for livestock heavy metal, the metal is divided into two essential and non essential. Metals essential needed in the physiological aspect of the animal so that the metal in this group are nutrients that if a deficiency can cause abnormalities of the physiological called mineral deficiencies, while the non-essential is a group of metals that are not useful or not known usefulness in an animal's body, therefore the presence of these elements more than normal can cause poisoning.
Furthermore, it can be said of the main food of the animal species of birds are the seeds that contain high carbohydrates.
This causes metal contamination on the type of grain and metal contamination of drinking water to be the main source of metal toxicity.
One of the leading Indonesian products from the field of fisheries for the purpose of export is shrimp and generally in utilization remains solid waste such as skin, head, and feet.
The solid waste is processed and it can provide additional benefits because it can be used as material for chitosan.
Lead mechanism becomes toxic due to replace Pb-active metal cations such as calcium, zinc and protein. Calmodulin binding four cations eg calcium and lead when replacing four cations calcium deficiency will occur enzim. Along with this enzyme deficiency will hamper plumbum total (delta -ALAD) and when the metal is zinc cation replace its single meal will disrupt the process of blood clotting so there will be severe anemia (Sutrisno, 2006).
Chitosan is a natural polymer that is non-toxic, more environmentally friendly and easily degradable in nature. Chitosan has the

properties of absorbing and clumping well, therefore these compounds can be used as an absorbent material of heavy metals such as Pb. Chitosan has amino ($NH_2$) relatively more than chitin so that more nucleophilic and alkaline.

Crystallinity of chitosan caused by intermolecular hydrogen bonds is lower than chitin making it easy to apply in some reagents. Chitosan has properties that are not soluble in water and some organic solvents such as dimethylsulfoxide (DMSO), dimethylformamide (DMF), organic alcohol solvent and pyridine; but chitosan is soluble in organic acid / mineral dilute through protonation free amino group on the pH less than 6.5. The good solvent for chitosan is an acid such as formic acid, acetic acid, and the acid glutamate and solubility decreases with increasing molecular weight of chitosan.

Lead levels in the liver and bone quail is a good indicator to indicate exposure, because the levels of Pb in the liver can describe the level of lead in the body. This is because Pb contained in the liver to be detoxified and accumulate in the bone or quail.

The liver is the organ that secretes bile which is channeled into the duodenum which weighs 3% of their body weight. The liver is the defense of life and plays a role in almost every function of the body's metabolism. The liver has a large reserve capacity and network functions to defend the body and the liver also has the ability to regenerate awesome. Liver damage mostly in most cases cell death or illness, it will be replaced with a new liver tissue.

Hepatic function in the body is essentially that as filter toxins, heavy metals that enter through food or drinking water will be filtered in the liver, but the liver also has a threshold in the poison screening. Pb unfiltered most will settle in the liver and in the long term will result in liver function. The series of processes that occur in the liver can result in severe damage to the liver with the result function and structure of the liver cells, which in turn could adversely affect the health and whole organ (Antoine et al, 2008).

Bone formation takes place continuously and can be lengthening and thickening of the bone. Rate of bone formation changes throughout life. Bone formation is influenced by hormonal stimulation, dietary factors, and the amount of stress imposed on a bone, and is the result of the activity of bone-forming cells.

The damage to the liver caused by lead exposure can be detected by screening biochemical and *histopathological* examination of the liver. One of the biochemical examination of the liver that are useful for this purpose is the examination of class transaminase enzyme levels, namely, serum glutamate oxaloacetate transaminase (SGOT) and serum glutamate pyruvate transaminase (SGPT). AST can be found at various places in the body, but more useful as a marker of liver damage and liver, while ALT is more concentrated in the liver.

Quail known as a model animal and a great potential to be developed, is due to the advantages of the properties owned by his quail as small, rounded, and the tail is very short; a relatively short reach sexual maturity at 42 days; egg production can reach 300 eggs per year; ration needs is not too big, ie 14-24 g per head per day and colored feathers brown spots; land needs not be so broad that quail cage can be nested to save the location and maintenance costs are relatively low compared to other poultry accordance smaller body size.

## MATERIALS AND METHODS

This research was conducted at the Faculty of Animal Husbandry, Padjadjaran University for 40 days. The material used quail females aged 2 weeks (phase grower) as many as 100 birds, consisting of five (5) treatments that were $P_0$ (without chitosan), $P_1$ (50 ppm chitosan), $P_2$ (100 ppm chitosan), $P_3$ (150 ppm chitosan), $P_4$ (200 ppm chitosan) with five replicates and each unit consisted of 4 tail treatment.

Experiment using a completely randomized design (CRD) and the data were analyzed by analysis of variance, significant treatment further continued with *orthogonal polynomial test.*

Variables measured were: Pb content of the liver and bone of quail exposed to acetate Pb in drinking water. Rations used during the experiment were purchased from Poultry Shop PT. Charoen Phokpand Indonesia Tbk with feed ingredients as follows: corn, bran, soybean meal, meat and bone meal, fish meal, coconut cake, broken wheat, canola, calcium, phosphorus, vitamins, trace minerals and anti-oxidants.

## RESULTS AND DISCUSSIONS

### (1) Effect of Chitosan on the Content of Pb Livers in Quail Exposed to Pb Acetate in Drinking water

Based on Table 1 it shows that there is a decrease of Pb in the livers of quail with the level of administration of chitosan.

The average of the highest concentrations of Pb obtained at $P_0$ (without administration of chitosan) that is equal to 1,131 and the lowest Pb contents obtained at $P_4$ is equal to 0.839 ppm (200 ppm chitosan). It shows that chitosan can absorb Pb so it does not accumulate in the liver.

Furthermore, the data were analyzed using analysis of variance and the results is significant ($P<0.05$).

Based on the results of the analysis showed that the chitosan gave effect to the content of Pb in the livers of quail.

Then proceed with the advanced test and orthogonal contrasts was obtained linear equation $y = -0,0014x + 1.1126$ (illustration 1).

Table 1. Effect of Chitosan on the Content of Pb Livers in Quail Exposed to Pb Acetate in Drinking Water

| Replication | Treatment (ppm) | | | | | Total |
|---|---|---|---|---|---|---|
| | P0 | P1 | P2 | P3 | P4 | |
| R1 | 1,136 | 1,045 | 0,957 | 0,926 | 0,822 | 4,886 |
| R2 | 1,141 | 1,035 | 0,945 | 0,907 | 0,825 | 4,853 |
| R3 | 1,138 | 1,043 | 0,952 | 0,907 | 0,866 | 4,906 |
| R4 | 1,109 | 0,993 | 0,950 | 0,909 | 0,842 | 4,803 |
| **Total** | **4,524** | **4,116** | **3,804** | **3,649** | **3,355** | **19,449** |
| **Average** | **1,131** | **1,029** | **0,951** | **0,912** | **0,839** | **4,862** |

*Notes: P0: Without chitosan; P1: 50 ppm chitosan; P2: 100 ppm chitosan; P3: 150 ppm chitosan; P4: 200 ppm chitosan*

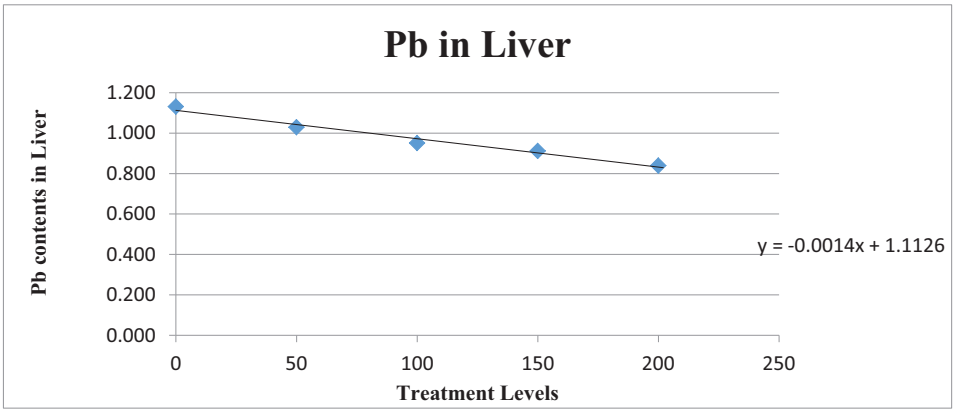


Figure 1. The content of Pb in the Liver Quail

Based on the chart above shows that decreasing levels of Pb in the liver caused by the level of giving chitosan. Chitosan concentration that could absorb Pb maximally obtained at a concentration of 200 ppm. On average 10-30% Pb inhaled absorbed through the lungs, and about 5-10% of ingested absorbed through the gastrointestinal tract (Palar, 1994). Furthermore, absorbed lead is transported by the blood to the organs as much as 95% Pb in the blood bound by erythrocytes. Most Pb plasma in a form that can diffuse and estimated in balance with pool Pb body which is divided into two, namely to soft tissue (bone marrow,

nervous system, kidneys, liver) and to the hard tissues bones, nails, hair, teeth.

Pb is not absorbed in the digestive tract and blood would bring his whole body and can accumulate in other organs including the liver. Alveolar cleaning function is carried particles to mucociliary escalators, through the layer of lung tissue and then towards the lymph nodes and blood stream. As many as 30-40% Pb in absorption through the respiratory tract will get into the bloodstream.

Pb excretion in several ways, the most important is through the kidneys and gastrointestinal tract. Pb excretion via urine as much as 75-80%, through the feces of 15% and more through the bile, sweat, hair, and nails (Palar, 1994). Pb excretion via the gastrointestinal tract is affected by the active and passive channel salivary glands, pancreas and other glands in the intestinal wall, the regeneration of epithelial cells and biliary excretion. Meawhile, the excretion process Pb through the kidneys is through glomerulus filtration. Pb in urine reflects recent exposure to Pb examination of urine used for occupational exposure (Goldstein & Kippen, 1994).

With the availability of chitosan can be expected that Pb can be absorbed by chitosan and can be excreted through urine and feces, because if it accumulates in the liver can cause liver damage. In general, Pb excretion is running very slow. Lead in the blood half-life of approximately 25 days, at 40 days, whiles the soft tissues in the bones of 25 years (Nordberg, 1998).

Hepatic function in the body is essentially as a toxin filter, heavy metals that enter through food or drink into the body will be screened at the liver, but the liver also has a threshold in the poison screening. Pb unfiltered most will settle in the liver and in the long term will result in liver function. The series of processes that occur in the liver can result in severe damage to the liver with the result function and structure of the liver cells, which in turn could adversely affect the health and whole organ (Antoine et al, 2008).

According to research Alifia and Djawad (2000) mentions that the milkfish (Chanos Chanos Forskall) exposed to metallic lead caused liver fatty degeneration. Fatty degeneration is characterized by the appearance of histological vacuole-vacuole. State of the network that has been damaged is due to liver has been exposed to toxic substances (lead). If the toxic substances that enter the body are relatively small or less and liver detoxification function well, then there is no damage, but if the toxic substances that enter in large numbers, the function of detoxification will be damaged (Lu, 1995).

**(2) Effect of Chitosan on the Content of Pb Bones in Quail Exposed to Pb Acetate in Drinking Water**

Table 2 shows that Pb decrease in bone of quail with the level of administration of chitosan. The average of the highest concentrations of Pb obtained at $P_0$ (without the administration of chitosan) that is equal to 0.788 ppm and averaging the lowest Pb contents obtained at $P_4$ is equal to 0.707 ppm (200 ppm chitosan). It shows that chitosan can absorb Pb so it does not accumulate excess bone.

Table 2. Effect of Chitosan on the Content of Pb Bones in Quail Exposed to Pb Acetate in Drinking Water

| Repeat | Treatment | | | | | Total |
|---|---|---|---|---|---|---|
| | P0 | P1 | P2 | P3 | P4 | |
| R1 | 0,810 | 0,746 | 0,734 | 0,722 | 0,707 | 3,719 |
| R2 | 0,776 | 0,752 | 0,737 | 0,732 | 0,707 | 3,704 |
| R3 | 0,794 | 0,741 | 0,731 | 0,730 | 0,707 | 3,703 |
| R4 | 0,772 | 0,750 | 0,739 | 0,725 | 0,707 | 3,693 |
| **Total** | **3,152** | **2,990** | **2,940** | **2,909** | **2,828** | **14,820** |
| **Rata-rata** | **0,788** | **0,747** | **0,735** | **0,727** | **0,707** | **3,705** |

*Notes: P0: Without chitosan; P1: 50 ppm chitosan; P2: 100 ppm chitosan; P3: 150 ppm chitosan; P4: 200 ppm chitosan*

Furthermore, the data were analyzed using analysis of variance and the results showed a significant (P<0.05). This suggests that the chitosan acts as a heavy metal adsorbent.

Then conducted further tests orthogonal contrast with the results of the linear equation y = -0,0004x + 0.7772 (illustration 2)

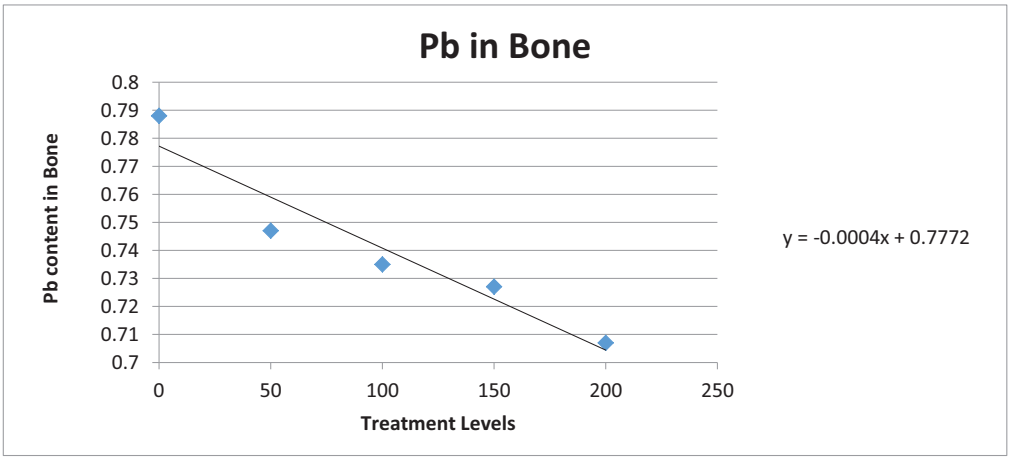


Figure 2. The content of Pb in bone quail

Based on the figure 2 can be seen that Pb accumulation in the bones can be decreased because it is absorbed by chitosan. Best chitosan concentration contained in $P_4$ (200 ppm). Provision of chitosan can bind heavy metals which accumulate in the body. In the body of the animal, the metal lead gets into the small intestine and is absorbed by the blood which then binds to blood proteins which are then distributed to all body tissues. Highest metal accumulation usually in the liver, soft tissue such as bone and teeth, and is excreted by the kidney (Darmono, 2001). Research Hasan and Seth (1981), reported that the administration of lead in mice can reduce the activity of enzyme δ -ALAD so that Pb can accumulate in bone marrow and become toxic.

In the bone formation process erythrocyte cells, red blood cells is a complex form chelate formed by metal Fe (iron) with a group of hemoglobin synthesis of the complex involves two enzymes, ie enzymes-ALAD (Amino levulinic acid Dehidrase) or amino acids levulinat dehidrase and ferrokhelatase enzyme. ALAD Enzyme is an enzyme type cytoplasm. This enzyme will react actively at an early stage during the synthesis and red blood cell circulation takes place. Hematopoietic system is very sensitive to the effects of Pb. Pb hematopoetic effect is to inhibit the majority of enzymes involved in heme biosynthesis. Among the enzymes involved in heme, an enzyme δ-aminolevulinik acid dehydrogenase (δ -ALAD) and ferrochelatase including enzymes are most susceptible to the inhibitory effect of Pb (Goldstein and Kipen, 1994).

Chitosan can absorb heavy metals based on the nature non-toxin and easily degraded. Chitosan is a polysaccharide amine process results deacetylation of chitin. Polycationic nature of chitosan compounds can be applied in various fields such as metal adsorbent, absorbent dye textiles, materials for cosmetics and antibacterial agents (Bhuvana, 2006).

Effect of chitosan can absorb Pb in bone because chitosan has amino ($NH_2$) relatively more than chitin so that more nucleophilic and alkaline. This makes the alkaline properties of chitosan: Soluble in dilute acid medium form a viscous solution so that it can be used in the manufacture of the gel. In some variations of configurations such as grain, membranes, coatings capsules, fibers and sponges.

Forming insoluble complexes with water with poly-anion can also be used to manufacture the gel granules, capsules and membranes. Can be used as chelating heavy metal ions in which the gel provides a production system to the effects of destruction of the ion (Meriaty, 2002).

## CONCLUSIONS

Based on the results of research and discussion, it can be concluded that the administration of chitosan in the diet lower levels of Pb in liver and bone of quail. Best dose obtained at a dose of 200 ppm ($P_4$). Administration of chitosan is able to absorb Pb contents in liver and bone quail.


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# CRYOGENIC CHANGES OF IONIC COMPOSITION AND STABILITY OF THE SPERM BIOCOMPLEXES OF AGRICULTURAL ANIMALS AT ITS CONSERVATION

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***Abstract***

*In the article are presented the literal references devoted to theoretical investigations of phase changes in biological membranes at the cryopreservation process, also the development and new experimental methods application in studying different physico-chemical manifestations that are reflected by such type of devices. Lowering the temperature causes the phase of reorganization of biological membranes. Dehydration of cells in the process of phase transition is accompanied by the deformation of biological membranes. The result of phase transitions in biological membranes there is a migration, aggregation, translocation, and other types of movement protein components. Low temperatures lead to destabilization of membrane structures, causing activation of peroxidation and changes in lipid composition, and this affects the functional state of cells. Own protective functions of the gametes aimed at the stabilization of lipids and lipid processes in the low-temperatures zone can be sufficiently manifested in conditions of purposeful regulation of them from the outside.*

***Key words****: lipids, phase transitions, biological membranes.*

## INTRODUCTION

At the influence of low temperatures on biological membranes is a series of structural rearrangements in their organization, among which of particular importance are phase transitions of lipids and their lateral separation in bilayer with the formation of clusters and aggregation of protein and lipid components. The role of the inducing element in the damage of biological objects at low temperature is to phase transitions of the lipids from liquid-crystal state into a gel (Белоус et al., 1982). The process of phase transition of lipids leads to the formation of a rigid membrane structure, the plasticity of which is markedly different from the state of the membrane at physiological temperatures, when the fatty acid chains of the lipids are in liquid crystalline state. At temperatures below the phase transition, in the gel phase the mobility of fatty acid chains is limited and the movement of them anisotropic. The phase transition temperature of lipids increases with their intensity. It depends on the length of the chemical bond of fatty acid chains and decreases with increasing of polar radical of lipids, the nature of the interaction of lipids with proteins, and to a large extent determined by the molar ratio of cholesterol:phospholipid in biological and model structures. The latter plays a dominant role in the regulation of structurally-dependent functions of cells related with state of lipid membranes. According to existing data, cholesterol has a specific effect on the packing of lipid membranes and in the certain concentration shifts the phase transition to the area of lower temperatures or completely eliminates it. Therefore, in the biological membrane of erythrocytes that contain more than 33% of cholesterol, the liquid-crystalline state of lipids is maintained until a temperature of -20°C. On the basis of the detected linear decrease of temperature of the apparent phase transition in cholesterol-phospholipid suspensions with increasing content of cholesterol, it was concluded that the phase transition disappears when the molar ratio of cholesterol-phospholipids is equal to 1:1.

Temperature-dependent phase transition from liquid-crystal state to a gel is accompanied by the formation of a very rigid membrane structures that differ from the state of membranes at physiological conditions. At temperatures below the phase transition in the gel phase the mobility of fatty acid chains is very limited and their movement is anisotropic. Lowering the temperature leads to the transition of lipids in the new phase, and also to conformational changes in their structures. The phase transition temperature of the lipids depends on the length, degree of saturation of fatty acid chains and the chemical nature of the polar radicals of lipids (Бондаренко et al., 2002).

In this regard, the objective of the research was to investigate the cryogenic changes in the ion composition of spermatozoa and stability of protein-cholesterol complexes of sperm of farm animals in the cryopreservation process.

## MATERIALS AND METHODS

The main experimental work was carried out in the laboratory of Cryosanocreatology "V. Nauc". The object of investigations was the sperm of roosters of the Rhode Island breed. The experimental material was frozen in the form of granules with a volume 0,1-0,2 ml on the fluoroplastic plate surface at a temperature of -110 – -120°C. In the study of mass transfer through the plasma membrane was determined the content of $Na^+$, $K^+$, $Li^+$ and $Ca^{+2}$ ions by flame photometry using the device ПФ-2. The allocation of plasma membranes using two-phase polymer system was performed according to the method of N. Ivanov, I. Porfirov in our modification (Наук В.А. et al., 1993). The stability of protein-cholesterol complexes were determined by the amount of loosely bound cholesterol (Кейтс, 1975).

Statistical processing of digital material was done by the method E. Merkurieva using the Student's t-test.

## RESULTS AND DISCUSSIONS

Cryogenic changes in biological membranes can be reduced to the realization of a number of physico-chemical mechanisms. Among them it should be noted: the phase transition of lipids, changes in the structure of water, segregation and aggregation of proteins and lipids, biochemical changes in the structure of membrane components, lipid peroxidation, the violation of the barrier properties of the membranes. The listed processes are risky in the maintenance of the functional state of defrosted material.

As a result of our research was established the change of the ions concentration of $Na^+$, $K^+$, $Li^+$ and $Ca^{+2}$ in the sperm of roosters in the cryopreservation process (Table 1).

Table 1. Cryogenic changes in ionic composition of the rooster sperm

| Concentration of ions | Investigated material | |
|---|---|---|
| | Spermatozoa | Plasma |
| Native material | | |
| $Na^+$ | 147.9 ± 5.48 | 407.0 ± 22.80 |
| $K^+$ | 156.8 ± 27.90 | 35.7 ± 2.52 |
| $Li^+$ | 0.73 ± 0.03 | 0.58 ± 0.01 |
| $Ca^{++}$ | 7.53 ± 0.16 | 12.0 ± 0.49 |
| Defrosted material | | |
| $Na^+$ | 187.6 ± 5.32* | 280.6 ± 19.0* |
| $K^+$ | 43.3 ± 4.58* | 47.8 ± 2.97* |
| $Li^+$ | 0.17 ± 0.04* | 0.30 ± 0.07* |
| $Ca^{++}$ | 13.16 ± 0.36* | 7.66 ± 0.74* |

Note: * Cryogenic changes are statistically authentic.

From table 1 it follows that the state of membrane lipids has a significant effect on the cell functions. It is at the stage of the phase transition of lipids occurs a sharp change in the membrane permeability for ions and metabolic products. In this regard, the nature of phase transitions of lipids in membranes is important not only to determine their cryoresistance, but

also for predicting the results of cryopreservation.

Accounting of the phase transition of lipids acquires special significance, if we take into account the fact that namely in the given case increases the level of membrane cryodamage.

Considering the nature of the phase transition of the lipids and the influence of temperature on these processes, it should be noted that the membrane lipids are amphipatic compounds, i.e. they contain both a polar hydrophilic end and a long hydrophobic part formed by fatty acid chains.

The general interest are the data about lipid mixtures wich containing cholesterol, in connection with the ability of the latter to regulate the liquid crystal compound in lipid bilayer (Балан et al., 2005).

According to the Belous (Белоус et al., 1982), lowering the temperature and reducing the level of cell hydration is accompanied by a deformation of the plasma membrane of cells and activation of phosphatidylinositol cycle. In the author's opinion, the increase of ionized $Ca^{++}$ greatly changes the polymeric and conformational state of the proteins cytosol and the cytoskeleton. Polymerization of cytoskeleton proteins is accompanied by the appearance on the plasma membrane of spinules. Via deforming the membrane, they change the function of ion pumps. The specified structural changes of proteins contribute to the processes of phosphorylation, which areactivated when the temperature drops. The formation of myosin hydration shells and its conformational state at the temperature changes - are closely related processes. The leading in the conformational rearrangements of the protein upon changing the temperature are the factors associated with the existence of thermolability of the physico-chemical parameters of water and end up with thermolability of the interactions of the protein macromolecule with the aqueous medium (Жилякова et al., 1991).

Cryogenic changes of cellular or other biological structures conditioned by phase transitions of proteins or lipids, are in direct dependence on the degree of membranes hydration. Therefore, the study of its role in maintaining the structure of membranes is one of the approaches to clarify the mechanisms of cryodamage and cryoprotection of biological objects.

Under the influence of the negative temperatures there is a change in the structure of water. The structure of vicinal water is close to the structure of liquid crystals. The vicinal water is characterized by temperature anomalies, which can be considered as phase transitions.

The existence of four regions of temperature anomalies (from +14 to +16°C, from +29 to +32°C, from +44 to +46°C and from +59 to +62°C) suggests, at least, phase transitions between the five structural types of water. It turns out that not only vicinal water influences the functioning of the membranes, but also the membrane structures determine the state of the vicinal water. According to Drost-Hansen (Drost-Hansen, 1973), the specific structure of water adjacent to the surface depends on the surface properties. The processes taking place in membranes during freezing of various fractions of water are of great importance for the successful implementation of the cryopreservation problem.

The extreme conditions of cryopreservation have a significant impact on the dynamics of components of the cells plasma membranes (Болдырев et al., 2006). With decreasing of temperature in the membranes take place the phase transitions.

Regulation of aggregation and segregation of proteins in the membrane is carried out by means of ionic bridges between charged groups of phospholipids and proteins, as well as the sulfhydryl-disulfide bonds, which condition the state of the protein complex. Through the method of fluorescent probes Belous with co-researchers (Белоус et al., 1982) found that in the case of changes in temperature and osmotic conditions of the medium, the nature of the protein-lipid interactions is modified. The authors suggest that the primary processes that induce sensibilization of cells to cooling, developing at the level of the protein cytoskeleton associated as a part of membrane lipids and with certain integral proteins. This is evidenced by the slow reversibility of structural changes of proteins, for which are characteristic more prolonged relaxation and reparation processes.

For biological membranes is characterized the

difference in the lipid composition on the both sides of the bilayer. The asymmetric distribution of phospholipids in the membrane is provided by the following three mechanisms. The first mechanism is related to the thermodynamic probability of distribution of phospholipids in accordance with the stereoconfiguration of their molecules. This is evidenced by the fact that the bilayer in the preparation of liposomes from a mixture of phospholipids is characterized by asymmetrical distribution of components: the outer part of the lipid bilayer during the formation of liposomes, by the predominance of phosphatidylcholine, and the internal - of phosphatidylethanolamine. This distribution of phospholipids contributes to the formation of bends and the formation of a gradient of flexibility.

The second mechanism is realized by differences in the composition of the medium, the surrounding bilayer in natural and experimental conditions. From the extracellular side of the membrane, the medium is characterized by a high content of $Mg^{++}$ and $Ca^{++}$. From the cytoplasmic side, the membrane is exposed to contact with $Mg^{++}$ and $K^{+}$ ions. Differences in the ionic composition of the extra- and intracellular medium also contribute to the mobility of the bilayer.

The third mechanism is due to the enzymatic factors (Юрченко et al., 2002). At the same time the asymmetry of bilayer is provided by enzymes of lipid metabolism and lipid-carrying proteins. The latter are a group of proteins of different specificity - from highly specific, providing the exchange of membrane components, to the relatively low-specific, binding and transferring to membranes, or vice versa lipids of different classes. The transfer of lipid molecules is realized in the form of complexes with these proteins-carriers. While protein-lipid complexes acquire a hydrophilic nature.

Elucidation of the mechanisms of cryogenic changes in biological systems at different levels of organization is one of the fundamental problems of modern Cryobiology. Numerous data of special literature indicate that one of the most cryolabile structures of the cell are biological membranes (Наук, 1991). Herewith the change of their barrier properties leads to a variety of cellular modifications (Holban et al., 2000). The study of barrier properties of membranes is due to the theoretical and practical significance of this issue.

A necessary condition of cellular homeostasis is to maintain the functional activity of biological membranes. Experimental studies performed in our laboratory demonstrate that at all stages of cryopreservation occur the membrane modification, the nature and intensity of which is determined by the composition of synthetic mediums and modes of technological processing of biological material (Table 2).

Table 2. The stability of protein-cholesterol complexes in the sperm of bulls depending on the conditions of cryopreservation

| The experimental variants | Content of loosely bound cholesterol, $\mu g/10^9$ spermatozoa |
|---|---|
| Fresh diluted semen | 274 ± 21 |
| After cooling and keeping the sperm at 2-4°C for four hours under conditions | |
| - anaerobic | 230 ± 22 |
| - aerobic | 207 ± 13 |
| - ordinary | 223 ± 24 |
| After freezing and thawing of semen, cooled in conditions | |
| - anaerobic | 207 ± 22*,** |
| - aerobic | 171 ± 17* |
| - ordinary | 159 ± 17* |

Note: * Cryogenic changes are statistically authentic.
** Statistically authentic changes in comparison with the ordinary conditions.

From Table 2 it follows that the change of cryopreservation conditions contributes to the preservation of protein-cholesterol complexes and thereby the functional state of the membranes.

The plasma membrane in hypertonic salt solutions and during freezing in the range of pre-eutectic temperatures are characterized by increased permeability only for cations while maintaining the barrier properties with respect to marker compounds of greater molecular weight such as sucrose. The authors showed that the increased permeability of membranes for cations is a reversible process, as evidenced by the absence of change in the permeability of plasma membranes for water molecules after freezing to different temperatures in the range (0) - (-16°C). Comparing the facts about the change of plasma membranes permeability for cations in the pre-eutectic temperature range, the authors concluded that hypertonic saline solutions in combination with a decrease of temperature are a risk factor leading to a change in the functional activity of plasma membranes at temperatures of the order of (0) - (-17°C).

Thus, it can be assumed that along with the ratio of cholesterol:phospholipids or cholesterol content their dynamics plays an important role in the process of cryopreservation as a possible mechanism of cells adaptation to low temperatures. However, the increase of this ratio can not be a positive phenomenon if it is associated only with the loss of phospholipids of the most important functional and structural components of biological membranes as the loss of phospholipids will lead to a significant deterioration of the physiological and morphological state of gametes (Борончук et al., 2003). Consequently, the positive effect of this mechanism can manifest only in the presence of exogenous lipids, for example lipids of seminal plasma, egg yolk and other components, and also may be due to the synthesis or resynthesis processes of endogenous substrates.

The presented material allows concluding that low temperatures lead to destabilization of membrane structures, causing activation of peroxidation and changes in lipid composition, and this affects the functional state of cells. Own protective functions of the gametes aimed at the stabilization of lipids and lipid processes in the low-temperatures zone can be sufficiently manifested in conditions of purposeful regulation of them from the outside.

## CONCLUSIONS

The researches allow making the following conclusions:

1. The decrease of temperature causes the phase reorganization of biological membranes.
2. In the result of a phase transition of lipids there is a risk of ion permeability violation of plasma membranes.
3. Dehydration of cells during phase transitions is accompanied by the deformation of biological membranes.
4. As a result of active phase transitions in biological membranes take place migration, aggregation, translocation and other types of protein components movement.
5. Regulation of the resistance of spermatozoa to the action of low temperatures is possible by the introduction of cholesterol into the composition of synthetic mediums.
6. The maintenance of the bonds stability in the protein-cholesterol complexes of sperm is better achieved in the anaerobic conditions of cryopreservation.
7. The cryoprotective properties of synthetic mediums are manifested under condition of mandatory presence of exogenous lipids.

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# THE EFFECT OF FERMENTED MILK, SOY MILK AND THE COMBINATION OF IT ON MEAT CHOLESTEROL AND INTESTINE PH OF BROILER

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***Abstract***

*This research is aimed to determine the effect of giving fermented milk, soymilk, and thecombinationof it on meat cholesterol and intestine pH of broiler. The research was conducted on December, 19th2016 to February, 16th 2017 at Cipacing Village, Jatinangor, Sumedang, West Java, Indonesia. Thisexperiment uses a Completelty Randomized Design (CRD) in five kinds of treatment. Those are T0 (Control), T1 (basal ration with cow's milk), T2 (basal ration with fermented milk), T3 (basal ration with fermented soymilk), T4 (basal ration with fermented milk and fermented soy milk combination in comparison of 1:1) done with four replications. There are 20 experimental units. In each unit, there are five heads with 100 broilers for the 35-day maintenance. Based on the statistical analysis of ANOVA, the result has no significant effect (P>0,05) on cholesterol content of meat. Besides, the result shows that the total of cholesterol content of meat and intestine pH of broiler tend to decrease equal to T0=0.00% (Control), T1= 2.24% (102.55±3,56), T2= 5.42% (99.22±3,06), T3= 2.29% (102.50±3,00), T4= 8.65% (95.83±17,88). The conclusion of this research is that giving of fermented milk, fermented soy milk, and combination of it can decrease meat cholesterol up to 8.65% and intestine pH of broiler.*

***Key words****: Fermented milk, Fermented soy milk, broiler, Meat cholesterol, Intestine pH.*

## INTRODUCTION

One of the animal protein sources that has high nutritional value is meat. Meat got a top rank as one of the most animal protein sources consumed by enormous number of people due to the fact of its delicious taste and high nutritional value. One of common sources of meat is broiler.

Based on the General Directorate of Livestock and Animal Health, data processed by the Agriculture Data and Information Services Center in 2015, the average daily feed consumption of broiler meat in Indonesia is 3.9733 kg per capita per year. Chicken meat production in Indonesia reached 1.62711 million tons with a total population of 1,497,625,658 chickens.

The average daily growth of broiler meat demand in the period time of 2015-2019 gainedto 1.90% per year (Direktorat Jenderal Peternakan, 2015).

Broiler as one of meat sources that has high nutritional value is the largest contributor of animal protein from livestock production a leading commodity. The growth of broiler gain peaked due to a meat producing in a relatively short time of five to six weeks.

Cholesterol content of broiler is relatively high compared to native chicken. Part of the broilercarcass that contains cholesterol are chest and thigh. This is because they contain lipids especially on oily skin (Setiawan, 2009).

Cholesterol is the main sterol in animal tissues. It is a typical product of the metabolism of animals. In result, all animal-based production foods such as yolk, meat, liver, and brain clearly contain cholesterol (Murray et. al., 1996 : 248).

Cholesterol in meat can be lowered by probiotic microbes used as feed additive. It can be profitable the host by improving the ecosystem in the digestive tract. One of probiotic products is a fermented milk that can be made from cows and soy milk.

In fermented milk product, there are groups of lactic acid bacteria that can lower cholesterol content. Lactic acid bacteria is found in

probiotics produce Bile Salt Hydrolise (BSH) enzymes through feces together with the cholesterol that causes reducing cholesterol levels (Sunarlim, 2009).

The use of cow's milk and fermented soy milk can lower the pH of the digestive tract of broiler. Increasing the use of probiotics can improve non pathogenic bacteria and reduce bacterial pathogen so that the balance of microflora in the digestive tract of broiler maintained.

When the lactic acid bacteria come into the system, it can reduce the bile acids and lower the pH in the digestive tract. In the acidic pH conditions, most of pathogenic bacteria will come out of the colon

## MATERIALS AND METHODS

## MATERIALS

The research used 100 broilers with 35-days treatment. The samples of meat are taken at the end of the research. This research has been carried out in 35-days. The broilers are randomly divided in to 20 units with 5 treatment rations, 4 repetitions for each containing 5 broilers.

This research is conducted using the experimental method of Completely Randomized Design (CRD) with 5 kinds of treatments, each treatment is repeated 4 times. Each experimental unit consists of five broilers. In fermented cow milk and soy milk using three kinds of lactic acid bacteria (*Streptococcus thermophillus, Lactobacillus bulgaricus* and *Lactobacillus acidophillus)*

The treatment consists of :

$T_0$ = Basal Ration (Control)
$T_1$ = Basal ration with cow milk (CM)
$T_2$ = Basal raton with fermented milk (FM)
$T_3$ = Basal ration with fermented soymilk (FSM)
$T_4$ = Basal ration with fermented milk + fermented soy milk (FCM)

## METHODS

The measured variables are:

1. **Meat cholesterol broiler**
   Cholesterol Test CHOD-PAP method (Cholesterol Amino OxidasePHenylperoxidase PHenozonpHenol) (Richmond, 1973).
   Setting up the tube. Filling the first tube with 10 mL of plasma plus 1 mL reagent, a second tube filled 10 mL standard cholesterol, and a third tube is a reagent blank containing 1 mL of color reagent, a standard 1 mL and 1 mL plasma. Incubate for 20 minutes at a temperature of 20-25$^{o}$C. Measuring the absorbance of the sample and standard absorbance against reagent blank daam 60 minutes. The measurements is using a spectrophotometer with a wavelength of 500 nm, with a calculation:

$$\text{Cholesterol(mg/dL)} = = \frac{AbsorbanSampel}{AbsorbanStandar} x standarkolesterol$$

2. **Intestine pH broiler**
   PH measurements performed with pH instructions (Bloom,1988).
   PH measurement principles that determine the condition of acids and bases. PH testing uses an electronic pH meter. before cleaning the cathode indicator with distilled water to neutral (pH 7 listed). Then clean with a tissue and then placing the cathode put the indicator on broiler intestine and colon.

Probiotics and meat samples are taken and analyzed at the Laboratory of Physiology and Biochemistry, Faculty of Animal Husbandry, Universitas Padjadjaran.

## RESULTS AND DISCUSSION

### 1. The Effect of Fermented Milk, Soy Milk and The Combination of it On Meat Cholesterol Broiler

Table 1. Average Meat Cholesterol in Broiler

| Repeat | Treatment | | | | |
|---|---|---|---|---|---|
| | T0 | T1 | T2 | T3 | T4 |
| | | | .....mg/dl..... | | |
| 1 | 103.02 | 98.44 | 97.81 | 103.65 | 101.98 |
| 2 | 103.86 | 101.56 | 95.93 | 105.53 | 111.37 |
| 3 | 110.32 | 106.99 | 103.02 | 102.40 | 99.90 |
| 4 | 102.40 | 103.23 | 100.10 | 98.44 | 70.07 |
| Average | 104.90 | 102.55 | 99.22 | 102.50 | 95.83 |

Based on the statistical analysis, the research has no significant($P>0.05$) result, but it shows that there is a tendency of cholesterol content of meat of broiler decreased. Based on Table 1, the average daily of the highest cholesterol content of meat to the lowest one are as follows; T0(Control) = 0, 00% (104.90 mg/dl), T1 (CM) = 2.24% (102.55 mg/dl), T3 (FSM) = 2.29% (102.50 mg/dl), T2 (FM) = 5.42% (99.22 mg/dl), T4 (FM+FSM) 8.65 % (95.83 mg/dl), below is the graph for giving informations more detail.

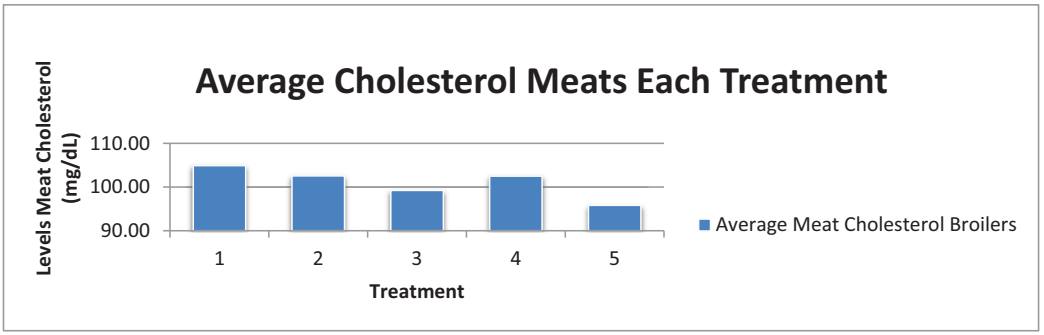


Figure 1. The effect of fermented milk, fermented soy milk and their combinations on meat cholesterol broiler

The lowest reductionis in the treatment of T4 (FM+FSM) (95.83 mg/dl) and the highest one is in the treatment ofT0 (Control) (104.90 mg/dl). The result of this research indicates that giving fermented cow's milk, fermentedsoy milk and their combinations led to a decreased cholesterol content of meat instead of having the untreated meat and cow's milk treatment. Cholesterol content of broiler meat in the treatment of T2(FM), T3 (FSM), and T4 (FM+FSM) produce lower cholesterol content of chicken meat than T0's (Control) and T1's (CM). The resulting of T4 (FM+FSM) treatment is the lowest cholesterol content which amount is 95.83 mg/dl decreased for 8.65% due to the fact that fermented milk can improve the balance of microorganisms in the digestive tract (Daud *et al*, 2007). Fermented milk can reduce the bile acids so that it can lower the pH of intestines in which the beneficial microbes will increase and suppress the growth of harmful microbes' mostly disease-causing microbes (pathogens). The use of probiotics has no negative effect to both livestock and humans who consume livestock (Budiansyah, 2004). In acidic conditions, the

pathogenic bacteria will be reduced so that the nutrients can be absorbed in the intestine optimally (Fuller, 1992).

Probiotics produce Bile Salt Hydrolise (BSH) enzymes to conjugate bile salts. This enzymes result conjugated bile salts and is excreted through feces together with the cholesterol leading to reduce cholesterol content (Sunarlim, 2009). Based on Lee, bile salts will be disposed of through the feces, where the conjugated bile saltthat is unabsorbed by the intestine is more easily removed from the digestive tract compared to the conjugated one. This explain that the more cholesterol is needed to synthesize, the more bile salt will lower cholesterol content as long as lactid acid bacteria binds cholesterol so that it prevents the absorption of cholesterol back to the liver (Lee et al., 2009). Cholesterol assimilation occurs through the mechanisms of cholesterol by lactic acid bacteria cell walls which then it will corporate the cholesterol with bacterial cell membranes, causing reduction in the number of free cholesterol in the body (Surono, 2004).

Maximally lipid absorption occurs in the distal and proximal ileum jejenum, also dekonjugasi bile salts in the ileum by *Lactobacillus* that can affect the efficiency of feed conversion because it has an important role in emulsify and absorption of lipids (Adriani et al., 2015). In addition, the giving of fermented milk products which contains a mixture of three bacterial interactions is better than the second bacterial mixture for fermentation since more bacteria will result more metabolites.

Fermented soy milk also contains lactic acid bacteria that has a very important role in improving the digestibility of isoflavones soy. The effects of isoflavones in decreasing cholesterol have been proven not only in animal testing such as mice and rabbits, but also in broiler and humans. Wider effects evident also are found in the treatment of soyflour; there is not only downing cholesterol content, but also triglycerides VLDL (very low density lipoprotein) and LDL (low density lipoprotein). On the other hand, soy flour can increase HDL (high density lipoprotein) (Amirthaveni and Vijayalakshmi, 2000). Zilliken (1987) elaborates that factor-II (6,7,4' tri-hidroksiisoflavon), isoflavone compounds the greatest effect. Another isoflavones-decreasead mechanism which is explained by its influence to the increasing catabolism of fat cells for energy production resulting in a decrease of cholesterol content.

This research shows that isoflavones from soy is an active substance which has a variety of useful biological activities. Therefore, the increasing of the fermentation of soy isoflavone content is due to the activity of β-glucosidase enzyme in the bacteria that can hydrolyzes isoflavone set to be free isoflavone compounds which is called aglycone (Larkin et al., 2009).Aglycone has higher activity in lowering total cholesterol. Ralston (2005) conductes a researchwhich shows that the enzymes produced by lactid acid bacteria can change flavanones into isoflavone compounds during the fermentation process.

Fermentation process can also hydrolyze aglycone flavone compounds into its glycoside which shows a higher antioxidant activity. Isoflavone compound is one component that is also metabolized. Another compound found in fermented soy milk that can inhibit the absorption of cholesterol is a flavonoid. Flavonoids are also capable of inhibiting the activity of the enzyme 3-hydroxy-3-methylglutaryl CoA that plays a role of the inhibition of cholesterol synthesis and enzyme actylCoA: cholesteryl aciltransferase takes a role in the decline of esterification of cholesterol in the intestine and liver (Fuhrman and Aviram., 2001).

The T4 (FM+FSM) treatment shows thatits cholesterol content is lower than T3 due to the combination of fermented milk and fermented soy milk. This is because of the type of carbohydrate contained in soy milk is not in the form of lactose found in cow's milk so thatlactic acid bacteria could not be taken as an advantage. Lactic acid bacteria does not grow in the fermented soy milk in resulting flavonoid compounds contained in soy cannot be converted into free isoflavone which is called aglycone.Flavonoids in the form of glycosides cannot be absorbed. So in order to make fermented milk requires a mixture of soy milk with cow's milk to get more benefits.Fermented soy milk can lower cholesterol content because of the presence of compounds such as an fatty acid-generated short chain from either fermentation of soy or milk products as a result

of the activity of probiotics in the digestive tract. Such compounds will compete with HMG CoA binding to the *reductase* enzyme of HMG CoA, so that cholesterol synthesis is inhibited (Hardiningsih and Nurhidayat, 2006).

## 2. The Effect of Fermented Milk, Soy Milk and The Combination of it On pH Intestine Broiler

Table 2. The average of Ileum pH in Broiler

| Repetition | Treatments | | | | |
|---|---|---|---|---|---|
| | T0 | T1 | T2 | T3 | T4 |
| | .....pH of ileum..... | | | | |
| 1 | 5.97 | 6.06 | 5.9 | 6.03 | 5.83 |
| 2 | 6.03 | 5.98 | 6.2 | 5.97 | 6.16 |
| 3 | 6 | 6.43 | 6.11 | 6.01 | 6.21 |
| 4 | 5.96 | 5.86 | 6.23 | 6.38 | 6.44 |
| Average | 5.99 | 6.08 | 6.11 | 6.10 | 6.16 |

Based on the result of statistical, there is a treatment which does not give a real effect (P> 0,55) to the intestine pH of broiler and pH of ileum. From above table, the lowest average pH on ileum can be seen as follows: T0 (5.99), T1 (6.08), T3 (6,10), T2 (6,11), T4 (6, 16). These results indicate that the treatment has no significant effect on pH of ileum. pH of the broiler's digestive tract ranged from 3.47 (gizzard) to 6.43 (small intestine) (Mabelebele et al., 2013). The broiler's digestive system in the small intestine is divided into 3 parts namely dudenum, jejenum and ileum. Duodenum is the small intestine that is grooved and united by the pancreas gland. The pancreas gland produces enzymes and bicarbonates that are channeled into the duodenum. Bicarbonate serves to neutralize the acidity or pH of the intestinal contents.

The non-acidic conditions of the broiler ileum can also be caused by the temperature in the broiler intestine which does not support the growth of lactic acid bacteria. This is in accordance with (Fardias, 1992) who elaborates that the environment which is suitable for living of lactic acid bacteria is temperature, hydrogen potential and nutritional content. The temperatures that are too high will damage the proteins which support the life of bacteria. This damage will result the bacteria being die. Temperatures that are too low will result BAL dormant and do not grow (Fardias, 1992). Lactic acid bacteria has an optimum temperature range of 37$^{o}$C – 42$^{o}$C (Husmaini et al., 2011) and can live at pH of 2 - 6.5 (Hardiningsih et al., 2006).

The giving of 1,25% Probiotics dose from broiler body weight is considered ineffective because it does not provide a meaningful effect on the pH conditions of broiler's intestine. Based on many studies, intestine ph will cause the decrease in broiler's colon because only the probiotic bacteria which can enter until the broiler's colon so the pathogenic bacteria in the broiler's digestive track can be reduced and come out with feces. Yet, this study does not analyze the colon ph of broiler.

## CONCLUSIONS

The conclusion of this research is giving fermented milk, fermented soymilk, and the combination of it can decrease cholesterol content of broiler meat up to 8.65% (95.83±17.88) at T4 containing fermented milk + fermented soy milk, T2=5.42% (99.22±3.06) fermented cow's milk, T3=2.29% (102.50±3.00) fermented soy milk, and T1=2.24% (102.55±3,56) cow's milk.

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# EFFECT OF GENOTYPE AND AGE OF LAYING HENS ON THE QUALITY OF EGGS AND EGG SHELLS

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***Abstract***

*The paper presents the results of testing of the egg and egg shell quality of two light line hen hybrids (Tetra SL and Bowans Brown). There were total of 4 tests. At the end of 35, 45, 55 and 65 weeks of age, the external and internal egg quality properties were tested as well as the quality of egg shell. Testing was conducted on a sample of 30 eggs for each hybrid. The weight and egg shape index were determined for each egg, as properties of the external quality, also internal quality properties such as albumen height and value of Haugh units (HU), and of the egg shell qualities - measured the egg shell deformation, the shell breaking force, the weight of raw shell and egg shell thickness were determined. The weight of the eggs did not differ significantly under the influence of said factors. Significantly higher mean values of the egg shape index at the age of 35 weeks were determined in comparison to 65 weeks of age. Under the influence of genotype, significantly higher ($p<0.05$) values of the albumen height in genotype Tetra SL were recorded. Albumen height in the 35th week of age was significantly higher ($p<0.01$) compared to other ages. Tetra SL layers showed significantly higher ($p<0.05$) HU values. With the increase of the age of hens the value of this parameter decreased, so, in the 35th week of age, statistically significantly higher HU value ($p<0.01$) was determined, compared to other ages. Deformation of the egg shell, breaking force and egg shell weight did not differ significantly influenced by genotype and age of laying hens. Significantly higher egg shell thickness values ($p<0.05$) were recorded for genotype Bowans Brown. With the increase of age of laying hens, there were no significant changes in egg shell thickness. Overall, it is concluded that both examined hybrids in terms of egg and egg shell quality traits have given satisfactory results. For most of the egg quality traits, better results were observed for Tetra SL hen genotype, while the most egg shell quality traits were in favour of hens of Bowans Brown genotype.*

***Key words:*** *genotype, age, egg quality, egg shell quality.*

## INTRODUCTION

In the conditions of intensive production of table eggs special attention should be paid to the egg quality traits. It is not enough to achieve a high laying capacity, it is necessary that the resulting products are of satisfactory quality. Important objectives of the leading breeding centres of light hybrid lines are aimed primarily at improving the egg quality properties and egg shell strength as well as maintaining the said quality properties during the production cycle. Given the prevalence of different hybrids of laying hens on the market, manufacturers of table eggs are often in a dilemma which hybrid to choose. End consumers require from manufacturers high standards in terms of quality of table eggs. It is necessary to produce healthy, safe and biologically correct product that will at the same time satisfy the criteria of freshness and good quality (Pavlovski et al., 2002).

Changes in individual traits of egg quality, in the case of the same genetic basis of laying hens, occur exclusively under the influence of the biological cycle of the laying hen (Roberts and Nolan, 1997; Perić et al., 1998). Zita et al. (2009) have found that, in addition to genotype, age of hens has a significant impact on the quality of the eggs. The quality of eggs and egg shell can be influenced by different factors: genotype, age, breeding system, lighting program, ambient temperature (Škrbić et al., 2006). Nutrition is also an important factor that can influence the individual quality properties, i.e. egg mass and egg shell strength (Supić et al., 1999; Vitorović et al., 2002; Petričević et al., 2014).

The poor egg quality traits represent a significant economic burden on commercial

producers of table eggs. Of the total number of eggs laid, on average 7-8% are broken on their way to the consumer. Egg shell must meet certain requirements in terms of resistance to various physical deformities; it is a mineral structure that maintains and preserves the contents of the egg to the final consumer.
The aim of this study was to determine the effect of genetic basis and age of hens on egg quality traits and egg shell quality traits in conditions of farm production.

## MATERIALS AND METHODS

The research was conducted at the experimental farm of the Institute of Animal Husbandry in Zemun, on laying hens of two light line hybrids: Tetra SL and Bowans Brown. 1000 birds of both hybrids were moved into the exploitation facility/building, of cage type, and evenly distributed, at the age of 18 weeks. During the duration of the trial, hens were provided the same conditions in terms of housing, environment and care.
Hens were fed ad libitum, with same mixtures for laying hens: in the period from 19-29 weeks of age, the mixture with a protein content of 17.4%; in the period from 30-50 weeks, the mixture with the protein content of 16.7%, and after 50 weeks the mixture with the protein content of 16.2%.
Randomly 30 eggs of both hybrids were taken at the end of 35, 45, 55 and 65 weeks of age. Egg quality has been tested on fresh eggs, immediately after collection, and thus a score of the initial quality of table eggs was obtained.
Egg quality traits are divided on the properties of the external and internal egg quality.
Determination of the external quality of eggs included:
-The egg mass was determined using the electronic scales of the accuracy $10^{-2}$ g.
-The egg shape index was determined by using the instrument that directly indicates the maximum width of the egg as a percentage of its length.
Testing the internal quality of eggs included:
-The albumen height, determined using the tripoid micrometre in the middle between the edges of the egg yolk and thick egg white accuracy of 0.1 mm.
-Value of Haugh units was determined as a logarithmic function of egg mass and thick albumen height.
Egg shell quality included the determination of the following properties:
-Deformation of the shell was determined by Marius instrument and expressed in μm, obtained as the mean value of 3 measurements.
-Egg shell breaking force expressed in kilograms.
-The egg shell mass with membranes measured by electronic scale with accuracy of $10^{-2}$ g.
-The egg shell thickness determined by micrometre on a part of the shell taken from the egg equator and after removing the membrane.
The software package STATISTICA, version 12 (StatSoft Inc.) was used for statistical analysis. The level of statistical significance of differences between groups was determined by the Tukey test.

## RESULTS AND DISCUSSIONS

Average values of egg quality properties obtained during the test are shown in Table 1. In layers of Tetra SL genotype, higher values for the egg mass were determined, however, in the statistical processing of the obtained data no significant differences for egg mass depending on the genotype and age of laying hens were observed. The egg shape index was not different under the influence of hens' genotype. A significant ($p<0.05$) effect of age of hens on egg shape index was recorded. Significantly higher mean values of shape index were measured at the age of 35 weeks in comparison to 65 weeks of age. Also, under the influence of genotype, significantly higher ($p<0.05$) values of the albumen height were recorded for genotype Tetra SL. The albumen height in the 35th week of age was significantly higher ($p<0.01$) compared to other ages. HU have less variability compared to the albumen height and therefore, for the purpose of objective assessment of the internal quality of eggs are more appropriate indicator. Significantly higher ($p<0.05$) HU values were found for genotype Tetra SL. With the increasing age of hens the value of the HU decreased, in the 35th week, statistically significantly higher value of this parameter ($p<0.01$) was recorded compared to other ages.

Table 1. Egg quality properties

| Genotype | Age (weeks) | Egg mass, g | | Egg shape index | | Albumen height, 0.1 mm | | HU | |
|---|---|---|---|---|---|---|---|---|---|
| | | ξ | SD | ξ | SD | ξ | SD | ξ | SD |
| Tetra | | 64.48 | 4.64 | 77.06 | 2.35 | 76.63$^{a}$ | 14.72 | 85.04$^{a}$ | 9.52 |
| Bowans | | 63.75 | 5.14 | 77.15 | 2.14 | 72.04$^{b}$ | 16.27 | 81.90$^{b}$ | 11.28 |
| | 35 | 64.43 | 4.96 | 78.07$^{a}$ | 1.91 | 87.47$^{A}$ | 12.16 | 91.67$^{A}$ | 6.34 |
| | 45 | 63.52 | 3.07 | 77.50$^{ab}$ | 1.69 | 74.28$^{B}$ | 11.54 | 84.06$^{B}$ | 7.41 |
| | 55 | 64.02 | 5.24 | 76.63$^{ab}$ | 2.37 | 68.23$^{B}$ | 15.07 | 79.50$^{B}$ | 10.65 |
| | 65 | 64.26 | 5.59 | 76.27$^{b}$ | 2.38 | 66.27$^{B}$ | 12.59 | 78.19$^{B}$ | 10.50 |
| Tetra | 35 | 64.72 | 4.16 | 77.80 | 2.18 | 86.47 | 12.23 | 91.13 | 6.25 |
| | 45 | 64.68 | 2.52 | 77.33 | 1.87 | 76.78 | 13.58 | 85.22 | 8.84 |
| | 55 | 64.81 | 5.65 | 76.87 | 2.13 | 73.53 | 15.77 | 82.80 | 10.67 |
| | 65 | 63.68 | 5.39 | 76.23 | 2.95 | 68.77 | 11.54 | 80.46 | 8.88 |
| Bowans | 35 | 64.15 | 5.78 | 78.33 | 1.63 | 88.47 | 12.44 | 92.20 | 6.60 |
| | 45 | 62.37 | 3.27 | 77.67 | 1.58 | 71.78 | 9.20 | 82.89 | 5.95 |
| | 55 | 63.23 | 4.85 | 76.40 | 2.64 | 62.93 | 12.72 | 76.20 | 9.89 |
| | 65 | 64.85 | 5.95 | 76.31 | 1.75 | 63.77 | 13.55 | 75.92 | 11.82 |
| Two-factorial variance analysis (p value) | | | | | | | | | |
| Genotype | | 0.413 | | 0.785 | | 0.025 | | 0.036 | |
| Age | | 0.938 | | 0.011 | | 0.008 | | 0.006 | |
| Genotype x Age | | 0.657 | | 0.835 | | 0.314 | | 0.398 | |

*a, b Average values in each column without common superscript are significantly different at the level of 5%
*A, B Average values in each column without common superscript are significantly different at the level of 1%

In accordance with our results, Tolimir et al. (1999) and Kocevski et al. (2011) have not found significant differences in the egg mass under the influence of the genotype. Tolimir et al. (1999) have found a significant effect of the age on the decrease of the values of albumen height and HU. Vračar et al. (1995) and Ledvinka et al. (2012) have determined a significant decrease in the egg shape index with the increase of age of hens. Silversides and Scott (2001) have recorded a significant impact of genotype on the albumen height.

Egg shell quality traits are presented in Table 2.

Table 2. Egg shell quality properties

| Genotype | Age (weeks) | Deformation, μm | | Breaking force, kg | | Egg shell mass, g | | Egg shell thickness, 0.01 mm | |
|---|---|---|---|---|---|---|---|---|---|
| | | ξ | SD | ξ | SD | ξ | SD | ξ | SD |
| Tetra | | 22.27 | 3.90 | 4.06 | 0.81 | 8.77 | 0.93 | 31.29$^{b}$ | 3.08 |
| Bowans | | 22.15 | 3.17 | 4.11 | 0.90 | 8.44 | 0.93 | 32.15$^{a}$ | 2.72 |
| | 35 | 21.97 | 3.79 | 4.22 | 0.72 | 8.61 | 1.04 | 31.40 | 2.90 |
| | 45 | 21.89 | 2.78 | 4.13 | 1.16 | 8.92 | 0.80 | 31.94 | 2.29 |
| | 55 | 22.80 | 3.61 | 4.03 | 0.76 | 8.38 | 0.87 | 31.77 | 3.48 |
| | 65 | 22.04 | 3.73 | 3.98 | 0.86 | 8.63 | 0.98 | 31.88 | 2.78 |
| Tetra | 35 | 22.07 | 4.59 | 4.22 | 0.74 | 8.71 | 0.95 | 31.13 | 2.56 |
| | 45 | 22.78 | 3.46 | 4.33 | 0.59 | 9.29 | 0.94 | 31.33 | 2.12 |
| | 55 | 22.47 | 3.02 | 3.93 | 0.76 | 8.70 | 0.89 | 31.47 | 4.27 |
| | 65 | 21.92 | 4.59 | 3.85 | 1.02 | 8.55 | 0.92 | 31.23 | 2.89 |
| Bowans | 35 | 21.87 | 2.95 | 4.22 | 0.73 | 8.51 | 1.15 | 31.67 | 3.27 |
| | 45 | 21.00 | 1.66 | 3.92 | 1.56 | 8.54 | 0.40 | 32.56 | 2.40 |
| | 55 | 23.13 | 4.21 | 4.12 | 0.78 | 8.07 | 0.75 | 32.07 | 2.58 |
| | 65 | 22.15 | 2.79 | 4.12 | 0.67 | 8.71 | 1.07 | 32.54 | 2.60 |
| Two-factorial variance analysis (p value) | | | | | | | | | |
| Genotype | | 0.710 | | 0.959 | | 0.058 | | 0.029 | |
| Age | | 0.769 | | 0.741 | | 0.291 | | 0.911 | |
| Genotype x Age | | 0.715 | | 0.590 | | 0.309 | | 0.948 | |

*a, b Average values in each column without common superscript are significantly different at the level of 5%

The deformation of the egg shell is a parameter which indirectly indicates the strength of egg shell. It represents the value that expresses how much the eggshell bends under the pressure of 500 g, at the equatorial part of the egg. Lower values of this egg shell quality parameter indicate its greater resistance to pressure, and a potentially stronger shell. Eggs of Bowans Brown genotype laying hens had lower values of egg shell deformation, but the differences were not statistically significant. Also, no significant differences in observed parameter were recorded under the influence of age of hens. The breaking force indicates a minimum load (in kg) that leads to the breaking of egg shell. Higher values of this parameter were measured in genotype Bowans Brown. Breaking force did not differ significantly between genotypes and ages of laying hens. The analysis of obtained results for egg shell mass showed no significant effect of genotype and age of laying hens on this parameter. Significantly higher ($p<0.05$) values for the egg shell thickness were determined for genotype Bowans Brown. The increase of hens' age had no significant influence on the egg shell thickness.

The obtained values for egg shell deformation for the hybrids are lower compared to those obtained by Perić et al. (1998) and Rajčić et al. (2008). Similar to our results, Solomon (2001) and Ledvinka et al. (2012) have found statistically significant differences in the egg shell thickness under the influence of genotype. Contrary to our results, Škrbić et al. (2006) have recorded statistically significant reduction in egg shell thickness with the increase of age of hens.

## CONCLUSIONS

Based on the results of this research, it can be concluded that the laying hens of genotype Tetra SL had better quality of eggs. The greater egg mass was determined, significantly higher ($p<0.05$) value of albumen height, and significantly higher values ($p<0.05$) of Haugh units. With increasing age of hens, statistically significant ($p<0.01$) decrease in the values for albumen height and Haugh units were recorded. Better results of egg shell quality were found in laying hens of genotype Bowans Brown, expressed through less egg shell deformation, greater breaking force and significantly greater ($p<0.05$) egg shell thickness.

Overall it can be concluded that none of the hybrids can be characterized as absolutely better and that with the increase of age of hens there is a gradual decline in the quality of eggs.

## ACKNOWLEDGMENTS

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# THE IMPACT OF THE LUNAR PHASES ON BOVINE CONCEPTION RATE

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***Abstract***

*The paper tried to make a connection between the lunar phases and the bovine conception rate both in dairy and beef farms. The goal of the study was to improve the efficiency of farming by sparing semen doses used for a successful insemination. 1481 pregnant cattle from seven different areas of Romania were the subject of this study. New moon and waxing crescent vs. full moon and waning crescent were associated with the insemination process. In one farm hormonal timing was used for inducing heats. In the others farmyards the oestrus was marked by spontaneous behaviour changing. The results confirmed a small influence of the lunar phases on the conception rate. The score was tight proportionally with the number of subjects. However the full moon offered a higher number of gestations than the new moon. The cows with hormonal timing heats offered a better connection between the lunar phase and fertility. Further studies could be done in this direction.*

***Key words****: lunar phases, biodynamic agriculture, cattle.*

## INTRODUCTION

The study started from the known influence of the earth's natural satellite on sexual behavior in humans, the development of plants and animals.
As the moon revolves around the Earth planet, approaching or moving away from it cyclically, producing tides, marine currents alterations, wind direction, it changes and having approved role in sexual behavior of aquatic species (Ramos, 2011).
Since enunciation of the biodynamic agriculture concept by Rudolph Steiner in 1924 (Vogt, 2007), more and more farmers have embraced this approach to agriculture. Currently almost 1 million hectares from 60 countries belonging to several continents are cultivated based on the principle of the interconnection of the solar system with soil, plants and animals.
One of the pillars of this naturist theory is based on the effect of the moon on the living world. Thus all the phenomena related to germination or fertilization are associated with the growth of the moon (Das, 2014).
On the other hand, the period of decrease of the Earth satellite has been found to favor higher quality crops, emergence of vigorous animals, etc. (Subrahmaniam, 1991).
If for vegetable crops: leaf crops, flowers crops, root crops or seed plants crops were created calendar days recommended for sowing, planting or harvesting (Ellis, 2010) for the biology of reproduction in animals, studies are controversial. (Martens, 1998).
On the contrary, some authors believe the entire theory of biodynamic agriculture is charlatanism (Smith, 2010).

## MATERIALS AND METHODS

Due to conflicting data in the literature we have tried to contribute with new elements for supporting this unconventional theory.
This paper followed 1481 bovine gestations from both dairy breeds (Holstein) as well as from beef breeds (Angus and Limousin black).
The study was spread over a period of five years. Farms were chosen from six different geographic areas in terms of soil and climate,

covering the historical lands of Romania. The main criterion for selecting a farm was the single insemination for a heat cycle.
The purpose of the research was to identify a potential model to maximize the rate of conception, depending on the period of waxing crescent or waning crescent.
The new moon and the full moon were handled separately. In one farm it was used the hormonal timing of heats.
Oestrus detection was otherwise practiced by animal behavior observation.
Each lunar phase was determined at midnight using the moon chart and the percentage of light.

## RESULTS AND DISCUSSIONS

In the dairy farms the differences between the waxing crescent and waning crescent was ranged between 10% and 20% (Figures 1, 2 and 3).

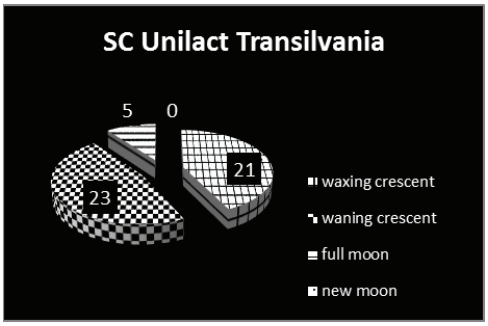


Figure 1. Pregnant dairy cows vs. moon phases. Alba county, 2016

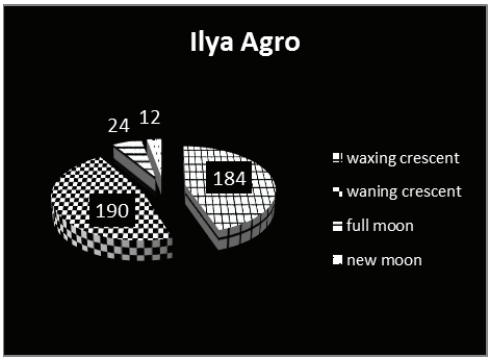


Figure 2. Pregnant dairy cows vs. moon phases. Calarasi county, 2015

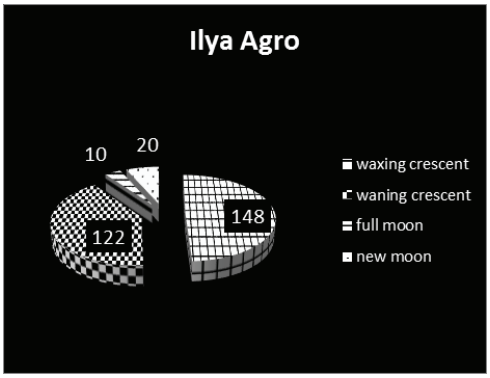


Figure 3. Pregnant dairy cows vs. moon phases. Calarasi county, 2016

As the number of studied animals increased, the differences between the two main phases decreased (Figure 4).

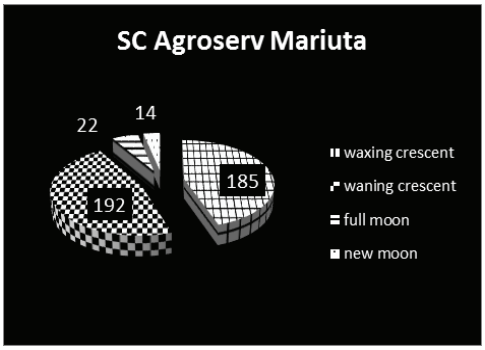


Figure 4. Pregnant dairy cows vs. moon phases. Ialomita county, 2016

Only in hormonal timing farm it was noticed an obvious ratio of 2:1 of the waxing crescent vs. waning crescent (Figure 5).

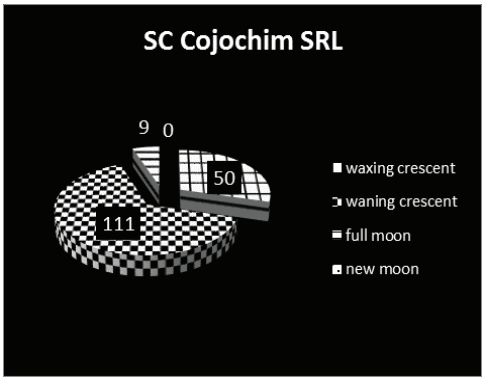


Figure 5. Pregnant dairy cows vs. moon phases. Timis county, 2013

The full moon phase offered a ratio of 2:1 vs. new moon (Figure 4). For the beef farms the waxing crescent was higher (Figure 6) with the exception of a small farm with 21 pregnant cows. In the last case the number was not significant for the general rule (Figure 7).

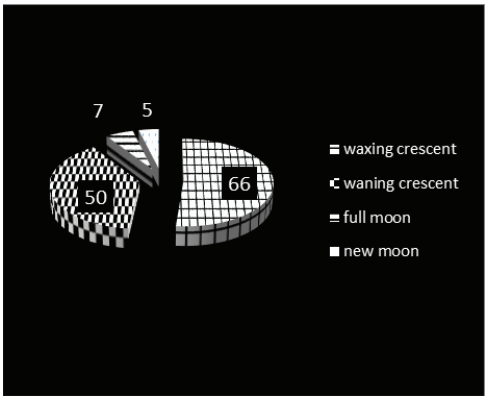


Figure 6. Pregnant beef cows vs. moon phases. Northern Transilvania, 2014

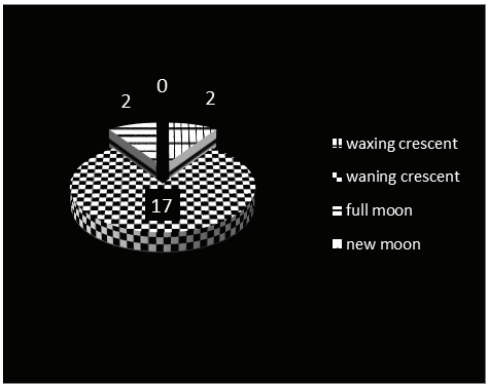


Figure 7. Pregnant beef cows vs. moon phases. Iasi county, 2012

## CONCLUSIONS

The full moon phase is the most favorable for high conception rate. On the contrary the new moon phase has the lowest rate. Economically speaking is no justification for sparing semen doses on decreasing moon phase. However grouping the heats with hormonal timing can offer a better solution for a model of inseminations in connection with lunar phases.

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# SPIN-SPIN PROTON TRANSVERSE RELAXATION TIMES STUDIES OF RED BLOOD CELL MEMBRANE IN RABBITS WITH EXPERIMENTAL ATHEROSCLEROSIS

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***Abstract***

*Nuclear magnetic resonance (NMR) is a modern and accessible technique for studies of erythrocyte membrane permeability in physiological and pathological conditions. In this paper we investigated in rabbits fed on high reach cholesterol diet, by nuclear magnetic resonance method (NMR) the following parameters: the proton life time in erythrocyte, in erythrocyte sediment proton transverse relaxation times (T2a), proton transverse relaxation times in plasma (T2b) and erythrocyte membrane permeability (EMPW). Investigations were carried out on 12 male rabbits aged 20 months old divided into two groups of 6 rabbits each: group A controls and group B fed on cholesterol reach diet (animal origin) for 8 weeks. 1H NMR measurements of the above parameters were performed with an Aremi`78 Spectrometer at 25 mHz frequency. There was a decrease in proton transverse relaxation times in red blood cells from rabbits fed on cholesterol reach diet which suggests an accelerated proton exchange. The activation energy of water exchange through erythrocyte membrane ($E_a$) is decreased in cholesterol fed rabbits versus controls. This means that at higher levels of cholesterol the exchanges of water become more accelerated and the processes of membrane exchange are partially disconnected under the influence of thermal processes with heat liberation. In other words, in controls the water exchange processes through erythrocyte membranes increases in parallel with the increase in local or global temperature due to metabolic reactions with heat liberation in intracellular environment. Erythrocyte membrane permeability to water can be taken into consideration as an index of cardiovascular system recovery, important in maintaining a dynamic equilibrium with vascular destruction phenomenon due to high blood pressure.*

***Key words****: erythrocyte membrane permeability, nuclear magnetic resonance (NMR), spin-spin proton transverse relaxation times, atherosclerosis, arterial hypertension.*

## INTRODUCTION

High blood pressure and its complications is one of the major problem of medical research, the attention being concentrated towards elucidation of path physiology mechanisms which interfere during evolutionary stages of the disease.

Recent investigations regarding atherosclerosis origin have initiated a strong debate upon the main preponderent role of hypercholesterolemia in the onset of this desease, in counterpart with the ideea that atherosclerosis could have the origin in an inadequate immune response to the appearance of vascular alterations. Despite the fact that the role of the immune system has been studied, an impresive quantity of experimental studies clearly have shown that atherogenesis is innitiated under the reciprocal influence between cholesterol, cytokine cellular secretion (esspecialy IL-6), apolipoprotein E and arterial wall (Balta, 2009).

Recent studies have shown that the cells posses two types of sensors for cholesterol:

- Ck receptors, which are sensitive for extracellular colesterol and initiate the sygnaling pathway responsible for gene regulations implicated in cell cycle, cell death and homeostasis of cell cholesterol and cytokines including ( IL-6) and
- LxR alfa receptors, which are sensitive to intracellular oxysterols and control genes implicated in cell death, cellular cholesterol homeostasis and cytokine IL-8 (Balta, 2009).

The understanding of the cell membrane permeability mechanisms to water and of changes in the intracellular water structure will might improve the actual view about various diseases in which water transport is directly involved, or the medication influences the cellular water state (Balta, 2009). These aspects are well revealed by the most modern nuclear magnetic resonance (NMR) techniques (Gatina et al., 1998; Petcu et al., 1995).

Water crosses cell membranes by two routes: by diffusion through the lipid bilayer and through water channels (namely aquaporins) (Benga, 2012), which are intrinsic membrane proteins that have been characterized as facilitators of water flux. Originally termed major intrinsic proteins (MIPs), they are now also known as water channels, glycerol facilitators and aqua-glyceroporins, yet recent data suggest that they facilitate the movement of other low-molecular-weight metabolites as well (Herrera et al., 2006; Zhang et al., 2007). Different aquaporins have different functionally important specialty (Rutkovskiy et al., 2013). The AQP-1 is found in the erythrocyte membranes, as well as in the epithelia, its expression being recently confirmed in the arterial and the capillary endothelia, in the smooth muscle vascular cells and in the atherosclerotic plaques (Shanahan et al., 2000). Taking into account this distribution we might suppose that the vascular cells and the erythrocyte membrane permeability to water are well correlated; they are modulated by the same AQP-1, controlled by the same circulating factors. Moreover, the role of arginin vasopressin and atrial natriuretic peptide in the aquaporine regulation of water channel activity (Schrier et al., 2001) consolidates this assumption. These aspects facilitate the evaluation of the cardiovascular status by NMR relaxometry measurements on blood.

Nuclear magnetic resonance (NMR) is an accessible technique for studies of erythrocyte membrane permeability in physiological and pathological conditions such as arterial hypertension experimentally induced feeding rabbits on reach cholesterol diet.

Erythrocyte membrane illustrates the functional state and the capacity of cell to renew during the life span (120 days) and imagistic methods (Stoian et al., 2012) allow the evidence for modifications in water permeability and the results may contribute to a better understanding of pathological mechanisms of arterial hypertension (Gatina et al., 1998).

The aim of this study was to investigate in an experimental model of arterial hyperthension induced in rabbits fed on cholesterol reach diet,the proton transverse relaxation times of intracellular water protons and membrane permeability for water, by 1H NMR method.

## MATERIALS AND METHODS

1. Biological material – 12 white male rabbits aged 20 months old divided into two groups of 6 rabbits each: (group A and B) for 8 weeks, with high reach cholesterol (animal origin).

2. Determinations of nuclear magnetic resonance

Biological material used was the peripherial blood harvested on heparin by exsanguination of rabbits and dopped with an adequate volume of $MnCl_2$ in such a way to obtain in extracellular compartment a concentration of 20 mM $MnCl_2$.

The method used consists in determination by means of 1 H NMR technique of proton transverse relaxation times of intra and extra erythrocyte water, determination of protons exchange time through erythrocyte membrane and the calculus of permeambility for water.[3]

The principle of the method consists in characterisation în a system composed of two compartments - A and B – of two relaxation times - $T_{2a}$ and $T_{2b}$ – of the same type of nuclei originating from the same compartment.

Nuclear relaxation times are the parameters which characterise the returning to the equilibrium of the nuclei after appling of an adequate perturbations of radiofrequency. For the system erythrocyte-plasma we are dealing with the same type of molecules distributed in A and B compartments which have corresponding relaxation times different $T_{2a}$ and $T_{2b}$. A compartment represents intra erythrocyte compartment, and B represents extracellular compartment, respectively blood

plasma, and nuclei of interest are water protons from the two compartments.

Because there is a relatively rapid exchange process between the two compartments, and the relaxation times have the closer values, the result is the perception of a single medium global relaxation time.

Therefore, for differentiation of relaxation times between the two compartments is necessary a method which makes $T_{2a}$ >>$T_{2b}$.One of the possibilities is doping with paramagnetic ions.

If a paramagnetic ion is added (for example Mn) to cell suspension, then $T_{2b}$ relaxation times of water molecules in suspension solution decreases considerably because of some processes of electron-proton, interactions resulting in such a way the possibility of separation of the two relaxation times.

Determinations of erythrocyte membrane proton transverse relaxation times ($T_{2a}$ and $T_{2b}$) Nuclear Magnetic Resonance were done on an Aremi`78 Spectrometer in impulses at 25 MHz frequency, using the standard sequence CARR-PURCELL-MEIBOOM-GILL with the interval between impulses of 1 ms.

The measurement of $T_{2a}$ and $T_{2b}$in intracellular compartment was done in the presence of water exchange between intracellular and extracellular compartments doped with $Mn^{2+}$ obtaining in such a way the apparent relaxation time $T_{2a}$'. Representation as a function of time of transverse magnetisation is:

$$M(t)=A^* \exp(-t/T_{2a}')+B^*\exp(-t/T_{2B}) \qquad [1]$$

Where the slow component of magnetisation with apparent relaxation time $T_{2a}$' separates significantly from the fast decreasing component, after introduction of experimental data in a filtering program of the two exponentials.

After 10 min centrifugation of blood samples at 1000 g, has been collected the supernatant for NMR measurements, obtaining in such a way the intrinsic relaxation time $T_{2b}$ of doped plasma which represents the extracellular water compartment. Then the erythrocytes have been washed 3 times with phosphate buffer saline (PBS) and centrifuged at the above mentioned parameters. The sediment has been measured in order to obtain the intrinsic relaxation time of intracellular compartment of water $T_{2a}$. Using these data, has been calculated the life time of a water molecule in intracellular compartment.

$$\frac{1}{\tau}=\frac{1}{T'_{2a}}(1-h)-\frac{1}{T_{2a}}(1-h)^2-\frac{1}{T_{2b}}h(1-h)\left[1+\frac{(1-h)\frac{\left(\frac{1}{T_{2a}}-\frac{1}{T_{2b}}\right)^2}{\frac{1}{T_{2b}}}}{\frac{1}{T'_{2a}}-h\frac{1}{T_{2a}}-\frac{1}{T_{2b}}(1-h)}\right] \qquad [2]$$

The h parameter represents the ratio between the intracellular water volume and the total volume of water in blood. It is obtained from hematocrit, taking into account that 71.5% from the medium erythrocyte volume (vem) and respectively 94.5% from the volume of blood plasma is occupied by water. In our experiments the integral blood samples have been reconstituted by resuspending erythrocytes in plasma, using in all cases a 55% proportion of erythrocyte pellet.

The value of erythrocyte membrane permeability is obtained from t using formula:

$$P=\left(\frac{V}{A}\right)\left(\frac{1}{\tau}\right) \qquad [3]$$

where V and A represent volume, and respectively erythrocyte area .

The mean erythrocyte volume has been calculate by formula:

$$V=\frac{h*10}{N} \qquad [4]$$

where h is the percentige measured value of haematocrite, and N is the number of erythrocyte/$mm^3$, experimentally determined.

The mean erythrocyte surface is obtained from:

$$A=\pi*\frac{D^2}{2}+2*\frac{V}{D} \qquad [5]$$

D is being the medium erythrocyte diameter measured.

RMN measurements have been done on a range of temperatures between 0-42$^0$C, respectively at 0$^0$C, 22$^0$C, 30$^0$C, 37$^0$C and

$42^0$C, and the obtained values for membrane permeability to water (PMEA) have been compared at $37^0$C.

There are many pathways of water transport (lipid and protein), to each being associated a certain specific activation energy of transmembrane water diffusion process ($Ea^L$ and $Ea^P$), in such a way that the determined transmembrane exchange time becames:

$$\frac{1}{\tau} = a * \exp\left(\frac{-Ea^L}{kT}\right) + b * \exp\left(\frac{-Ea^P}{kt}\right) \quad [6]$$

*a* and *b* being constants dipending on the membrane structure.

In this context is defined the activation global energy of transmembrane water exchange processes (Ea) as being:

$$\frac{1}{\tau_{\exp}} = c * \exp\left(\frac{-Ea}{kT}\right) \quad [7]$$

where *c* is a constant. By logaritming the expression, results:

$$\ln\left(\frac{1}{\tau_{\exp}}\right) = \ln c - \frac{Ea}{kT}$$

$$\text{so } \ln\tau_{\exp} = \ln c - \frac{Ea}{kT} \text{ or } \ln\tau_{\exp}\frac{Ea}{kT} - \ln c \quad [8]$$

From the graph $\ln\tau_{\exp} = functie\left(\frac{1}{kT}\right)$ is calculated Ea, after filtering with a (line) of experiemntaly obtained points.

In all above formula *k* is the Boltzmann constant.

Using the logarithmic representations of variations of proton relaxation times of plasma water and respectively of erythrocytes, as a function of temperature, we have deduced analogous activation energies of water relaxation processes from extracellular and intracellular water compartment.

## RESULTS AND DISCUSSIONS

*Nuclear Magnetic Resonance data*

The study of experiemtaly induction of arterial hyperthension by overdosing cholesterol in feding the rabbits was intended to point out vascular system dysfunctions, respectively at the level of red blood cells membrane permeability for water.

In our laboratory, the previous research data (Gatina et al., 1998) have pointed out that any of the modifications produced at the level of coronary or periferic circulation, are accompanied by changing in the permeability for water of vascular walls, which brings about a modification in hydration state of tissues supply by the vascular affected bed.

These deviations from the equilibrium state is reflected in modifications of proton transverse relaxation times parameters which are accesible for NMR for the investigated biological material.

Modern studies in the molecular biology field have pointed out the presence of some proteins which are implicated in water channels 9, aquaporins (Gatina et al., 1998).

Aquaporin AQP1 is the most widely found in organism being present in erythrocyte membrane, in artery, arterioles, venes, cappilaries endothelium, as well as in certain smooth vascular muscle from human atherosclerotic plaques and which assure the active water transport through cell membranes, is responsible for water exchange through vascular walls (Benga, 2012).

Because the aquaporine synthesis is altered, the action of some hormones, such as arginin-vasopresin (activator of synthesis) or natriuretic peptide (inhibitor of synthesis), result in erythrocyte membrane permeability to water alteration which are sincronous with those from the cardiovascular system and are produced in the same way.

Also, the permeability to water can be modified by chainging the proportion and distribution of lipid membranes.

The proton transverse relaxation time of intraerythrocyte water ($T_{2a}$) decreases very slightly, in hypercholesterolemic rabbits, versus controls, while Proton transverse relaxation time in case of plasma erythrocyte water ($T_{2b}$) increases slightly in cholesterol fed rabbits (Figure 1).

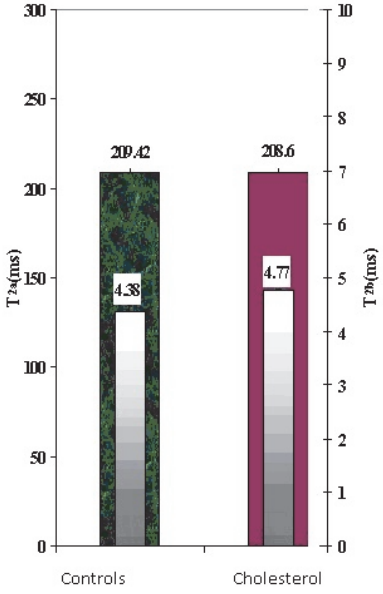


Figure 1. Proton transverse relaxation time of intra erythrocyte water (T2a) and of plasma water (T2b) from controls and cholesterol fed rabbits

Figure 2 presents the aspects related to the dynamics of protons through erythrocyte membrane and modifications of water exchange energetics.

There is a decrease of erythrocyte proton life time ($\tau$), which suggests an accelerated proton exchange, in cholesterol fed rabbits of group B. Activation energy of water exchange through erythrocyte membrane ($E_\tau$) is decreased in cholesterol fed rabbits versus controls.

This means that at higher levels of cholesterol the exchanges of water became more accelerated, and the process of membrane exchange is partially deconected under the influence of thermic processes with heat liberation.

In other words, in controls the water exchange processes through erythrocyte membrane increases in paralell with the increase in local or global temperature due to metabolic reactions with heat liberation in intracellular environment.

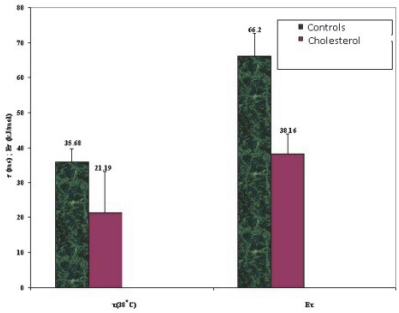


Figure 2. Exchange time of water through erythrocyte membrane($\tau$) and activation energy of water exchange through erythrocyte membrane ($E_\tau$) in controls and cholesterol fed rabbits

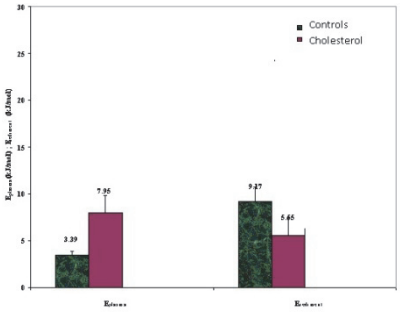


Figure 3. Activation energy of water exchange through plasma and activation energy of water exchange through erythrocyte pellet in controls and cholesterol fed rabbits

When specific energetic processess of an exchange between the two compartments are affected, this means that either one of these compartments is responsable for a certain „turn" on of energetic domain or, both compartments are implicated in this process.

Investigating the situation of biocompartimental system plasma-erythrocyte from the point of view of activation energy of proton relaxation processes (Figure 3), it is observed a significant increase of activation energy in plasma ($E_{plasma}$) of cholesterol fed rabbits versus controls. There is a significant decrease in activation energies inside erythrocytes from cholesterol fed rabbits versus controls. Erythrocyte membrane permeability to water (PMEA) is a parameter which accounts for the exchange of water through erythrocyte membrane, as well as for those processes which take place in vascular

walls. This correlation is allowed by the presence of the same type of aquaporine-AQP1- both in erythrocyte membrane and in vascular endothelial membranes at all levels.

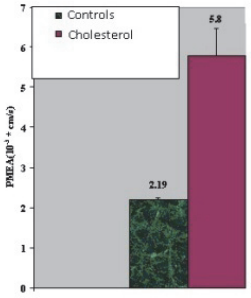


Figure 4. Permeability to water increases very much in hyper cholestorelemic rabbits (for example: from the value of 2,19cm-s*$10^{-3}$ in controls, to 5,8cm-s*$10^{-3}$ in hyper cholesterolemic rabbits

The study of experiemtaly induction of arterial hyperthension by overdosing cholesterol in rabbits feding diet was intended to point out vascular system dysfunctions, respectively at the level of blood.

In our laboratory, the research data have pointed out that any of the modifications produced at the level of coronary or periferic circulation, are accompanied by changing in the permeability to water of vascular walls, which brings about a modification in hydration state of tissues supply by the vascular bed affected. These deviations from the equilibrium state is reflected in modifications of proton transverse relaxation time, parameters which are accesible for NMR for the investigated tissue.

Modern studies in the molecular biology field have pointed out the presence of some proteins which are implicated in water channels 9 aquaporins. Aquaporin AQP1 is the most widely found in organism being present in erythrocyte membrane, in artery, arterioles, venes, capillaries, endothelium, as well as in certain smooth vascular muscule from human atherosclerotic plaques, and which assure the active water transport through cell membranes, is responsible for water exchange through vascular wallls (Benga, 2012).

Because the aquaporine synthesis is altered, the action of some hormones, such as arginin-vasopresin (activator of synthesis) or natriuretic peptide (inhibitor of synthesis), determine modifications in membrane permeability to water at the level erythrocyte which are sincronous with those from the cardiovascular system which are produced in the same way. Also, the permeability to water can be modified by chainging the proportion and distribution of lipid membranes.

There is a decrease of erythrocyte proton life time ($\tau$), which suggests an accelerated proton exchange, in group B (Figure 2). Activation energy of water exchange through erythrocyte membrane ($E_\tau$) is decreased in cholesterol fed rabbits versus controls.

This means that at higher levels of cholesterol the exchanges of water becames more accelerated, and the process of membrane exchange is partially deconected under the influence of thermic processes with heat liberation. In other words, in controls the water exchange processes through erythrocyte membrane increase in investigations in pararel with the increase in local or global temperature (in presence of metabolic reactions with heat liberation in intracellular environment.

From our previous data (Gatina et al., 1998) resulted the fact that permeability to water is increased in the onset stages of the disease as an adapting mechanism to the increased arterial hypertension value and if after maintaining it at an increased level occurs a sudden decrease, this is correlated with an increased risk for stroke onset.

Therefore, not always the reduction to normal values of an increased physiological parameter in the context of a pathological state is indicated because the adaptation of organism has created a new state of equilibrium; if the primary cause of the disturbance of normal equilibrium is not corrected then a more severe situation is achieved.

It is mentioned that a single dose administration of drugs in hypertensive patients which decreases permeability to water of red blood cell membranes is risky and it is recommended that this administration to be associated with drugs that have an effect on membrane permeability.

## CONCLUSIONS

Modern investigations with NMR methodology have pointed out modifications in erythrocyte membrane function in hyperthensive aging rabbits fed on cholesterol reach diet versus age matched controls.

The proton transverse relaxation time of intraerythrocyte water ($T_{2a}$) decreases very slightly, in hypercholesterolemic rabbits, versus controls, while Proton transverse relaxation time in case of plasma erythrocyte water ($T_{2b}$) increases slightly in cholesterol fed rabbits

There is a decrease of proton life time in erythrocyte, which suggest an accelerated proton exchange in hypercholesterolemic rabbits.

In controls, water exchange through erythrocyte membrane is accelerated as a function of increase in local or global temperature (determined by the activation of metabolic reactions with heat liberation from intracellular medium.)

Membrane permeability to water (PMEA) increases significantly in cholesterol fed rabbits versus controls. This may be taken into account as an index of recovery of cardiovascular system, important in mentaining a dynamic equilibrium with phenomena of vascular destruction due to the increased arterial blood pressure.

NMR relaxometric could be a useful tool in functional evaluation of erythrocyte membrane permeability in normal and pathological conditions and these results bring a valuable contribution to a better understanding of aging process as well as of pathological mechanisms of arterial hypertension.

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# TECHNOLOGIES OF ANIMAL HUSBANDRY

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# SOME FACTORS AFFECTING BREED SELECTION OF LIVESTOCK FARMS IN SIIRT PROVINCE

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***Abstract***

*This study investigates factors such as location, altitude, number of animals and number of employees influential in breed preferences of sheep and goat farms in and around Siirt. Research material was formed from a survey carried out in 286 enterprises in Siirt and 6 districts. Data were analysed using SPSS 20.0 software package. Breeds reared in farms are varying by location, in such districts as Centrum, Baykan and Kurtalan with low altitude, hair goats were not preferred, while in places like Eruh, Pervari and Şirvan with rich pasture and high altitude hair goats were preferred intensely. Inbreed preference of farms animal number was found significant ($p<0.01$). It was found 42.5% of hair goats and 64.3% of Awassi ewes in farms with 26-50 animals, while in farms with 250 and more animals no hear goats was found. Satisfaction case in farms was different according to education level of farmers. Hence, 94.7% of uneducated farmers were satisfied with the reared breed. This rate was 98.3% in primary school graduates, 96.6% in secondary school graduates and increased to %100 in high school graduates. In cattle breed preferences Kurtalan and Pervari districts preferred exotic cattle in the close rates (%35.9, %33.3), also it was pointed out that the both districts had different altitudes. Again, the low percent of native breed and zero crossbreed cattle in Kurtalan district was found significant. Influential factors as location, number of employees in the farm, education, farming duration ($p<0.01$), altitude and age ($p<0.05$) were found significant in farms' cattle breed preferences. With this study made in sheep and goat farms located in Siirt, the impact of the investigated factors in breed selection was found significant. Considering the potential of the livestock in Siirt, it is concluded that in giving directions to the livestock in Siirt, these factors must be taken into account.*

***Key words****: sheep and goat breeds, cattle breeds, Siirt.*

## INTRODUCTION

Sheep and goats come to the forefront with their ability to adapt to insufficient pasture and unfavourable climatic conditions, and this situation is especially important for developing countries. Sheep and goats that can benefit from the pasture in the best way, utilise the pasture in the best way and can use the pasture in every period of the year are suitable for the geographical structure of Turkey and Siirt. The fact that sheep and goats adapt to areas where agricultural production is unproductive in a short time turns this disadvantage into an opportunity in developing countries like Turkey (DAKA, 2012). Ovine-caprine animal breeding is commonly performed in Siirt province as well as throughout the Southeastern region.

Holstein cattle, with dairy features, constitute a significant part of culture breed cattle raised in Turkey, and it is followed by Brown Swiss, Jersey and Simmental cattle. Native Black, Eastern Anatolian Red and Southeastern Anatolian Red cattle breeds constitute a significant portion of the indigenous breeds. Cross-breed genotypes are usually obtained by crossing culture breeds with indigenous breeds (TİGEM, 2013).

This study was carried out to determine the breed preferences of sheep-goats and bovine animal enterprises in Siirt province and its districts and to investigate some factors such as altitude, location, the number of animals, the number of employees in the enterprise that affect breed preferences.

## MATERIALS AND METHODS

Siirt province has an area of 11,003 $km^2$, and the surface area of Siirt Province decreased to 6,186 $km^2$ after the change in the borders in 1990. Siirt, which is located on $41^{o}57^{'}$ east longitude and $37^{o}55^{'}$ north latitude in the Southeastern Anatolia Region, is surrounded by the provinces of Şırnak and Van from the east, Batman and Bitlis from the north, Batman from

the west, Mardin and Şırnak from the south (Figure 1). Most of the province's territories are covered with mountains. The Muş South Mountains in the north and the Siirt East Mountains in the east are the mountain ranges that form the natural borders of the province.


Figure 1. The map of Siirt Province (Saygılı, 2015)

The villages representing the provinces to determine the breed preferences of the livestock owners were determined by the opinion of veterinarians working in the region. The 57 villages surveyed and 286 questionnaires were determined to best represent the districts of Merkez, Kurtalan, Baykan, Pervari, Eruh, Şirvan and Tillo in Siirt province. Data were collected by questionnaire during May - December 2015 period from migration and permanent livestock enterprises registered in Siirt region. The questionnaire forms prepared for the purpose of the research were filled in personally by the researchers themselves. Survey analysis was performed by SPSS Statistical package version 20.0.

## RESULTS AND DISCUSSIONS

Location, the number of animals, the number of workforce in the enterprise, and altitude were determined to be significant factors in the breed selection of enterprises ($p<0.01$). Hair goat is not preferred in districts such as Merkez (Central), Baykan and Kurtalan, which are lower in altitude than the other districts. However, hair goat is preferred more intensively in regions such as Pervari, Şirvan and Eruh, which are high in altitude and rich in pasture. While Awassi sheep are raised intensively in Baykan district, it is observed that Hamdani sheep are raised only by 5.6% in this district.

Hamdani sheep are raised in Şirvan, Pervari and Eruh districts, which are relatively high in altitude compared to the others and rich in pasture.

The number of animals is observed to be a significant factor in the breed preference of enterprises. While hair goat (42.5%) and Awassi sheep (64.3%) are intensively present in enterprises with 26-50 animals, hair goat is not present in enterprises with the number of animals over 250.

Since Hamdani sheep are the most preferred breed in the region, it is present in all groups although it is intensively raised in herds with 51-100 animals by 31.5% (Table 1). In a study carried out in Van province, while hair goat is raised in ovine-caprine animal enterprises by 79.68%, Norduz goat is raised by 20.32% (Karakuş and Akkol, 2013).

Table 1. The effect of location and animal numbers to the breed of sheep and goats of Siirt Province

| | | Location** | | | | | | | Total | Animal number** | | | | | | Total |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Centre | Baykan | Şirvan | Pervari | Tillo | Eruh | Kurtalan | | 0-25 | 26-50 | 51-100 | 101-250 | 251-500 | 500 and above | |
| Hair goat | N | 2 | 2 | 7 | 15 | 0 | 11 | 3 | 40 | 6 | 17 | 9 | 8 | 0 | 0 | 40 |
| | % | 5 | 5 | 17.5 | 37.5 | 0 | 27.5 | 7.5 | 100 | 15 | 42.5 | 22.5 | 20 | 0 | 0 | 100 |
| Awassi | N | 0 | 12 | 2 | 0 | 0 | 0 | 0 | 14 | 2 | 9 | 1 | 2 | 0 | 0 | 14 |
| | % | 0 | 85.7 | 14.3 | 0 | 0 | 0 | 0 | 100 | 14.3 | 64.3 | 7.1 | 14.3 | 0 | 0 | 100 |
| Hamdani hybrid | N | 23 | 13 | 48 | 58 | 11 | 41 | 38 | 232 | 14 | 60 | 73 | 48 | 19 | 18 | 232 |
| | % | 9.9 | 5.6 | 20.7 | 25 | 4.7 | 17.7 | 16.4 | 100 | 6 | 25.9 | 31.5 | 20.7 | 8.2 | 7.8 | 100 |
| Total | N | 25 | 27 | 57 | 73 | 11 | 52 | 41 | 286 | 22 | 86 | 83 | 58 | 19 | 18 | 286 |
| | % | 8.7 | 9.4 | 19.9 | 25.5 | 3.8 | 18.2 | 14.3 | 100 | 7.7 | 30.1 | 29 | 20.3 | 6.6 | 6.3 | 100 |

**:p<0.01, *:p<0.05 significance level

Altitude is an important factor in breed preference ($p<0.01$). According to this, hair goat is grown intensely (42.5%) in the 1312-1590 m altitude enterprises, whereas there are no Awassi sheep in the enterprises at this altitude. While Hamdani sheep was grown at 35.8% at this altitude, it has been determined that there are different rates at each altitude. While hair goat is grown at 52.5% in the enterprises where the number of employees is 1-2, hair goat breeding rate decreases as the number of employees increases and sheep rate increases rapidly. While Awassi sheep breeding centred in the number of employees with 5-6 employees, Hamdani breeding is centred in 1-4 groups (Table 2).

Table 2. The effect of altitude and employee numbers to the breed of sheep and goats of Siirt Province

| | | Altitude** | | | | | Total | Employee numbers** | | | | | Total |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 475-753 | 754-1032 | 1033-1311 | 1312-1590 | 1591-1869 | | 1-2 | 3-4 | 5-6 | 7-8 | 9+ | |
| Hair goat | N | 4 | 10 | 9 | 17 | 0 | 40 | 21 | 11 | 6 | 0 | 2 | 40 |
| | % | 10 | 25 | 22.5 | 42.5 | 0 | 100 | 52.5 | 27.5 | 15 | 0 | 5 | 100 |
| Awassi | N | 12 | 0 | 2 | 0 | 0 | 14 | 1 | 3 | 7 | 0 | 3 | 14 |
| | % | 85.7 | 0 | 14.3 | 0 | 0 | 100 | 7.1 | 21.4 | 50 | 0 | 21.4 | 100 |
| Hamdani hybrid | N | 26 | 78 | 36 | 83 | 9 | 232 | 90 | 84 | 33 | 11 | 14 | 232 |
| | % | 11.2 | 33.6 | 15.5 | 35.8 | 3.9 | 100 | 38.8 | 36.2 | 14.2 | 4.7 | 6 | 100 |
| Total | N | 42 | 88 | 47 | 100 | 9 | 286 | 112 | 98 | 46 | 11 | 19 | 286 |
| | % | 14.7 | 30.8 | 16.4 | 35 | 3.1 | 100 | 39.2 | 34.3 | 16.1 | 3.8 | 6.6 | 100 |

**:$p<0.01$, *:$p<0.05$ significance level

Breeders' satisfaction with the current breeds in their enterprises was examined. Accordingly, while 97.2% of the breeders expressed satisfaction with the current breeds, only 2.8% of them said that they were not satisfied. The state of satisfaction in enterprises differed by the educational levels of managers. Accordingly, while 94.7% of managers without education were satisfied with the breed they raised, this ratio increased to 98.3% in primary school, 96.6% in secondary school and 100% in high school. Furthermore, satisfaction varied by the level of altitude at which the enterprises were located. While all managers at low altitudes (475-753 m) were satisfied with the breed they raised, the state of satisfaction decreased to 94% when the altitude level increased to 1312-1590 m. Managers' states of dissatisfaction with the breed they raised were examined. Accordingly, while the yield problem ranked first by 88.9%, feed supply and price were determined to be the second factor by 11.1%.

While Kurtalan and Pervari districts preferred culture breed in the preference of cattle breeds at close ratios (35.9%, 33.3%), it was remarkable that both districts were different from each other in terms of the settlement. It was also found to be significant that Kurtalan district did not demand cross breeds and demanded indigenous breeds at a low rate. The fact that indigenous breeds are intensively preferred in Eruh district is thought to be associated with the socio-economic nature of the district. It is observed that the demand for culture breeds decreases as the number of employees in the enterprise increases and that the enterprises with 1-2 and 3-4 employees demand culture breeds at most. It was found out that the enterprises demanding cross breeds were intensively the enterprises with 1-2 employees, and that the demand for cross breeds decreased with the increase in the number of employees (Table 3).

Table 3. The effect of location and employee numbers to the breed of cattle of Siirt Province

| | | Location** | | | | | | | Total | Employee numbers** | | | | | Total |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Centre | Baykan | Şirvan | Pervari | Tillo | Eruh | Kurtalan | | 1-2 | 3-4 | 5-6 | 7-8 | 9+ | |
| Culture breed | N | 1 | 5 | 13 | 28 | 2 | 3 | 26 | 78 | 29 | 24 | 13 | 5 | 7 | 78 |
| | % | 1.3 | 6.4 | 16.7 | 35.9 | 2.6 | 3.8 | 33.3 | 100 | 37.2 | 30.8 | 16.7 | 6.4 | 9 | 100 |
| Hybrid | N | 1 | 0 | 1 | 3 | 3 | 1 | 0 | 9 | 6 | 2 | 0 | 1 | 0 | 9 |
| | % | 11.1 | 0 | 11.1 | 33.3 | 33.3 | 11.1 | 0 | 100 | 66.7 | 22.2 | 0 | 11.1 | 0 | 100 |
| Native breed | N | 8 | 10 | 18 | 1 | 0 | 37 | 2 | 76 | 25 | 41 | 9 | 0 | 1 | 76 |
| | % | 10.5 | 13.2 | 23.7 | 1.3 | 0 | 48.7 | 2.6 | 100 | 32.9 | 53.9 | 11.8 | 0 | 1.3 | 100 |
| Total | N | 10 | 15 | 32 | 32 | 5 | 41 | 28 | 163 | 60 | 67 | 22 | 6 | 8 | 163 |
| | % | 6.1 | 9.2 | 19.6 | 19.6 | 3.1 | 25.2 | 17.2 | 100 | 36.8 | 41.1 | 13.5 | 3.7 | 4.9 | 100 |

**:p<0.01, *:p<0.05 significance level

A significant (p<0.05) relationship was found between the altitude at which the enterprises were located and the preferred breed. Accordingly, culture and cross breeds were determined to be raised in enterprises in the districts such as Pervari and Şirvan with a relatively higher altitude at close ratios. It is observed that indigenous breeds are preferred in enterprises with a very high altitude (1592-1869 m) at a lower ratio (3.9%) and are generally raised at close ratios in enterprises with other altitudes. When it was taken into account that the educational backgrounds of managers generally consisted of primary, secondary education graduates and illiterate and that there were only 7 high-school graduate managers, it was found out that 64.1% of those who preferred culture breeds were primary school graduates. The fact that those without education did not raise cross breeds attracted attention (Table 4). In a study in which the breed preferences of feeder cattle enterprises in Ergani district were investigated, while illiterate managers preferred indigenous breeds (48.5%), it was observed that the preference for indigenous breeds increased from primary school to high school (52.6%, 75.0% and 92.3%) (Han and Bakır, 2009).

Table 4. The effect of altitude and education to the breed of cattle of Siirt Province

| | | Altitude* | | | | | Total | Education** | | | | Total |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 475-753 | 754-1032 | 1033-1311 | 1312-1590 | 1591-1869 | | Not educated | Primary school | Secondary school | High school | |
| Culture breed | N | 6 | 30 | 7 | 32 | 3 | 78 | 15 | 50 | 8 | 5 | 78 |
| | % | 7.70 | 38.50 | 9.00 | 41.00 | 3.80 | 100.00 | 19.20 | 64.10 | 10.30 | 6.40 | 100.00 |
| Hybrid | N | 1 | 3 | 1 | 4 | 0 | 9 | 0 | 4 | 4 | 1 | 9 |
| | % | 11.10 | 33.30 | 11.10 | 44.40 | 0.00 | 100.00 | 0.00 | 44.40 | 44.40 | 11.10 | 100.00 |
| Native breed | N | 16 | 24 | 17 | 16 | 3 | 76 | 20 | 52 | 3 | 1 | 76 |
| | % | 21.10 | 31.60 | 22.40 | 21.10 | 3.90 | 100.00 | 26.30 | 68.40 | 3.90 | 1.30 | 100.00 |
| Total | N | 23 | 57 | 25 | 52 | 6 | 163 | 35 | 106 | 15 | 7 | 163 |
| | % | 14.10 | 35.00 | 15.30 | 31.90 | 3.70 | 100.00 | 21.50 | 65.00 | 9.20 | 4.30 | 100.00 |

**:p<0.01, *:p<0.05 significance level

The duration of performing animal husbandry was a significant (p<0.01) factor in breed selection. It was found out that the enterprises with the duration of performing animal husbandry of 41 years and over preferred indigenous breeds. Accordingly, while young breeders do not prefer indigenous breeds, a few of them demand cross breeds. It is observed that the breeders demanding culture breeds are over the age of 25 years (Table 5). In a previous study carried out in Ergani district, the preference for cross breeds in all groups of the duration of performing animal husbandry draws attention, and the young breeders with little

experience and those with the experience of 8-13 years preferred indigenous breeds by 36.4% and 38.1% (Han and Bakır, 2009).In a study carried out in bovine animal enterprises in Muş province, it was determined that the enterprises were engaged in indigenous, cross breeding and culture breeding by 46.9%, 37.2% and 15.9%, respectively (Şeker et al., 2012). The age of managers was found to be a significant factor in the breed preference.

Table 5. The effect of duration of performing animal husbandry and age to the breed selection of cattle of Siirt Province

| | | Duration of performing animal husbandry ** | | | | | Total | Age* | | | | | Total |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 1-10 | 11-20 | 21-30 | 31-40 | Above 41 | | 16-25 | 26-36 | 37-48 | 49-59 | 60 and older | |
| Culture breed | N | 1 | 17 | 28 | 17 | 15 | 78 | 1 | 18 | 29 | 21 | 9 | 78 |
| | % | 1.3 | 21.8 | 35.9 | 21.8 | 19.2 | 100 | 1.3 | 23.1 | 37.2 | 26.9 | 11.5 | 100 |
| Hybrid | N | 1 | 3 | 3 | 2 | 0 | 9 | 1 | 3 | 3 | 2 | 0 | 9 |
| | % | 11.1 | 33.3 | 33.3 | 22.2 | 0 | 100 | 11.1 | 33.3 | 33.3 | 22.2 | 0 | 100 |
| Native breed | N | 0 | 10 | 24 | 16 | 26 | 76 | 0 | 7 | 28 | 22 | 19 | 76 |
| | % | 0 | 13.2 | 31.6 | 21.1 | 34.2 | 100 | 0 | 9.2 | 36.8 | 28.9 | 25 | 100 |
| Total | N | 2 | 30 | 55 | 35 | 41 | 163 | 2 | 28 | 60 | 45 | 28 | 163 |
| | % | 0.6 | 18.4 | 33.7 | 21.5 | 25.2 | 100 | 1.2 | 17.2 | 36.8 | 27.6 | 17.2 | 100 |

**:p<0.01, *:p<0.05 significance level

It was determined that the location was a significant factor (p<0.01) in the cattle breed selection. According to this, it is noteworthy that only one breeder's request for Simmental from the culture breed, and the others and the breeders of Eruh demand the South Anatolian Red breed from the indigenous breed in the rates of 60% and 75.6%. In addition, breeders from Baykan and Şirvan mainly preferred Native Black breed in similar proportions (46.7%, 46.9%). It was found significant, that the breeders in Pervari district prefer the Brown Swiss breed in the rate of 75.6% among the culture breeds (Table 6). The proportion of culture breed cattles raised in Muş province was found as 17.2% Simmental, 12.5% Holstein and 70.3% Brown Swiss (Şeker et al., 2012).

Table 6.Cattle breeds preferred in Siirt Province

| Location | | Cattle breeds** | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Simmental | Holstein | Brown Swiss | SAR | EAR | Native Black | Hybrid | Total |
| Centre | N | 1 | 0 | 0 | 6 | 0 | 2 | 1 | 10 |
| | % | 10 | 0 | 0 | 60 | 0 | 20 | 10 | 100 |
| Baykan | N | 2 | 0 | 3 | 3 | 0 | 7 | 0 | 15 |
| | % | 13.3 | 0 | 20 | 20 | 0 | 46.7 | 0 | 100 |
| Şirvan | N | 4 | 1 | 8 | 3 | 0 | 15 | 1 | 32 |
| | % | 12.5 | 3.1 | 25 | 9.4 | 0 | 46.9 | 3.1 | 100 |
| Pervari | N | 3 | 0 | 26 | 0 | 0 | 0 | 3 | 32 |
| | % | 9.4 | 0 | 81.3 | 0 | 0 | 0 | 9.4 | 100 |
| Tillo | N | 2 | 0 | 0 | 0 | 0 | 0 | 3 | 5 |
| | % | 40 | 0 | 0 | 0 | 0 | 0 | 60 | 100 |
| Eruh | N | 0 | 3 | 1 | 31 | 3 | 2 | 1 | 41 |
| | % | 0 | 7.3 | 2.4 | 75.6 | 7.3 | 4.9 | 2.4 | 100 |
| Kurtalan | N | 15 | 1 | 10 | 0 | 0 | 2 | 0 | 28 |
| | % | 53.6 | 3.6 | 35.7 | 0 | 0 | 7.1 | 0 | 100 |
| Total | N | 27 | 5 | 48 | 43 | 3 | 28 | 9 | 163 |
| | % | 16.6 | 3.1 | 29.4 | 26.4 | 1.8 | 17.2 | 5.5 | 100 |

**:p<0.01, *:p<0.05 significance level, SAR: Sourtheastern Anatolian Red, EAR: Eastern Anatolian Red

Village type was found to be a significant factor in the cattle breed preference ($p<0.01$). Accordingly, while the enterprises located in mountain villages preferred the Southeastern Anatolian Red cattle breed from among indigenous breeds, and the Brown Swiss cattle breed from among culture breeds, it was observed that the enterprises in slope villages were more willing in the preference of the Brown Swiss breed from among culture breeds compared to those in mountain villages. The enterprises in lowland villages raise the Simmental breed in addition to the Brown Swiss cattle breed from among culture breeds (Table 7).

Table 7. The effect of village types to the cattle breed selection of Siirt Province

| Village type | | Cattle breeds** | | | | | | | Total |
|---|---|---|---|---|---|---|---|---|---|
| | | Simmental | Holstein | Brown Swiss | SAR | EAR | Native Black | Hybrid | |
| Mountain villages | N | 12 | 4 | 18 | 29 | 3 | 14 | 6 | 86 |
| | % | 14 | 4.7 | 20.9 | 33.7 | 3.5 | 16.3 | 7 | 100 |
| Slope villages | N | 1 | 0 | 16 | 11 | 0 | 9 | 2 | 39 |
| | % | 2.6 | 0 | 41 | 28.2 | 0 | 23.1 | 5.1 | 100 |
| Lowland villages | N | 14 | 1 | 14 | 3 | 0 | 4 | 1 | 37 |
| | % | 37.8 | 2.7 | 37.8 | 8.1 | 0 | 10.8 | 2.7 | 100 |
| Migrant villages | N | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 1 |
| | % | 0 | 0 | 0 | 0 | 0 | 100 | 0 | 100 |
| Total | N | 27 | 5 | 48 | 43 | 3 | 28 | 9 | 163 |
| | % | 16.6 | 3.1 | 29.4 | 26.4 | 1.8 | 17.2 | 5.5 | 100 |

**:$p<0.01$, *:$p<0.05$ significance level, SAR: Sourtheastern Anatolian Red, EAR: Eastern Anatolian Red

Educational background was significant $p<0.05$ in the breed preference of enterprises, while illiterate ones preferred SAR from indigenous breeds and the Brown Swiss breed from culture breeds, the ratio of SAR decreased in primary school graduates, and it was observed that the demand for the Brown Swiss and Simmental breeds from culture breeds increased, and the demand for Native Black cattle also increased. While there was a rapid increase (33.3%) in the Simmental breed, a similar situation was observed in the cross breed in secondary school graduates compared to others. Although the fact that the number of enterprises with high school graduates was small was taken into account, it was remarkable that the Brown Swiss breed was preferred at the highest rate (Table 8).

Table 8.The effect of education to the cattle breed selection of Siirt Province

| Education | | Cattle breeds* | | | | | | | Total |
|---|---|---|---|---|---|---|---|---|---|
| | | Simmental | Holstein | Brown Swiss | SAR | EAR | Native Black | Hybrid | |
| Not educated | N | 5 | 2 | 8 | 14 | 0 | 6 | 1 | 36 |
| | % | 13.9 | 5.6 | 22.2 | 38.9 | 0 | 16.7 | 2.8 | 100 |
| Primary school | N | 16 | 3 | 32 | 28 | 3 | 20 | 3 | 105 |
| | % | 15.2 | 2.9 | 30.5 | 26.7 | 2.9 | 19 | 2.9 | 100 |
| Secondary school | N | 5 | 0 | 4 | 0 | 0 | 2 | 4 | 15 |
| | % | 33.3 | 0 | 26.7 | 0 | 0 | 13.3 | 26.7 | 100 |
| High school | N | 1 | 0 | 4 | 1 | 0 | 0 | 1 | 7 |
| | % | 14.3 | 0 | 57.1 | 14.3 | 0 | 0 | 14.3 | 100 |
| Total | N | 27 | 5 | 48 | 43 | 3 | 28 | 9 | 163 |
| | % | 16.6 | 3.1 | 29.4 | 26.4 | 1.8 | 17.2 | 5.5 | 100 |

**:$p<0.01$, *:$p<0.05$ significance level, SAR: Sourtheastern Anatolian Red, EAR: Eastern Anatolian Red

In the preference of breeds raised in enterprises, while beginners in animal husbandry preferred indigenous breed and SAR, this ratio decreased to 25.2% in those who performed it as a predecessor job, and the culture breed ratios increased to 30.5% and 17.2% in the Brown Swiss breed and Simmental breed, respectively. Accordingly, it is observed that the beginners appear to be more distant to culture breeds (Table 9).

Table 9. The effect of career to the cattle breed selection of Siirt Province

| Career | | Cattle breeds** | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Simmental | Holstein | BrownSwiss | SAR | EAR | Native Black | Hybrid | Total |
| Beginner | N | 1 | 2 | 2 | 5 | 2 | 0 | 0 | 12 |
| | % | 8.3 | 16.7 | 16.7 | 41.7 | 16.7 | 0 | 0 | 100 |
| Predecessor job | N | 26 | 3 | 46 | 38 | 1 | 28 | 9 | 151 |
| | % | 17.2 | 2 | 30.5 | 25.2 | 0.7 | 18.5 | 6 | 100 |
| Total | N | 27 | 5 | 48 | 43 | 3 | 28 | 9 | 163 |
| | % | 16.6 | 3.1 | 29.4 | 26.4 | 1.8 | 17.2 | 5.5 | 100 |

**:p<0.01, *:p<0.05 significance level, SAR: Sourtheastern Anatolian Red, EAR: Eastern Anatolian Red

The number of employees in the enterprise was found to be a significant (p<0.01) factor in the preference of cattle breeds in enterprises. Accordingly, while SAR from indigenous breeds and the Brown Swiss breed from culture breeds were intensively preferred in the enterprises with 1-2 employees, the ratio of SAR from indigenous breeds increased and the ratio of the Brown Swiss breed decreased as the number of employees increased to 3-4 people. While the SAR breed was not demanded if the number of employees was 7 and over, significant increases (50% and 66.7%) were determined in the Simmental breed among culture breeds. It was found remarkable that the demand for the Holstein breed among culture breeds was low in this region (Table 10).

Table 10. The effect of number of employees in the farm to the cattle breed selection of Siirt Province

| Number of employees in the farm | | Cattle breeds** | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Simmental | Holstein | Brown Swiss | SAR | EAR | Native Black | Hybrid | Total |
| 1-2 | N | 6 | 1 | 23 | 13 | 0 | 11 | 6 | 60 |
| | % | 10 | 1.7 | 38.3 | 21.7 | 0 | 18.3 | 10 | 100 |
| 3-4 | N | 8 | 4 | 13 | 27 | 3 | 10 | 1 | 66 |
| | % | 12.1 | 6.1 | 19.7 | 40.9 | 4.5 | 15.2 | 1.5 | 100 |
| 5-6 | N | 5 | 0 | 8 | 3 | 0 | 6 | 1 | 23 |
| | % | 21.7 | 0 | 34.8 | 13 | 0 | 26.1 | 4.3 | 100 |
| 7-8 | N | 4 | 0 | 1 | 0 | 0 | 0 | 1 | 6 |
| | % | 66.7 | 0 | 16.7 | 0 | 0 | 0 | 16.7 | 100 |
| 9+ | N | 4 | 0 | 3 | 0 | 0 | 1 | 0 | 8 |
| | % | 50 | 0 | 37.5 | 0 | 0 | 12.5 | 0 | 100 |
| Total | N | 27 | 5 | 48 | 43 | 3 | 28 | 9 | 163 |
| | % | 16.6 | 3.1 | 29.4 | 26.4 | 1.8 | 17.2 | 5.5 | 100 |

**:p<0.01, *:p<0.05 significance level, SAR: Sourtheastern Anatolian Red, EAR: Eastern Anatolian Red

Significant differences were found in the breed preferences according to the altitude at which the enterprises were located (p<0.01). Accordingly, while an increase was determined in the demand for the Brown Swiss breed, which is resistant to high altitudes, among culture breeds along with the increase in altitude, a decrease was determined in the

Simmental breed. It was found out that the Native Black cattle were preferred in enterprises with a lower altitude, and there was a decrease in the demand at higher altitudes (Table 11).

Table 11. The effect of altitude to the breed selection in Siirt Province

| Altitude | | Cattle breeds** | | | | | | | Total |
|---|---|---|---|---|---|---|---|---|---|
| | | Simmental | Holstein | BrownSwiss | SAR | EAR | Native Black | Hybrid | |
| 475-753 | N | 3 | 1 | 3 | 9 | 1 | 5 | 1 | 23 |
| | % | 13 | 4.3 | 13 | 39.1 | 4.3 | 21.7 | 4.3 | 100 |
| 754-1032 | N | 17 | 1 | 12 | 12 | 2 | 10 | 3 | 57 |
| | % | 29.8 | 1.8 | 21.1 | 21.1 | 3.5 | 17.5 | 5.3 | 100 |
| 1033-1311 | N | 4 | 2 | 1 | 8 | 0 | 9 | 1 | 25 |
| | % | 16 | 8 | 4 | 32 | 0 | 36 | 4 | 100 |
| 1312-1590 | N | 3 | 0 | 30 | 11 | 0 | 4 | 4 | 52 |
| | % | 5.8 | 0 | 57.7 | 21.2 | 0 | 7.7 | 7.7 | 100 |
| 1591-1869 | N | 0 | 1 | 2 | 3 | 0 | 0 | 0 | 6 |
| | % | 0 | 16.7 | 33.3 | 50 | 0 | 0 | 0 | 100 |
| Total | N | 27 | 5 | 48 | 43 | 3 | 28 | 9 | 163 |
| | % | 16.6 | 3.1 | 29.4 | 26.4 | 1.8 | 17.2 | 5.5 | 100 |

**: p<0.01, *: p<0.05 significance level, SAR: Sourtheastern AnatolianRed, EAR: Eastern Anatolian Red

## CONCLUSIONS

This study was carried out to determine the breed preferences of sheep-goats and bovine animal enterprises in Siirt province and its districts and to investigate some factors such as altitude, location, the number of animals, the number of employees in the enterprise that affect breed preferences.

Location, the number of animals, the number of workforce in the enterprise, and altitude were determined to be significant factors in the breed selection of enterprises.

With this study made in sheep and goat farms located in Siirt, the impact of the investigated factors in breed selection was found significant. Considering the potential of the livestock in Siirt, it is concluded that in giving directions to the livestock in Siirt, these factors must be taken into account.

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# PREDICTION OF CARCASS WEIGHT OF HOLSTEIN AND BROWN SWISS CATTLE GROWN IN A 12-MONTHS INTENSIVE BEEF PRODUCTION SYSTEM BY USING REAL-TIME CARCASS MEASUREMENTS

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***Abstract***

*In this study, it was aimed to evaluate the use of some morphometric carcass measurements to predict carcass weight of Holstein and Brown Swiss cattle grown in a 12-months intensive beef production system. Associations between carcass weights (CW) and some carcass measurements such as carcass heart girth (CHG), carcass length (CL) and carcass depth (CD) were examined for prediction ability, using the data with 134 observations for each traits.*
*The linear, quadratic and cubic regression models were performed to predict CW for both breeds and since there were no statistically significant (P >0.05) differences in carcass measurements between breeds. The data of these breeds were combined and found that CL and CHG would be the best possible traits in predicting CW ($R^2$=57.9 and 50.7% respectively) among the other measurements. The highest $R^2$ values were obtained from both the equation contained all carcass traits ($R^2$=65.5%) and the equation that included only CHG and CL ($R^2$=65.4%). All type of regressions showed that addition of quadratic and cubic terms contributed little benefit in predicting CW. Therefore, all linear terms of all carcass measurements were considered for analysis and they were significant (P <0.05) and the $R^2$ value for other carcass measurement CD was approximately 20.8%.*
*It can be concluded that in management situations where CW cannot be measured it can be predicted accurately by measuring CL and CHG alone and different models may be needed to predict CW in different feeding and environmental conditions and for other breeds.*

***Key words:*** *Prediction, Carcass weight, Carcass measurements, Brown Swiss and Holstein cattle, Feedlot.*

## INTRODUCTION

Small-scale agriculture is characterized by weak resources and investments, especially in developing countries. Decisions on agricultural activities are primarily based on small farming level trials and errors. Body measurements of beef cattle are used for a variety of purposes, especially since ration preparations are based on the body weight of the animal and are important for predicting body weight, including growth rate, body condition, and conformation (Wilson et al., 1997; Fourie et al., 2002)
Especially in developing countries, often animal marketing to farmers is based on visual evaluation.
Most veterinary medicines are prescribed according to live weight criteria. However, prescriptions and drug estimations are often made with approximate estimates. The use of ration formulation, drug estimation, body condition score and live weight criteria in marketing requires expensive and realized at less suitable and less advanced facilities of many small scale farmers.
The use of live weight criteria in ration formulation, drug estimation, body condition score and marketing requires sophisticated facilities which are expensive and hardly affordable to many small-scale farmers.
A simple and logical technique should be considered in management decisions, as scientists appreciate the importance of correct estimation of the body weight of the animal. Some studies have indicated a relationship between some body measurements and body weight (Peters and Ball, 1995; Nesamvuni et al., 2000). It is important to know the weight of a cattle carcass for a variety of reasons, especially for breeding, selection, nutrition,

feeding strategies and health care (antibiotics, anthelmintics and other treatment doses).

The results of most investigators have indicated that the accuracy of predicting body weight from heart girth or other body characteristics can be influenced by the breed types, animal species, age, size and condition of the animal (Heinrichs et al., 1992) and also by different environmental conditions (Enevoldsen et al., 1997).

It was indicated by Bozkurt et al. (2007) and Bozkurt et al. (2008) that the prediction ability of digital image analysis system was very promising to predict body weight and hot carcass weight.

Therefore, the objective of this study was to gain further information about the relationship between carcass weight and some carcass measurements of different breeds such as Brown Swiss and Holstein cattle such as CHG, CL, CD, and also to determine the value of using more than one carcass measurement as a single variable entry to the model to predict carcass weight and to validate the potential of this method as a means of predicting carcass weight under small scale farming conditions.

## MATERIALS AND METHODS

*Animals*

The animals used in this study were comprised of Brown Swiss and Holstein male cattle previously grown in a 12-month feedlot beef system. The average carcass weight was 254.4 and 262.5 kg for Brown Swiss and Holstein groups respectively. The carcass measurements of the slaughtered cattle were collected at Gulkoy slaughterhouse near Isparta province. Data were collected from December 2012 to March 2013 and a total of 134 observations were used for each trait measured. The carcasses were weighed using a mobile weighing bridge. Carcass weights were recorded to the nearest kilogram (kg). All carcass measurements were taken by the same individuals throughout the experimental period.

*Carcass Measurements*

Carcass measurements were taken while carcasses were strap in a bascule before weighing. A plastic tape marked in centimetres (cm) was used for the measurement of most carcass traits except carcass depth, which was measured by measuring stick (Hauptner, Germany).

Carcass weight was measured in kilograms and the carcass measurements in centimetres.

*Statistical Analysis*

The best prediction equations for carcass weight from other traits as independent variables, including CHG, CL and CD were determined. Descriptive statistics and regression analysis of CW on each of the independent variables was performed using the General Linear Models procedure of Minitab, 16 Inc. (Minitab, 2016).

Correlation coefficients were also obtained between carcass traits. Polynomial regression analysis of carcass weight on CHG, CL and CD were performed.

Linear, quadratic and cubic effects of independent variables on CW were included in the following model:

$$y_i = b_0+b_1X_i+b_2X_i^2+b_3X_i^3+e_i$$

Where

$y_i$= CW observation of an i' th carcass,

$b_0$= intercept, $b_1$, $b_2$, $b_3$= corresponding linear, quadratic and cubic regression coefficients I,

$X_i$ = Carcass measurement (CHG, CL, CD) and

$e_i$ = residual error term

Several different regression analyses were conducted;

1- All three carcass measurements, expressed as linear functions, were combined in CW prediction equation

2- Each carcass measurement was included separately in regression analysis as linear, quadratic and cubic expressions to predict CW; and

3- The linear regression of each other carcass measurement was then also added to the model as described previously.

## RESULTS AND DISCUSSIONS

There were no statistically significant differences in carcass measurements between breeds ($P > 0.05$).

Therefore, data of these breeds were combined for all statistical analysis.

Descriptive statistics of carcass weight and carcass traits on basis are shown in Table 1.

Table 1. Descriptive statistics of carcass weight and carcass traits by weight means

| Weight Means [SE] | CW (kg) | CHG (cm) | CL (cm) | CD (cm) |
|---|---|---|---|---|
| | 258.46 [3.38] | 179.85 [0.99] | 170.18 [1.37] | 66.29 [0.613] |

CW: Carcass Weights, CHG: Carcass Heart Girth, CL: Carcass Length, CD: Carcass Depth, SE: Standard Error

The average values for CW 258.5 kg. The corresponding ranges for CHG, CL, and CD were 179.85 cm, 170.18 cm and 66.29 cm respectively.

Regressions models of animal carcass weight on various carcass measurements using individual observations are shown in Table 2.

Table 2. Prediction equations of carcass weight and the linear effects of other carcass traits

| Models With Three Variable | $R^2$ % |
|---|---|
| CW= -185+1.20CHG+1.27CL+0.191CD | 65.5 |
| Models With Two Variable | |
| CW = -184+1.26CHG+1.28CL | 65.4 |
| CW = -180+2.29CHG+0.408CD | 51 |
| CW = -90.7+1.72CL+0.861CD | 59.8 |
| Models With One Variable | |
| CW = - 179 + 2.43 CHG | 50.7 |
| CW = - 62 + 1.88 CL | 57.9 |
| CW = 91.6 + 2.52 CD | 20.8 |

As Table 2 shows models with one variable together with determination coefficients it was found that CL and CHG would be the best possible traits in predicting CW ($R^2$=57.9% and 50.7% respectively) among the other carcass measurement. In other words, the $R^2$ values in the models with one predictor shows the proportion of variation in the dependent variable that is predictable from the independent variable. Therefore, in this study 57.9% of the variation in CW can be explained by CL.

It was observed that in every steps of regression analysis, inclusion of CL and CHG in the equation increased $R^2$ greatly. It was also found that when all variables were included in the regression CD was not significant while the rest gave significant slope values. The table containing the equations with all combinations of all carcass traits were cumbersome therefore it was not shown in the paper. However, the highest $R^2$ values were obtained from the equation contained all carcass traits ($R^2$=65.5%) and the equation that only CHG and CL ($R^2$=65.4%) and those equations that included CL and CD ($R^2$=59.8 %), CHG and CD ($R^2$ =51 %). These results were in line with the findings of Tuzemen et al. (1993), Ulutas et al. (2001), Bozkurt et al. (2007), Bozkurt et al. (2008).

However, in this study, the individual equations with one predictor CD had the lowest $R^2$ values as 20.8% (Table 2).

Results of regression analysis of carcass weight on the linear, quadratic and cubic effects of each carcass measurement are presented in Table 3.

Table 3. Regressions of carcass weight on the linear, quadratic and cubic effects of each carcass measurement[#]

| Carcass Measurements | Model | Intercept | $b_1$ | $b_2$ | $b_3$ | $R^2$ % |
|---|---|---|---|---|---|---|
| Carcass Heart Girth (CHG) | Linear | -178.6 | 2.43 | - | - | 50.7 |
| | Quadratic | -6037 | 68.35 | -0.185 | - | 72.5 |
| | Cubic | -33750 | 539.9 | -2.857 | $0.005039^{ns}$ | 73.3 |
| Carcass Length (CL) | Linear | -62.02 | 1.88 | - | - | 57.9 |
| | Quadratic | -1403 | 18.12 | -0.049 | - | 71.6 |
| | Cubic | 2483 | -53.38 | 0.387 | $-0.000881^{ns}$ | 72.3 |
| Carcass Depth (CD) | Linear | 91.6 | 2.51 | - | - | 20.8 |
| | Quadratic | -2422 | 79 | -0.58 | - | 46.9 |
| | Cubic | -19355 | 861.3 | -12.59 | 0.0613 | 55.5 |

[#]Only non-significant regression coefficients had superscripts (ns), the rest were significant at P<0.05

It was observed in this study that a 1 cm change in CD resulted in almost 2.51 kg change in carcass weight. Similarly, a 1 cm change in CHG, CL and resulted in 2.43 and 1.88 kg change in carcass weight respectively (Table 3).

Higher order polynomial equations were examined. The $R^2$ values from the regression models indicate that carcass length and carcass heart girth to be the most highly related to carcass weight considering all linear, quadratic and cubic coefficient terms. For all carcass traits, addition of the cubic term increased the $R^2$ slightly. In this study, CL and CHG contributed 57.9% and 50.7% of variation respectively.

However, while all linear, quadratic terms of CL, CHG and CD were significant (P <0.05); only the cubic terms of CHG and CL were not significant (P >0.05). However, Heinrichs et al.

(1992) reported that none-significant cubic term for heart girth and significant term for wither height. In contrast Heinrichs et al. (1992) found that quadratic term of body length was significant. The results in this study also showed that linear, quadratic and cubic expressions of both CL and CHG are the most useful predictors, and support the findings of Wilson et al. (1997), Bozkurt (2006), Bozkurt et al. (2007) and Bozkurt et al. (2008). All linear terms of all body measurements were significant ($P < 0.05$). These results were in line with Heinrichs et al. (1992), Wilson et al. (1997), Ulutas et al. (2001), Bozkurt (2006), Bozkurt et al. (2007) and Bozkurt et al. (2008).
It can be noted that, in the correctness of the carcass weight estimates, the additional carcass measurements of the equations provide a slight increase except CL alone.
Correlation coefficients of the traits are shown in Table 4.

Table 4. Pearson correlations between carcass traits in both breed cattle

| Variables | CW | CHG | CL |
|---|---|---|---|
| CHG | 0.71 | | |
| CL | 0.76 | 0.67 | |
| CD | 0.46 | 0.57 | 0.43 |

All correlation values were found to be statistically significant ($P < 0.05$). Amongst all the carcass measurements, the highest correlation was found between CL and CW ($r=0.76$). The second highest correlation was between CHG and CW ($r=0.71$). In addition the correlation value between CL and CHG ($r=0.67$) was higher than the correlation between the rest of the traits. It was expected that CL would give higher correlation coefficient value than the other carcass measurements since the $R^2$ value between CW and CL was also high.

## CONCLUSIONS

As most of the previous studies have shown, this study also showed that carcass length and carcass heart girth can be used accurately to predict carcass weight of Brown Swiss and Holstein cattle grown in small-scale farming conditions. The carcass length (CL) showed the highest correlation with the carcass weight of the other carcass properties examined.

When using any of the other three carcass measurements in the models that contained linear, quadratic and cubic terms, CL usually provided the most important contribution compared with other carcass dimensions. CHG can be considered the second best predictor.
For this reason, the use of carcass length and carcass heart girth provides a simple way of estimating carcass weight. This is the general purpose of applying the technique to practice. However, there is always a need for further research in this work and for other breeds, as well as for identifying different model parameters and developing different models to predict carcass weight in different management and environmental conditions. It is also important to be very careful when measuring carcass dimensions to reduce experimental errors.

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# PREDICTION OF BODYWEIGHT OF HOLSTEIN AND BROWN-SWISS MALE CATTLE BY USING DIGITAL IMAGES

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***Abstract***

*This research aimed to develop prediction models for accurate estimation of performance and body measurements of beef cattle grown in feedlot beef system by using Digital Image Analysis (DIA). For this purpose, 40 animals were used in total and composed of 20 animals of the Brown Swiss breed and 20 animals of the Holstein breed with the age of about 4-5 months at the beginning of the experiment. Animals were fed the same dietary rations throughout the experimental period of 12 months. When the animals reached 500-550 kg bodyweights (BW), they were slaughtered.*
*The digital images of each live animal were taken and the same parameters (digital wither height (DJWH), digital body length (DJBL), digital body depth (DJBD), digital hip width (DJHW), digital hip height (DJHH) and digital pin bone length (DJPL) were also determined from the images, using the data with 1069 observations for each traits. Then, prediction models were developed by DIA.*
*The linear, quadratic and cubic regression models were performed to predict BW for both breeds and since there was no statistically significant differences ($P>0.05$) in body measurements between breeds. The data of these breeds were combined and found that DJBL and DJWH would be the best possible traits in predicting BW ($R^2$=93.9% and 90.7% respectively) among the other measurements. The linear terms of all body measurements by DIA were considered for analysis and they were significant and $R^2$ values for other body measurements DJHW, DJBD, DJHH and DJPL were approximately 78.4, 81.4, 87.7 and 67.7% respectively.*
*It can be concluded that in management situations where BW cannot be measured it can be predicted accurately by measuring DJBL and DJWH alone or both DJBD and even DJHH and different models may be needed to predict BW in different feeding and environmental conditions and breeds.*

***Key words:*** *Prediction, Body weight, Digital Body Measurements, Digital Image Analysis.*

## INTRODUCTION

The decisions on agricultural activities are primarily depended on trials and errors at small farming level, especially in developing countries where small scale farming is characterised by poor resources and investments.
Body measurements of beef cattle are used for several purposes, especially since ration preparations are based on animal's body weight and very important for prediction of body weight, including growth rate, body condition and conformation (Wilson et al., 1997; Fourie et al., 2002)
Generally, marketing of animals between farmers is based on visual assessment in especially developing countries. Most of the veterinary medicines are prescribed on the basis of live weight criteria. However, prescriptions and dose of drug estimation is mostly performed by approximate estimations. The use of live weight criteria in ration formulation, drug estimation, body condition score and marketing requires sophisticated facilities which are expensive and hardly affordable to many small-scale farmers.
As long as scientists appreciate the importance of accurate prediction of animal's bodyweight, on managerial decisions a simple and reasonable technique should be considered. Several studies have indicated that there is a relationship between some body measurements and body weight (Peters and Ball, 1995; Nesamvuni et al., 2000). It is also important to know the bodyweight of cattle for a number of

reasons, related to breeding especially for selection, feeding and health care.

The results of the most studies have recognised that the accuracy of estimating body weight from heart girth or other body traits may be affected by breed, type, age, size and condition of the animal (Heinrichs et al., 1992) and also by different environmental conditions (Enevoldsen et al., 1997).

Therefore, the objective of this study was to gain further information about the relationship between body weight and some digital body measurements of different breeds such as Brown Swiss and Holstein cattle and also to determine the value of using one body measurement as a single variable entry to the model to predict body weight and to validate the potential of this method as a means of predicting body weight under small scale farming conditions by using DIA.

## MATERIALS AND METHODS

*Animals*

The animals used in this study were comprised of 40 Brown Swiss and Holstein cattle in total, divided into two groups on the basis of weight. The average weight was 132 and 158 kg for Brown Swiss and Holstein groups respectively. The digital images of various measurements were collected using a digital camera (canon) and a reference card to eliminate the distance between the object and the camera. Data were collected starting from December 2011 from the animals experimented on Suleyman Demirel University research farm and lasted for 12 months. A total of 1069 observations were used for each trait measured. The animals were weighed using a mobile weighing bridge once fortnight. Body weights were recorded to the nearest kilogram (kg) and the digital body measurements in centimetre (cm).

*Digital Body Measurements*

Digital images and digital body measurements were taken by the same person throughout the experimental period to avoid the experimenter error in measuring the digital parameters which are as follows:

1- Digital Wither Height (DJWH)- was the distance from the ground beneath the animal to the top of the withers directly above the centre of the shoulder,

2- Digital Body Length (DJBL)- was the distance from the point of the shoulders to the ischium; in other words, from the sternum (manubrium) to the aitchbone (tuber ischiadicum),

3- Digital Hip Width (DJHW)- was the widest point at the centre of the stifle,

4- Digital Body Depth (DJBD)- from sternum area immediately caudal to the forelimbs to top of the thoracic vertebra.

5- Digital Pin Bone Length (DJPL)- was the distance between two pin bones at the back

6- Digital Hip Height (DJHH)- was the distance from the ground beneath the animal rear legs to the top of the vertebra.

*Statistical Analysis*

The best prediction equations for body weight from other traits as independent variables, including DJBL, DJWH, DJHW, DJBD, DJHH, and DJPL were determined. Descriptive statistics on a monthly basis and regression analysis of BW on each of the independent variables was performed using the General Linear Models procedure of Minitab, 16 Inc. (Minitab, 2016).

Correlation coefficients were also obtained between digital body traits. Polynomial regression analysis of body weight on DJWH, DJBL, DJHW, DJBD, DJPL and DJHH were performed.

Linear, quadratic and cubic effects of independent variables on BW were included in the following model:

$y_i = b_0+b_1X_i+b_2X_i^2+b_3X_i^3+e_i$

Where

$y_i$= BW observation of an i' th animal,

$b_0$= intercept, $b_1$, $b_2$, $b_3$= corresponding linear, quadratic and cubic regression coefficients I,

$X_i$ = Digital body measurement (DJBL, DJWH, DJHW, DJBD, DJPL, DJHH) and

$e_i$ = residual error term

Several different regression analyses were conducted;

1- All seven digital body measurements, expressed as linear functions, were combined in BW prediction equation

2- Each digital body measurement was included separately in regression analysis as

linear, quadratic and cubic expressions to predict BW; and

3- The linear regression of each other digital measurement was then also added to the model as described previously.

## RESULTS AND DISCUSSIONS

There were no statistically significant differences in digital body measurements between breeds (P >0.05). Therefore, data of these breeds were combined for all statistical analysis. Descriptive statistics of body weight and digital body traits on a monthly basis are shown in Table 1.

The average values for BW increased throughout the experimental period from 144.7 kg to 507.8 kg with 363.1 kg difference.

The corresponding ranges for DJHW, DJBL, DJWH, DJBD, DJHH and DJPL were 27.92 cm to 42.8 cm with 14.88 cm difference, 102.77 cm to 153.16 cm with 50.39 cm difference, 98.56 cm to 134.19 cm with 35.63 cm difference, 50.11 cm to 72.64 cm with 22.53 cm difference, 103.38 cm to 138.13 cm with 34.75 cm difference and 19.77 cm to 30.98 cm with 11.21 cm difference, respectively.

Table 1. Descriptive statistics of body weight and digital body traits by weighing times on 1-12 months intervals

| Weighing Time (month) | Holstein | | | Brown Swiss | | | Both Breeds Means | | |
|---|---|---|---|---|---|---|---|---|---|
| | 1. | 12. | Difference | 1. | 12. | Difference | 1. | 12. | Difference |
| BW(kg) | 158.37 | 520.32 | 361.95 | 131.07 | 495.2 | 364.19 | 144.7 | 507.8 | 363.1 |
| DJWH (cm) | 101.14 | 137.15 | 36.01 | 96.05 | 131.24 | 35.19 | 98.56 | 134.19 | 35.63 |
| DJHH (cm) | 105.62 | 140.5 | 34.88 | 101.14 | 135.76 | 34.62 | 103.38 | 138.13 | 34.75 |
| DJHW (cm) | 29.11 | 44.5 | 15.39 | 26.73 | 41.1 | 14.37 | 27.92 | 42.8 | 14.88 |
| DJBL (cm) | 105.59 | 155.89 | 50.3 | 99.95 | 150.43 | 50.48 | 102.77 | 153.16 | 50.39 |
| DJBD (cm) | 52.87 | 74.74 | 21.87 | 47.35 | 70.54 | 23.19 | 50.11 | 72.64 | 22.53 |
| DJPL (cm) | 20.24 | 31.07 | 10.83 | 19.29 | 30.89 | 11.6 | 19.77 | 30.98 | 11.21 |

BW: Body Weights, DJWH: Digital Wither Height, DJBL: Digital Body Length, DJBD: Digital Body Depth, DJHW: Digital Hip Width, DJHH: Digital Hip Height, DJPL: Digital Pin Bone Length

Table 2. Prediction equations of body weight and the linear effects of other digital body traits

| Models With One Variable | $R^2$ % |
|---|---|
| BW = - 738 + 8.92 DJWH | 90.7 |
| BW = - 525 + 6.49 DJBL | 93.9 |
| BW = - 401 + 19.3 DJHW | 78.4 |
| BW = - 536 + 13.7 DJBD | 81.4 |
| BW = - 215 + 19.7 DJPL | 67.7 |
| BW = - 793 + 9.09 HH | 87.7 |

Regression models of animal body weight on various digital body measurements using individual observations are shown in Table 2. As Table 2 shows models with one variable together with determination coefficients it was found that DJBL and DJWH would be the best possible traits in predicting BW ($R^2$=93.9% and 90.7% respectively) among the other digital measurements. In other words, the $R^2$ values in the models with one predictor shows the proportion of variation in the dependent variable that is predictable from the independent variable. Therefore, in this study 93.9% of the variation in BW can be explained by DJBL.

It was observed that in every steps of regression analysis inclusion of DJBL and DJWH in the equation increased $R^2$ substantially. It was also found that when all variables were included in the regression DJPL, DJHH and DJBD were not significant while the rest gave significant slope values. The table containing the equations with all combinations of all digital body traits were cumbersome therefore it was not presented in this paper. However, the highest $R^2$ values were obtained from the equation contained all digital body traits ($R^2$=95.6%) and the equation that included all digital body measurements except DJWH, DJHW and DJBD ($R^2$=95.2%) and those equations that included DJWH, DJBL and DJHH ($R^2$=95.2 %), DJWH and DJBL ($R^2$ =95 %) and only DJBL ($R^2$=93.9 %). These results were in line with the findings of Tuzemen et al. (1993), Ulutas et al. (2001) Bozkurt et al. (2007), Bozkurt et al. (2008) and

Bozkurt (2006) who reported high $R^2$ value from the equation including all body traits. Bozkurt (2006) found that when considering individual equations with one predictor hip width and body depth have the lowest $R^2$ values; 69% and 66.2%, respectively. Heart girth and wither height had the highest $R^2$ values, approximately 90% and 77% respectively. However, in this study, the individual equations with one predictor DJHW and DJPL had the lowest $R^2$ values; 78.4% and 67.7%, respectively. DJBL and DJWH had the highest $R^2$ values, approximately 93.9% and 90.7% respectively (Table 2).

Results of regression analysis of body weight on the linear, quadratic and cubic effects of each digital body measurement are presented in Table 3.

Table 3. Regressions of body weight on the linear, quadratic and cubic effects of each digital body measurement[#]

| Measurements | Model | Intercept | $b_1$ | $b_2$ | $b_3$ | $R^2$ % |
|---|---|---|---|---|---|---|
| Digital Hip Width (DJHW) | Linear | -401.3 | 19.3 | - | - | 78.4 |
| | Quadratic | -354.8 | 16.7 | 0.03565 $^{ns}$ | - | 78.4 |
| | Cubic | 2839 | -255.6 | 7.637$^{ns}$ | -0.06958 | 81 |
| Digital Body Length (DJBL) | Linear | -525.4 | 6.49 | - | - | 93.9 |
| | Quadratic | -124 | 0.1772 | 0.02439 | - | 94.3 |
| | Cubic | 813.6 | -22.22 | 0.2007 | -0.000457 | 94.3 |
| Digital Wither Height (DJWH) | Linear | -737.8 | 8.921 | - | - | 90.7 |
| | Quadratic | 109.9 | -5.658 | 0.06201 | - | 91.3 |
| | Cubic | 6998 | -184 | 1.591 | -0.004336 | 91.8 |
| Digital Body Depth (DJBD) | Linear | -535.6 | 13.68 | - | - | 81.4 |
| | Quadratic | -318.7 | 6.566 | 0.05747 | - | 81.5 |
| | Cubic | 4389 | -227.3 | 3.884 | -0.02062 | 83 |
| Digital Pin Bone Length (DJPL) | Linear | -214.7 | 19.67 | - | - | 67.7 |
| | Quadratic | -578.5 | 48.15 | -0.5266 | - | 69 |
| | Cubic | 570.3 | -84.89 | 4.445 | -0.06054 | 69.8 |
| Digital Hip Height (DJHH) | Linear | -793.1 | 9.089 | - | - | 87.7 |
| | Quadratic | 262.8 | -8.431 | 0.07198 | - | 88.5 |
| | Cubic | 12363 | -311.9 | 2.592 | -0.00693 | 89.9 |

[#]Only none significant regression coefficients had superscripts (ns), the rest were significant at $P<0.05$.

It was observed in this study that a 1 cm increase in DJBL resulted in almost 6.5 kg increase in weight. Similarly, a 1 cm change in DJWH, DJHH, DJBD, DJHW, DJPL, and resulted in 8.92, 9.09, 13.7, 19.3 and 19.7 kg change in weight respectively (Table 3).

Higher order polynomial equations were examined. The $R^2$ values from the regression models indicate that digital body length and digital wither height to be the most highly related to body weight considering all linear, quadratic and cubic coefficient terms. For all digital body traits, addition of the cubic term increased the $R^2$ slightly. In this study DJBL and DJWH contributed 93.9% and 90.7% of variation respectively. However, while all linear, quadratic and cubic terms of DJBL and DJWH were significant ($P <0.05$) DJHW has not significant quadratic term ($P >0.05$). Moreover, DJBL produced the highest quadratic and cubic terms with $R^2$ of 94.3% for both. However, Heinrichs et al. (1992) reported that non-significant cubic term for heart girth and significant term for wither height. The quadratic term of body length was not significant ($P >0.05$). In contrast Heinrichs et al. (1992) found that quadratic term of body length was significant. Digital body depth has not significant both quadratic and cubic coefficients term either. All linear terms of all body measurements were significant ($P <0.05$). These results were in line with Heinrichs et al. (1992), Wilson et al. (1997), Ulutas et al. (2001), Bozkurt (2006), Bozkurt et al. (2007) and Bozkurt et al. (2008). The results in this study also showed that linear, quadratic and cubic expressions of both DJBL and DJWH are the most useful predictors, and support the

findings of Wilson et al. (1997), Bozkurt (2006), Bozkurt et al. (2007) and Bozkurt et al.(2008).

It can be noted that, in the correctness of body weight estimates, the additional digital body measurements of the equations provide a slight increase except DJBL alone.

Correlation coefficients of the traits are shown in Table 4.

Table 4. Pearson correlations between digital body traits in both breed cattle

| Variables | BW | DJWH | DJBL | DJHW | DJBD | DJPL |
|---|---|---|---|---|---|---|
| DJWH | 0.95 | | | | | |
| DJBL | 0.97 | 0.95 | | | | |
| DJHW | 0.89 | 0.86 | 0.89 | | | |
| DJBD | 0.90 | 0.90 | 0.91 | 0.88 | | |
| DJPL | 0.82 | 0.78 | 0.81 | 0.85 | 0.77 | |
| DJHH | 0.94 | 0.94 | 0.93 | 0.84 | 0.89 | 0.76 |

All correlation values were found to be statistically significant ($P < 0.05$). Amongst all the digital body measurements, the highest correlation was found between DJBL and BW ($r=0.97$). The second highest correlation was between DJWH and BW ($r=0.95$). In addition the correlation value between DJBL and DJWH ($r=0.95$) was higher than the correlation between the rest of the traits. It was expected that DJBL would give higher correlation coefficient value than the other digital body measurements since the $R^2$ value between BW and DJBL was also high.

## CONCLUSIONS

As the most of previous studies showed, this study also indicated that digital body length and digital wither height can be used with great accuracy in predicting the body weight for Brown Swiss and Holstein cattle grown under small-scale farming condition. Digital body length and digital wither height exhibited the highest correlation to bodyweight of the traits studied.

When any of the other six digital measurements were used in the models that contained linear, quadratic and cubic terms, DJBL generally made the most important contribution compared with other digital body dimensions. DJWH can be considered the second best predictor. Therefore, the use of digital body length and digital wither height provide a simple way of predicting body weight confidently which is the overall purpose applying the technique in the practice.

However, there is always a need for further studies for the breeds in this study and other breeds as well to determine and develop different models to predict bodyweight in different management and environmental conditions. It is also important to pay a great attention when measuring digital body dimensions to reduce the experimental errors.

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# WORLDWIDE TRENDS DEVELOPMENT OF SHEEP BREEDING

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***Abstract***

*The purpose of this paper was the revelation of sheep breeding development trends worldwide in the past 14 years. The research was conducted on sheep herds worldwide of all breeds, in the profile of countries and continents. Based on FAOSTAT data, the volumes of sheep breeding production (meat, milk, wool, skins of Karakul) were analyzed during the years 2000-2014. It was found that on the whole globe, the sheep herds increased by 10.7%, the amount of meat by 14.4%, the milk by 27.8%, the skins by 8.8% and the wool production decreased by 8,0%. Conclusions were deduced that: social and economic importance of sheep breeding is due mainly to ensure food security of the population with animal protein such as meat and milk; the increasing of sheep herd occurred on those continents, regions and countries of the world with important human populations but underdeveloped (Africa, Asia), who live in the arid zones with plains and scanty vegetation, where the sheep are kept the whole year in natural conditions, without capital investments, with minimal cost, for which the sheep are a crucial source of existence and survival; on the continents with developed countries and rural populations (Europe, North America, Oceania), for which the breeding and exploiting of sheep is seen as an economic business for obtaining a profit, sheep were reduced because, the ovine species, in conditions of modernization, industrialization and intensification of the zootechnic sector, economically cannot compete with other animal species (birds, pigs, bovines).*

***Key words****: trends, development, sheep, meat, milk, wool, Karakul skins.*

## INTRODUCTION

The sheep breeding, worldwide, is a branch of traditional livestock by major socio-economic importance. Multiple researches in this domain demonstrate that importance of this branch is due, at thirst, to ensure food security of the human population with protein products of animal origin such as meat (Bradford, 2001; FAO, 2017 a, 7; Pop et al., 1976; Ştefănescu et al., 1973; Taftă et al., 1997; Бастаев и др., 2003; Шайдулин и др., 2016) and milk (Barillet et al., 1974; Faostat, 2017, 8, Pop et al., 1976; Ştefănescu et al., 1973; Taftă et al., 1997; Ильев, 1969; Миллз, 1985), and, secondly, by providing natural raw materials, such as: wool (Ghiță et al., 1996; Faostat, 2015; Rider et al., 1968), skins (Adametz, 1927; Васин и др., 1971; Гигинейшвили, 1975; Дъячков, 1980; Закиров и др., 1987; Иванов, 1964: Кошевой, 1975), sheep fur and leather, for the processing industry and manufacturing of confection large consumer (clothes, fabrics, carpets, leather goods etc.). All these make ovine species indispensable for human society. It mentioned, that on the continents of the world the sheep production has been obtained from a wide range of breeds of sheep with various skills production: wool, meat, milk, wool-meat, wool-meat-milk, skins-milk etc. Better known in the world are about 600 breeds of sheep (Вениаминов, 1984), but after other data, newer of the FAO (FAO, 2016) in the world is growing more than 1129 sheep breeds, including less known.

In certain geographic areas of the world were created by humans, raised and spread those breeds of sheep at different stages of development of human society, satisfying the demands of society, they corresponded most appropriate traditions and local pedo-climatic conditions.

Throughout the ages, breeds of sheep have been perfected, ameliorated and specialized. Thus, in developed European countries (England, France, Holland, Germany) with favorable pedo-climatic conditions for intensive technologies, spread specialized breeds for meat, milk, meat, wool and prolific.

In underdeveloped countries (Africa, Asia), difficult conditions for intensive technologies, have spread breeds specialized for milk, wool-meat-fat, wool-milk-meat wool-milk-skins etc. Following, in the advanced stages of human society development in order to cover the

increasing demand on the world market to some specific sheep products, were created and in the Asian countries (in extensive) breeds performances, specialized in skins (Karakul), meat-tallow (Ghisar), wool (Australian Merino) and others.
In most cases, creating breeds and production skills development of sheep were driven by climatic and socio-economic conditions existing in the concerned areas (regions, countries). Those breeds and types of sheep that do not correspond to the requirements of the respective areas gradually disappeared.
Taking into account the climatic and socio-economic conditions quite varied in different parts and regions of the world, for elaboration measures of development of sheep branch under certain conditions, it is extremely necessary to know the development trends of sheep breeding at global and regional level.
In this context, the present work was proposed for research of sheep breed development trends worldwide in the last 14 years, regarding the dynamics of the sheep herds and production volumes of sheep meat, milk, wool and Karakul skins.

## MATERIALS AND METHODS

The research was conducted on sheep herds worldwide of all breeds, in the profile of countries and continents, based on FAOSTAT data (FAO 2015a).
Based on data of this institution has been, also, analyzed information on production volumes of sheep meat (FAO, 2017a), production volumes of sheep milk (FAO, 2017 b), quantities of raw wool (FAO, 2015 b) and quantity of Karakul skins, deducted based on data information from the international fur auctions (Kopenhagen Fur, 2015) and communication of the official representative of the first FAO International Symposium on Karakul from Vienna, 1967 (Ştefanescu et al., 1973).
Based on the analysis data above, were been deducted the main development trends of worldwide sheep breeding, including various continents and countries. Finally those conclusions have been made.

## RESULTS AND DISCUSSIONS

As a result of our research (Buzu, 2016), based on the FAOSTAT data, 2015, we found that worldwide herd of sheep in 2013 was 1172.8 million heads), of which the most numerous effective were been in Asia (526.6 mil heads) and Africa (325.3 mil heads), followed by Europe (129.6 mil heads) and Oceania (106.3 mil heads). The less sheep are on the American continent (84.9 mil heads) (Table 1).
Analysis distribution of sheep herds from Asia and Africa, on the countries, shows that this is largely linked to human population in those countries.
Thus, most numerous Asian sheep have been registered in China (185.0 mil sheep, with 1408.0 mil people), India (75.5 mil sheep, with 1236.7 mil people), Iran (50.2 mil sheep, with 76.4 mil people). On the African continent, the most important sheep herds are in Sudan (52.5 mil sheep, with 37.2 mil people) and Nigeria (39.0 mil sheep, with 168.8 mil people).
In Europe, the size of sheep herds in major countries is based not so with the number of population, but more with specific traditions of these countries.
Thus, the most numerous sheep herds from Europe are in the United Kingdom (32.8 mil heads), the development of human society throughout history have been created most numerous and powerful in the world breeds of sheep and wool textile industry and sheep meat consumption in food of human population in this country are some millennial traditions.
The important herds sheep are, also, in Turkey (27.4 mil heads), Russia (22.0 mil heads), Spain (16.1 mil heads), Greece (9.5 mil heads) and Romania (8.8 mil heads).
In Oceania, the largest herds of sheep are in Australia (75.5 mil heads) and New Zealand (30.8 mil heads), not in relation to the population of these countries, but rather with colonial traditions of Britain, peoples which imposed breeding of sheep in these colonies.

Table 1. Evolution of worldwide herds sheep during the 2000-2013 period (thousand heads)

| Nr d/o | Country, continent, part of the world | Years | | | 2013 % to 2000 |
|---|---|---|---|---|---|
| | | 2000 | 2007 | 2013 | |
| **WORLD TOTAL** | | **1 059 082.3** | **1 138 486.5** | **1 172 833.2** | **110.7** |
| **1.** | **EUROPE total** | **146 694.2** | **135 525.3** | **129 650.5** | **88.4** |
| | inclusive: United Kingdom | 42 264.0 | 33 946.0 | 32 856.0 | 77.7 |
| | Turkey | 30 256.0 | 25 616.9 | 27 425.2 | 90.6 |
| | Russia | 12 603.0 | 17 508.1 | 22 061.0 | 175.0 |
| | Spain | 23 965.0 | 22 194.2 | 16 118.6 | 67.3 |
| | Greece | 8 951.0 | 8 831.0 | 9 520.0 | 106.4 |
| | Romania | 8 121.0 | 7 678.0 | 8 833.8 | 108.8 |
| | Azerbaijan | 5 279.7 | 7 523.0 | 7 979.4 | 151.1 |
| **2.** | **ASIA total** | **414 248.8** | **491 031.5** | **526 590.6** | **127.1** |
| | inclusive: China | 131 095.0 | 171 961.1 | 185 000.0 | 141.1 |
| | India | 59 447.0 | 71 560.0 | 75 500.0 | 127.0 |
| | Iran | 53 900.0 | 53 800.0 | 50 220.0 | 93.2 |
| | Pakistan | 24 084.0 | 26 794.0 | 28 800.0 | 119.6 |
| | Mongolia | 15 191.3 | 16 990.1 | 17 500.0 | 115.2 |
| | Syria | 13 505.2 | 22 865.4 | 14 000.0 | 103.7 |
| **3.** | **AFRICA total** | **246 505.8** | **294 957.0** | **325 338.8** | **132.0** |
| | inclusive: Sudan | 46 095.0 | 50 944.0 | 52 500.0 | 113.9 |
| | Nigeria | 26 000.0 | 33 080.4 | 39 000.0 | 150.0 |
| | Ethiopia | 10 950.7 | 26 117.3 | 26 500.0 | 242.0 |
| | Algeria | 17 615.9 | 20 154.9 | 25 500.0 | 144.7 |
| | Morocco | 17 299.7 | 16 894.0 | 19 956.4 | 115.4 |
| | Kenya | 7 939.5 | 16 308.1 | 18 500.0 | 233.0 |
| **4.** | **AMERICA total** | **90 805.1** | **92 785.3** | **84 902.0** | **93.5** |
| | inclusive: Brasilia | 14 784.9 | 16 239.4 | 17 022.0 | 115.1 |
| | Argentina | 13 561.6 | 16 180.0 | 14 000.0 | 103.2 |
| | Peru | 14 686.3 | 14 580.2 | 12 434.3 | 84.7 |
| | Bolivia | 7 352.9 | 8 237.7 | 9 287.6 | 126.3 |
| | Mexico | 6 046.0 | 7 478.5 | 8 477.0 | 140.2 |
| | SUA | 7 032.0 | 6 120.0 | 5 335.0 | 75.9 |
| **5.** | **OCEANIA total** | **160 828.3** | **124 187.4** | **106 351.0** | **66.1** |
| | inclusive: Australia | 118 552.0 | 85 711.1 | 75 547.8 | 63.7 |
| | New Zeeland | 42 260.0 | 38 460.4 | 30 786.7 | 72.8 |

The sheep herds worldwide increased significantly in the period 2000-2013, from 1059.1 million heads in 2000 to 1172.8 million, in 2013 with 113.7 million heads (10.7%). The highest growth rhythm were been recorded in Africa and Asia, with 32.0 and 27.1% respectively.

In profile on the country, the sheep herds from Mali, Kenya and Ethiopia have increased during this period, by 2.02 to 2.42 times. From Asian countries, the sheep herds from China increased by 41.1%, in Pakistan, Iraq and Mongolia - with 19.6 to 15.2%.

An entirely different situation is in Europe, America and Oceania. In these continents the sheep herds, during the years 2000-2013, have been permanent decreased. For example, in Europe the sheep herds fell from 146.7 million heads in 2000 to 129.6 million heads in 2013 or with 17.0 million heads (11.6%). However, on the background of the herd's reduction, some countries in Eastern and Southern Europe recorded significant increases of sheep number. Thus, the sheep herds increased in Russia - with 9.4 million head, or 75.0%, Azerbaijan - with 2.7 million head, or 51.1%, in Romania - with 0.7 million heads, or 8.8%, Greece - with 0.6 million heads, or 6.4%.

On the American continent herds of sheep decreased, during that period, it was more moderate, only the 6.5%. This is due to countries in Central and Latin America, with important herds of sheep. Among these are Mexico, which registered an increase of sheep herds by 40.2%,

Bolivia – 26.3%, Brasilia – 15.1% and Argentina – 3.2%.

The most drastic reductions of sheep herds during 2000 - 2013 occurred in countries of Oceania, the herds are concentrated mainly in Australia and New Zealand. The herds of sheep from this continent have been reduced from 160.8 million heads in 2000 to 106.3 million heads, or with 54.5 million heads (33.9%), including: in Australia, the number of sheep decreased from 118.5 million heads in 2000 to 75.5 million heads in 2013, or with 43.0 million heads (36.3%) and in New Zealand herds of sheep decreased from 42. 3 million heads in 2000 to 30.8 million heads in 2013, or with 11.5 million (27.2%).

The evolution of the sheep worldwide reflects a similar picture in the evolution of sheep production volumes in this period, such as meat, milk, wool, skins

Thus, world production ***of sheep meat***, according to FAOSTAT data, 2017, in the 2000-2014 period has increased from 7829.1 thousand tons in 2000 to 8960.3 thousand tons in 2014, or with 1131.2 thousand tons (14.4%) (Table 2).

Table 2. Evolution worldwide of sheep meat production during the 2000-2014 period (thousand tons)

| Nr d/o | Country, continent, part of the world | Years | | | 2014 % to 2000 |
|---|---|---|---|---|---|
| | | 2000 | 2006 | 2014 | |
| **W O R L D T O T A L** | | **7 829.1** | **8 392.1** | **8 960.3** | **114.4** |
| **1.** | **E U R O P E t o t a l** | **1 268.7** | **1 309.5** | **1 139.7** | **89.8** |
| | inclusive: United Kingdom | 383.0 | 332.0 | 298.0 | 77.8 |
| | Turkey | 320.7 | 289.5 | 312.5 | 97.4 |
| | Russia | 119.1 | 139.3 | 186.4 | 156.5 |
| | Spain | 238.0 | 216.0 | 113.6 | 47.7 |
| | France | 130.4 | 139.6 | 110.2 | 84.5 |
| | Greece | 69.4 | 90.0 | 58.4 | 84.1 |
| | Romania | 60.5 | 54.7 | 67.7 | 111.9 |
| | Azerbaijan | 35.1 | 44.6 | 68.7 | 195.7 |
| **2.** | **A S I A t o t a l** | **3 433.5** | **3 928.6** | **4 453.9** | **129.7** |
| | inclusive: China | 1 478.1 | 1 939.1 | 2 184.0 | 147.7 |
| | India | 220.8 | 255.5 | 235.2 | 106.5 |
| | Syria | 206.1 | 252.2 | 161.3 | 78.3 |
| | Pakistan | 157.4 | 149.0 | 164.0 | 104.2 |
| | Iran | 329.1 | 276.3 | 147.9 | 44.9 |
| | Kazakhstan | 91.1 | 98.7 | 138.6 | 152.1 |
| **3.** | **A F R I C A t o t a l** | **1 254.6** | **1 483.4** | **1 757.2** | **140.1** |
| | inclusive: Sudan | 272.7 | 316.0 | 251.0 | 92.0 |
| | Algeria | 164.1 | 184.7 | 290.9 | 177.3 |
| | Nigeria | 111.5 | 138.6 | 139.5 | 125.1 |
| | Morocco | 125.3 | 120.4 | 120.3 | 96.0 |
| | Ethiopia | 36.4 | 78.9 | 88.1 | 242.0 |
| **4.** | **A M E R I C A t o t a l** | **419.3** | **416.7** | **401.7** | **95.8** |
| | inclusive: Brasilia | 68.7 | 77.0 | 85.9 | 125.0 |
| | SUA | 116.4 | 87.5 | 72.9 | 62.6 |
| | Mexico | 24.3 | 44.9 | 58.2 | 239.5 |
| | Argentina | 49.6 | 49.7 | 60.3 | 121.6 |
| **5.** | **O C E A N I A t o t a l** | **1 323.8** | **1 253.9** | **1 207.8** | **91.2** |
| | inclusive: Australia | 790.6 | 710.9 | 720.6 | 91.1 |
| | New Zeeland | 533.2 | 542.9 | 487.1 | 91.3 |

The biggest volumes of this production is in Asia (4453.9 thousand tons) and Africa (1757.2 thousand tons), followed by Oceania (1207.8 thousand tons) and Europe (1139.7 thousand tons). The lowest amount of sheep meat has been produced in America (401.7 thousand tons) and maintained at the same level with a downward trend. In Asia and Africa is found increase of meat production volumes.

In Asia, the meat production has been increased from 3433.5 thousand tons in 2000 to 4453.9

thousands tones in 2014, or with to 1020.4 thousand tons or (29.7%).

From Asian countries, the biggest quantities of meat has been produced in China (2184.0 thousand tons), India (235.2 thousand tons), Syria (161. 3 thousand tons), Pakistan (164.0 thousand tons) and Iran (147.9 thousand tons). The biggest growth rhythms production of sheep meat in the examined period was recorded in China (47.7%) and Kazakhstan (52.1%).

In Africa, the volume of sheep meat production increased from 1254.6 thousand tons in the 2000 year to 1757.2 thousand tons in 2012 year, or 502.6 thousand tons (40.1%). The highest total meat production increases occurred in areas of East, West and North Africa. Thus, sheep meat production increased during 2000-2014 period in Ethiopia by 2.42 times, in Egypt and Mali with 76.5 -74.7%, in Algeria and Nigeria with 77.3-25.1%.

In Europe, the sheep meat production volume decreased from 1268.7 thousand tons in 2000 to 1139.7 thousand tons in 2014, or with 10.2%. It is caused by a significantly reduced of sheep meat production in the most countries with major effective from Europe: United Kingdom - with 22.2%, Spain - with 52.3% France - with 15.5%, Greece - with by 15.9%.

The biggest volume of sheep meat production remain in Turkey (312.5 thousand tons), United Kingdom (298.0 thousand tons), Russia (186.4 thousand tons), Spain (113.6 thousand tons) and France (110.2 thousand tons). In some countries has been recorded an increase of meat production: in Azerbaijan – with 95.7%, Russia - with 56.5%, Romania – with 11.9%.

In Oceania it occurred, also, a decrease of sheep meat production level. In Australia sheep meat production decreased from 790.6 thousand tons in 2000 to 720.6 thousand tones in 2014 or with 70.0 thousand tons (8.9%), New Zealand - from 533.2 thousand tones in 2000 to 487.1 thousand tones in 2014.

***Sheep milk,*** with particularly valuable nutritional qualities, has a vital interest in the human food from worldwide. According to FAOSTAT data, 2017, the volume of world production of sheep milk in 2014 was 10429.2 thousand tons (Table 3).

The biggest volumes of sheep milk has been produced in Asia (4854.0 thousand tons) and Europe (3080.7 thousand tons), followed by Africa (2451.4 thousand tons).

In America, the sheep milk production is insignificant. In Oceania the sheep, in general, are not milked.

From Asian countries, the most considerable volumes of milk produced in China (1537.0 thousand tons/year), which is situated after this index first in the world, Syria (685.1 thousand tons) and Iran (445.0 thousand tons).

Be noted that sheep milk production evolution in this part of the world has been progressed permanent in the examined period, from 3534.0 thousand tons in 2000 to 4854.0 thousand tons in 2014, or with 1320.0 thousand tons (37.4%). The most accelerated growth rhythms of milk production volumes were recorded in Jordan (by 2.1 times), China (with 81.5%) and Syria (with 53.7%).

If after the sheep herds and meat production volumes, Europe ranks third in the world (after Asia and Africa), then milk production volume ranks second after Asia. This is explained by the fact that sheep milk production in Europe is not just an occupation to ensure the food needs of the rural population, but has become a profitable economic business due to the substantial increase in market demand.

Therefore, specifically in Europe have been created the most advanced breeds of sheep for milk production (Oestfriză, Lacaune) and produces the most delicious assortment of cheeses (Roquefort), which ensures competitiveness and profitability of this production.

From European countries, the highest annual volumes of milk produced is in Turkey (1113.0 thousand tons), Greece (772.0 thousand tons), Romania (673.4 thousand tones), Spain (592.8 thousands tones) Italy (372.5 tones) and France (266.5 thousand tones). In some countries of Eastern Europe, the milk production volumes have been vertiginous growth evolution.

Table 3. Evolution of worldwide production of sheep milk during the 2000-2014 period (thousand tons)

| Nr d/o | Country, continent, part of the world | Years | | | 2014 % to 2000 |
|---|---|---|---|---|---|
| | | 2000 | 2006 | 2014 | |
| **WORLD TOTAL** | | **8 159.9** | **9 017.3** | **10 429.2** | **127.8** |
| **1.** | **EUROPE total** | **2 880.9** | **3 098.8** | **3 080.7** | **106.9** |
| | inclusive: Turkey | 774.4 | 794.7 | 1 113.0 | 143.7 |
| | Greece | 743.2 | 753.5 | 772.0 | 103.9 |
| | Romania | 320.8 | 650.8 | 673.4 | 209.9 |
| | Spain | 392.0 | 424.3 | 592.8 | 151.2 |
| | Italia | 741.9 | 548.3 | 372.5 | 50.2 |
| | France | 253.9 | 262.8 | 266.5 | 105.0 |
| | Albania | 78.0 | 75.0 | 89.0 | 114.1 |
| **2.** | **ASIA total** | **3 534.0** | **4 156.0** | **4 854.0** | **137.4** |
| | inclusive: China | 847.0 | 1 091.0 | 1 537.0 | 181.5 |
| | Syria | 445.6 | 824.1 | 685.1 | 153.7 |
| | Iran | 555.0 | 543.9 | 445.0 | 80.2 |
| | Afghanistan | 225.0 | 138.0 | 216.3 | 96.1 |
| | Iraq | 160.0 | 64.0 | 35.5 | 22.2 |
| | Jordan | 30.1 | 84.5 | 63.8 | 212.0 |
| **3.** | **AFRICA total** | **1 731.4** | **2 045.5** | **2 451.4** | **141.6** |
| | inclusive: Somalia | 445.0 | 573.5 | 503.5 | 113.1 |
| | Sudan | 462.0 | 492.0 | 402.0 | 87.0 |
| | Algeria | 180.0 | 228.2 | 363.2 | 201.8 |
| | Mali | 88.1 | 123.6 | 301.6 | 342.3 |
| | Niger | 92.9 | 114.2 | 131.4 | 141.1 |
| **4.** | **AMERICA total** | **35.1** | **35.6** | **43.0** | **122.5** |
| | inclusive: South America | 35.1 | 35.6 | 43.0 | 122.5 |
| | Bolivia | 29.0 | 29.2 | 35.8 | 123.4 |

Thus, in Armenia, Ukraine, Azerbaijan and Romania the sheep milk production volume produced in the 2000-2014 period rose by 4.14 - 2.1 times. In Turkey, Republic of Moldova and Spain, the sheep milk production volume increased with 30.4 - 51.2%.

On the African continent sheep milk production volumes increased from 1731.4 thousand tons in 2000 to 2451.4 thousand tons in 2014, or with 720.0 thousand tons (41.6%).

From African countries, the biggest volumes of sheep milk produce: Somali (503.5 thousand tons), Sudan (402.0 thousand tons), Algeria (363.2 thousand tons) and Mali (301.6 thousand tons).

On the American continent, the sheep milk is produced in insignificant quantities, only in Latin America. Of the total of 43.0 thousand tons, in Bolivia has been produced 35.8 thousand tons sheep milk. The volume of milk production during the reference period has been increased with 6.8 thousand tons or 23.4%. This country is one of the few states where the tradition was preserved colonial specific of population (Spain) on the sheep milk production and cheese preparation.

***The wool*** production worldwide, according to FAOSTAT data, 2015, in the examined period has been a quantitative decreasing evolution, from 2311.4 thousand tons in 2000 to 2126.8 thousand tons in 2013, or with 184.6 thousand tons (8.0%) (Table 4).

This decrease is provoked by significant reduction of wool quantities produced in Oceania (43.4%) and on the American continent (27.0%). If in 2000 year, the countries of Oceania (Australia and New Zealand) ranks first in the world after the amount of wool produced, then, since 2006 year until now, they have to be second after Asian countries that produce a total 950.2 thousand tons.

In Asia, the biggest quantities of wool produce China (471.1 thousand tons), Iran (61.5 thousand tons), India (46.5 thousand tons) and Pakistan (43.6 thousand tons). The biggest growth rhythms, during this period, of the wool quantity produced in gross mass were recorded in Kazakhstan (64.2%), Turkmenistan (69.6%), China (61.0%) and Pakistan (12.1%).

Table 4. Evolution of worldwide wool production during the 2000-2013 period (thousand tons)

| Nr d/o | Country, continent, part of the world | Years | | | 2013 % to 2000 |
|---|---|---|---|---|---|
| | | 2000 | 2006 | 2013 | |
| **WORLD TOTAL** | | **2 311.4** | **2 213.8** | **2 126.8** | **92.0** |
| **1.** | **EUROPE total** | **257.1** | **256.2** | **265.8** | **103.4** |
| | inclusive: United Kingdom | 64.0 | 57.6 | 68.0 | 106.2 |
| | Russia | 39.2 | 50.3 | 54.7 | 139.5 |
| | Turkey | 43.1 | 46.8 | 51.2 | 118.3 |
| | Spain | 32.1 | 30.4 | 22.9 | 71.3 |
| | Romania | 18.0 | 19.4 | 18.6 | 103.3 |
| **2.** | **ASIA total** | **719.2** | **857.3** | **950.2** | **132.1** |
| | inclusive: China | 292.5 | 388.8 | 471.1 | 161.0 |
| | Iran | 75.0 | 75.0 | 61.5 | 82.0 |
| | India | 48.4 | 45.1 | 46.5 | 96.1 |
| | Pakistan | 38.9 | 40.1 | 43.6 | 112.1 |
| | Kazakhstan | 22.9 | 32.4 | 37.6 | 164.2 |
| **3.** | **AFRICA total** | **205.4** | **216.1** | **238.3** | **116.0** |
| | inclusive: Sudan | 45.5 | 54.7 | 56.0 | 123.1 |
| | Morocco | 40.0 | 48.9 | 56.0 | 140.0 |
| | Republic South-African | 52.7 | 44.0 | 39.9 | 75.7 |
| | Algeria | 17.7 | 20.4 | 27.0 | 152.5 |
| **4.** | **AMERICA total** | **201.5** | **186.9** | **147.1** | **73.0** |
| | inclusive: Argentina | 58.0 | 67.8 | 45.0 | 77.6 |
| | Uruguay | 57.2 | 46.7 | 36.0 | 62.9 |
| | SUA | 20.7 | 16.3 | 14.0 | 67.6 |
| | Brasilia | 13.3 | 10.9 | 12.0 | 90.2 |
| **5.** | **OCEANIA total** | **928.2** | **697.2** | **525.5** | **56.6** |
| | inclusive: Australia | 671.0 | 472.5 | 360.5 | 53.7 |
| | New Zeeland | 257.2 | 224.7 | 165.0 | 64.2 |

The Oceania countries produce in total 525.5 thousand tons of wool. Australia and New Zealand, having huge grazing fields, developed in the not too distant past, under the influence of their metropolis (United Kingdom), a whole industry of sheep breeding and wool and meat production with minimal expenses, obtaining huge profits.

With the development in the world of fibers industry and synthetic fabrics, it fell sharply wool demand on the world market and as a result, its competitiveness was compromised, causing sudden reduction of the sheep herds and the quantity of wool.

In Europe, total raw wool produced in the period under review remains practically at the same level (265.8 thousand tons) with a slight increase of 3.4%.

The biggest quantities of raw wool are produced in the United Kingdom (68.0 thousand tons), Russia (54.7 thousand tons), Turkey (51.2 thousand tons) and Spain (22.9 thousand tons).

The slight increase in the quantity of wool in Europe is due to the increase in the quantity of wool produced in some countries in the east: in Azerbaijan - with 51.4%; Russia - with 39.5%; Turkey - with 18.3% and other countries.

Increasing the quantity of wool in the United Kingdom - with 6.2% due to exploitation of performing sheep breeds specialized for meat-wool or wool-meat competitive in the livestock sector.

In Africa, the quantity of raw wool increased from 205.4 thousand tons in 2000 to 238.3 thousand tons in 2013, or with 32.9 thousand tons (16.0%).

This is due to respective growth of the sheep herds on this continent.

The American continent ranks last after the amount of wool produced in raw mass with 147.1 thousand tons/year. In the period 2000-2013 on this continent has produced a substantial decrease of wool quantity - with 54.4 thousand tons or 27.0%.

Production of ***Karakul skins***, with regret, is not systematized by FAOSTAT, therefore, concrete data on the number of Karakul skins products worldwide does not exist.

However, based on information of international tenders (Kopenhagen Fur, 2015) with furs and

sheep herds from country that grow Karakul, the global volume of Karakul skins could be estimated at 13.3 million pieces, with an increase by 33.0% compared to 1967, when it produced about 10.0 million pieces annually (Table 5).

Table 5. Total herds of sheep in some countries with biggest number of Karakul sheep (thousand heads)

| Nr d/o | Country name | The total herds of sheep | | | 2013 % to 2000 | Estimated herd of Karakul sheep | |
|---|---|---|---|---|---|---|---|
| | | 2000 | 2007 | 2013 | | share in total, % | thousand heads |
| 1 | Uzbekistan | 8 000.0 | 10 383.0 | 14 077.5 | 176.0 | 84 | 11 825.1 |
| 2 | Kazakhstan | 8 725.4 | 12 813.7 | 15 197.7 | 174.2 | 40 | 6 079.1 |
| 3 | Turkmenistan | 7 500.0 | 13 758.0 | 14 000.0 | 186.7 | 90 | 12 600.0 |
| 4 | Afghanistan | 15 000.0 | 8 105.0 | 13 141.0 | 87.6 | 40 | 5 256.4 |
| 5 | Namibia | 2 446.1 | 2 652.6 | 2 930.0 | 119.8 | 98 | 2 871.4 |
| 5 | Republic South-Africa | 28 550.7 | 25 082.0 | 25 000.0 | 87.6 | 5 | 1 250.0 |
| 6 | Tajikistan | 1 472.2 | 1 955.2 | 2 959.5 | 201.0 | 34 | 1 006.2 |
| 7 | Romania | 8 121.0 | 7 678.0 | 8 833.8 | 108.8 | 8 | 706.7 |
| 8 | Republic of Moldova | 930.2 | 835.1 | 695.1 | 74.7 | 50 | 350.0 |

If we distribute this growth at the period of 45 years, get an annual increase of 0.73%. Therefore, in the period of 2000-2012, it may be expected to increase the amount of Karakul skins at a rate of 8.8%. The biggest quantities of skins have been produced in Turkmenistan, Uzbekistan, Kazakhstan, Afghanistan and Namibia.

Generalizing analysis of the evolution of the sheep herds and the total amount of the main sheep productions (meat, milk, wool, skins) worldwide during the years 2000-2013, in profile on countries and continents, we find that this (evolution) is in concordance with Васин Б. Н., 1971 affirmation, that „*distribution of sheep herds and breeds on the world is conditioned largely by human economic activity, but the division will depend not only by human will, but also by various natural factors, existing in different regions of the world"*.

From the data above-shown we can deduce the conclusion that the increase on the total number of sheep herds held in those regions, parts and continents of the world with underdeveloped countries (Africa, Asia) and with in developing (in Eastern Europe), with important rural human populations living in arid areas of plains, semi deserted with poor vegetation where sheep are kept whole year without capital investments in natural conditions, with minimal cost. For these populations, sheep are indispensable source of existence and survival in difficult condition of nature.

Also, on continents with developed countries and rural populations (Europe, North America, Oceania), for the growth and exploitation of sheep is seen as an economic business of making a profit, the sheep herds in this period were reduced.

In our opinion, this situation is explains that sheep species in terms of modernization, industrialization and intensification of livestock sector in developed countries cannot makes economic compete with other animal species (birds, pigs, cattle), becoming uncompetitive. This is confirmed by the fact that in developed countries in Europe (United Kingdom, Germany, France, Spain etc.) quantity of sheep meat production was sharply reduced with 22.2 to 52.3% in the 2000-2014 period.

The data demonstrated that, from all sheep productions only milk production, thanks to its exceptional and specific nutrient application market remains competitive compared to other productions of animal species. This is confirmed by the fact that the quantity of sheep milk has continuously increased during the examined period considered in all parts of the world, including the developed countries of Western Europe and the Americas.

Therefore, when drawing up measures for the development of sheep breeding in different countries and regions of the world requires a comprehensive approach for assessing the conditions for growth and upkeep of sheep, traditions of the peoples inhabit the socio-

economic conditions and competition in the market of sheep species with other species of animals.

In these situations, both in the world and in our country, are in need actions of improvement (genetic amelioration) of existing breeds, and to create new breeds, intra and inter-racial types, competitive, with mixed productivity (milk meat) and high potential, that adequate corresponded the socio-economic provocations of human populations from different parts of the world.

## CONCLUSIONS

Sheep breeding, worldwide, is a traditional zoo technical branch, which permanently is in ascendant development. This is due to major socio-economic importance, expressed through to ensure necessity security food of the population with animal protein such as meat and milk, as well as by supplying natural raw materials (wool, skins, fur sheep, leather etc.) of processing and manufacturing industry of wide consumer confections (clothes, fabrics, carpets, leather goods etc.). All of these make the ovine species indispensable for human society.

The herds of sheep in the world have increased during the years 2000-2013, from 1059.1 million heads in 2000 to 1172.8 million in 2013, or by 10.7%. In Asia and Africa it has been registered essential growth rhythms of the sheep population - with 27.1 and 32.0%. While in Europe, America and particularly in Oceania, there was registered a decrease of the sheep population, respectively, with 11.6; 6.5 and 33.9%.

The increasing of total effective of sheep has occurred in the regions, parts and continents of the world with underdeveloped (Africa, Asia) and developing countries (in Eastern Europe), with significant rural human population, living in arid zones with plains and scanty vegetation, where the sheep are kept the whole year in natural conditions, without capital investments, with minimal cost. For these populations, sheep are indispensable source of existence and survival in difficult conditions of nature.

On the continents with developed countries and rural population (Europe, North America, Oceania), where the breeding and exploiting of sheep is seen as an economic business of obtaining a profit, sheep livestock in this period had been reduced. This situation can be explained by the fact that the ovine species in the conditions of modernization, industrialization and intensification of the zootechnic sector in developed countries, economically cannot compete with other animal species (birds, pigs, bovines), becoming uncompetitive.

Worldwide, from sheep products in the period 2000-2014 increased more significant volume of food production, such as meat - with 14.4% and milk - with 27.8%, and less, nonfood, such as skins Karakul, which rose with 8.8%, and wool, which suffered a decline with 8.0%.

From all sheep productions, only milk production, due to specific exceptional nutritional qualities and market demand, remains competitive on all continents, compared with production of other animal species. This is confirmed by the increase in the period of 2000-2014, of volumes of this production: with 6.9% in Europe, with 37.4% in Asia, with 41.6% in Africa and with 22.5% in America.

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# ESTIMATION OF THE EFFICIENCY OF POLLINATION BY BEES OF SUNFLOWER CULTURE FOR HYBRID SEED PRODUCTION

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**Abstract**

*The purpose of the research was to estimate the efficiency of pollination by bees of some parental sunflower varieties, depending on the distance between the selected sectors with inflorescences and hives with bees, placed for pollination, was carried out. The experiments were performed on a field of sunflowers, with an area of 36 ha, where maternal variety "Express" and paternal variety "NS-X 6006" (both from Republic of Serbia), destined for crossing to obtain hybrid seeds, were sown alternatively. Around chain have been located 144 bee families, so that there were 4 families per ha. For verifying the self-pollination capacity of sunflower, 4 experimental sectors were selected, at different distances from the beehives - 10, 100, 300 and 500 m. As a control 8 inflorescences were selected in each sector, which before flowering, were covered with gauze. In the phase of full ripening of seeds, from each experimental sector the seeds were collected manually - 8 control-inflorescences and 9 experimental inflorescences (naturally pollinated). For each inflorescence in part were examined: the total number of seeds, number of fertile and sterile seeds, total weight of seeds, weight of fertile and sterile seed (weighed on electronic scales accurate to 0.1 g) and degree of seed's fertility. A result of researches was found that sunflower crops of varieties "Express" and "NS-X 6006" researched by us, are typically entomophilous, because in our experience, self-pollination (isolated) did not occur. The degree of seed's fertility of pollinated by bees inflorescences, varied depending on the distance between experimental inflorescences to the beehives, from 85.7% to 90.3%. The average total mass of the seeds from free pollinated inflorescence was higher compared to that of the self-pollinated inflorescences with 36.6 to 54.0 g or 260-740% (td = 7.5 to 16.3; P < 0.001), hence, pollinating by bees of parental varieties of sunflower "Express" and "NS-X 6006" is quite effective and increases the harvest of seeds from 3.6 to 8.4 times.*

***Key words:*** *efficiency, pollination, bees, sunflower.*

## INTRODUCTION

One of the most important benefits brought to human by bees is additional product obtained from increased productivity of cultivated and spontaneous entomophile flora, as a result of their pollination, thus ensuring the perpetuation of nature biodiversity. Especially, the increasing of productivity occurs at crops.

In the Republic of Moldova approximately 350 thousand ha of lands with agricultural plants are pollinated by bees, which allow increasing the harvest by 20-30%, the annual value consisting over 700 mil. lei. Unfortunately, agricultural producers (farmers, agronomists), mostly ignores the fact that the bees are the main pollinators of entomophilous crops and can serves as key factor in increasing of their harvest. Both farmers and beekeepers until the present didn't realized the fact that only by increasing the yield of harvest per unit of land and quality of agricultural products by using honeybees for directed pollination of entomophilous crops and producing of ecologycal agricultural and beekeeping products, can ensure economic efficiency and sustainable development of the respective branches in the country. Traditionally, the sunflower (*Helianthus annuus)* crop is considered typical entomophilous, whose pollination is performed exclusively by means of insects (Alexandru et al., 2007). Numerous researches carried out in this field (Falaleev et al., 1973; Frediani, 1973; Furgala, 1973; Hociota, 1973; Ion, 2012; Ion et al., 2008; Ion et al., 2007; Ion et al., 2006; Yadav et al., 2003; Фаркаш, 1987) shows that sunflower pollination by bees increases considerably the production (harvest) of seeds with 35-70% compared to the free pollination, without bees. Moreover, some researchers (Frediani,

1973) states that "*after cross-fertilization there is a considerable increases not only of seed's weight, but also oil concentrate in sunflower*".

Also there is information that in some modern hybrid varieties of sunflower, the phenomenon of self-pollination occurs in 18-98% of cases (Ion, 2012). Based on this information in recent years, the traders of sunflower seeds had spread among farmers and seed producers of this crop, hypothesis that most modern hybrid varieties are self-pollinating and does not require pollination by insects.

The pollination of the flowers, in general, consists in transporting of the pollen from anthers of the stamens on the flower's stigmas. Sunflower is considered as allogamous but entomophilous flower, because the process of self-pollination of flowers in nature is very difficult. This is explained by the fact that "*there is a difference of maturity between male organs (stamens) and female organs (stigma), the stamens grow and mature before the stigma, which means that the pollen is set free long before the stigma is receptive*" (Ion, 2012). For these reasons, the necessity of entomophilous pollination of the sunflower crop is not researched enough (Лайко и др., 1987).

Unlike developed countries, in the Republic of Moldova honeybees are underutilized for directed crop pollination, in particular for sunflower. This is explained by the fact that it was extremely widespread in agronomy information that most contemporary varieties of sunflower are self-fertilized and do not requires entomophilous cross pollination.

In this context, our research was aimed to check the phenomenon of self-pollination and the necessity of pollination by bees of some varieties of sunflower, grown in our country.

## MATERIALS AND METHODS

To solve the purpose, an experiment to estimate the efficiency of pollination by bees of some parental sunflower varieties, depending on the distance between the selected sectors with inflorescences and hives with bees, placed for pollination, was carried out. The experiments were performed on a field of sunflowers, with an area of 36 ha, where maternal variety "Express" and paternal variety "NS-X 6006" (both from Republic of Serbia), destined for crossing to obtain hybrid seeds, were sown alternatively. Around chain have been located 144 bee families, so that there were 4 families per ha. For verifying the self-pollination capacity of sunflower, 4 experimental sectors were selected, at different distances from the beehives - 10, 100, 300 and 500 m. As a control 8 inflorescences were selected in each sector, which before flowering, were covered with gauze, thus, isolation of flowers from pollinating insects was ensured. In the phase of full ripening of seeds, from each experimental sector the seeds were collected manually - from 8 control-inflorescences and 9 experimental inflorescences (naturally pollinated). For each inflorescence in part were examined: the total number of seeds, number of fertile and sterile seeds, total weight of seeds, weight of fertile and sterile seed (weighed on electronic scales accurate to 0.1 g) and degree of seed's fertility. Obtained data were statistically analyzed using computer software "STATISTICA-12" and appreciated their significance, according to biometric variational statistics after the methods of Плохинский Н. (1969).

## RESULTS AND DISCUSSIONS

Given that to the natural pollination of meliferous plant participate several species of insects, and *A. mellifera* prevail in its activity, covering about 80% of all pollination, in our experience, the notion of "pollination by bees" means directed pollination, by placing bee families next to sunflower crop with free participation of various species of entomophilous insects, existing in respective area. The experimental results showed that the varieties of sunflowers "Express" and "NS-X 6006" are typical entomophilous because self-pollination (isolated), in our experience, has not been determined (Table 1). The degree of fertility of seeds at the pollinated inflorescences had varied depending on the distance between the experimental sectors and beehives. Thus, seed's fertility in the sector no. 4, located at 500 m from the beehives, was 85.7% in the sector no. 1, located at a

distance of 10 m of the beehives - up to 90.3%, and at the inflorescences pollinated isolate (self-pollinated) seed fertility varies from 0 to 0.8%.

Table 1. The results of pollination by bees sunflower varieties "Express" and "NS-X 6006", grown for hybrid seed production

| Or. No. | Studied indices | Pollination by bees, M ± m | Self-pollination M ± m | Bees pollination *versus* self-pollination | | |
|---|---|---|---|---|---|---|
| | | | | d | % | $t_d$ |
| Sector no. 1 (10 m) | | | | | | |
| 1 | Number of investigated inflorescences | 10 | 8 | x | x | x |
| 2 | Total number of seeds per 1 inflorescence | 1217 ± 75 | 1041 ± 51 | +176 | +16,9 | 1.9 |
| 3 | Total mass of seeds per 1 inflorescence, g | 68.7 ± 5.7 | 14.7 ± 1.5 | +54.0 | +4.7 time | 9.2 |
| 4 | Total number of fertile seeds per 1 inflorescence | 1104 ± 78 | 9.6 ± 9.0 | +1094 | +115 time | 13.9 |
| 5 | Fertile seeds, % | 90.3 ± 1.9 | 0.8 ± 0.7 | +89.5 | +112.9 time | 44.3 |
| 6 | Mass of fertile seeds (harvest), g | 68.0 ± 5.7 | 0.7 ± 0.7 | +67.3 | 97.1 time | 11.7 |
| Sector no. 2 (100 m) | | | | | | |
| 1 | Number of investigated inflorescences (N) | 9 | 8 | x | x | x |
| 2 | Total number of seeds per 1 inflorescence | 1010 ± 73 | 1224 ±147 | -214 | 0.83 | 9.1 |
| 3 | Total mass of seeds per 1 inflorescence, g | 61.9 ± 5.1 | 13.6 ± 1.4 | +48.3 | +4.6 time | 9.1 |
| 4 | Total number of fertile seeds per 1 inflorescence | 879 ± 70 | 0.0 ± 0.0 | +879 | 100 | 100 |
| 5 | Fertile seeds, % | 87.1 ± 2.6 | 0.0 ± 0.0 | +87.1 | 100 | 100 |
| 6 | Mass of fertile seeds (harvest), g | 61.0 ± 4.9 | 0.0 ± 0.0 | +61.0 | 100 | 100 |
| Sector no. 3 (300 m) | | | | | | |
| 1 | Number of investigated inflorescences (N) | 9 | 8 | x | x | x |
| 2 | Total number of seeds per 1 inflorescence | 947 ± 79 | 1081 ± 108 | -134 | 0,88 | 1,0 |
| 3 | Total mass of seeds per 1 inflorescence, g | 50.4 ± 4.8 | 13.8 ± 1.1 | +36.6 | +3.6 time | 7.5 |
| 4 | Total number of fertile seeds per 1 inflorescence | 858 ± 88 | 0.0 ± 0.0 | +858 | 100 | 100 |
| 5 | Fertile seeds, % | 89.9 ± 2.2 | 0.0 ± 0.0 | +89.9 | 100 | 100 |
| 6 | Mass of fertile seeds (harvest), g | 49.6 ± 4.9 | 0.0 ± 0.0 | +49.6 | 100 | 100 |
| Sector no. 4 (500 m) | | | | | | |
| 1 | Number of investigated inflorescences (N) | 9 | 8 | x | x | x |
| 2 | Total number of seeds per 1 inflorescence | 1041 ± 41 | 754 ± 60 | +287 | +38.1 | 3.9 |
| 3 | Total mass of seeds per 1 inflorescence, g | 56.6 ± 3.0 | 6.7 ± 0.6 | +49.9 | +8.4 time | 16.3 |
| 4 | Total number of fertile seeds per 1 inflorescence | 890 ± 35 | 0.0 ± 0.0 | +890 | 100 | 100 |
| 5 | Fertile seeds, % | 85.7 ± 1.8 | 0.0 ± 0.0 | +85.7 | 100 | 100 |
| 6 | Mass of fertile seeds (harvest), g | 54.8 ± 3.1 | 0.0 ± 0.0 | +54.8 | 100 | 100 |

For comparison, in our previous research (Cebotari et al., 2015), the degree of fertility of seeds of sunflower hybrid variety EL PASSO-199, pollinated by bees, was depending on the distance between the experimental sectors and beehives from 88.6 to 92.1%.

Based on these results we can say that self-pollination of researched by us varieties, completely is lacking, because the seeds from isolated inflorescences remained practically sterile (empty).

In various sectors, the total weight of seeds in a inflorescence was different, depending on the distance between the sector and beehives. The highest total seeds weight per inflorescence was registered at inflorescences from the sector no.1, which is at a distance of 10 m from beehives, and constituted 68.7 ± 5.7 g. The lowest total weight of seeds per inflorescences, was registered in the sector no. 3, 300 m distant from the beehives, and it was 50.4 ± 4.8 g. The weight of seeds from sector no.1 is higher compared to the sector no. 3 with 18.3 g or 36.3%, ($t_d = 2.5$; $P < 0.05$) and compared to sector no. 4, with 12.1 g or 21.4% ($t_d = 1.9$; $P < 0.1$).

Seeds of inflorescences pollinated by bees were voluminous and hard to the touch because they were filled with core. At the same time, the seeds of isolated from insects inflorescences were small and empty to touch, because they had no core.

The average weight of one seed from pollinated by bees inflorescences has constituted 53-61 mg, but seeds from the

isolated inflorescences - only 9-14 mg. So, the average weight of a seed from bee-pollinated inflorescences was 4.1 to 6.0 times higher compared to that from self-pollinated inflorescences. On average, total weight of the seeds of one inflorescence (harvest) pollinated by the bees exceeded the average weight of the seeds of an self-pollinated inflorescence by 3.6-8.4 times.

The increase of the sunflower harvest after pollination by bees is presented in histogram.

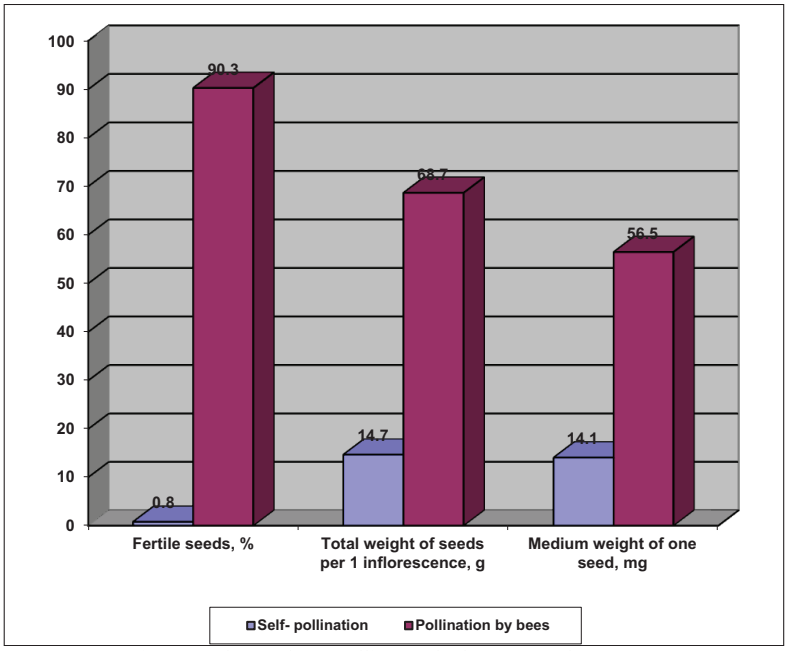


Figure 1. The results of pollination by bees of sunflower crop

The results have shown that total weight of the seeds is related to the degree of fertility of the seeds, since the weight of fertile seeds represents 96.8 to 99.0% of the total weight of the seeds.

Accordingly, the seeds collected from sector No.1, having the highest fertility also have had the highest total weight, compared to other sectors. The lowest total mass of seeds and, respectively, the lowest degree of fertility was recorded at the seeds collected from the sector no. 4. It was observed that with increasing of the fertility of seeds from 85.7%, in the sector no. 4, up to 90.3%, in the sector no. 1, there are an increasing of their total mass, from 96.8% to 99.0% respectively.

Given the fact that fertile seeds in proper represent the harvest, we can say that it (the harvest) is entirely due to the entomophilous pollination, especially pollination by bees.

Also, we found that the harvest of sunflower seeds pollinated by bees is depending on the distance between inflorescences and beehives. The closer the inflorescences of sunflower are to the beehives, the higher is the harvest of seeds.

Therefore, pollination with bees of the parental varieties of sunflowers "Express" and "NS-X 6006" is effective both from practical and economic viewpoint, since it increases the harvest of the seeds from 3.6 to 8.4 times.

## CONCLUSIONS

Sunflower crops of varieties "Express" and "NS-X 6006" researched by us, are typically entomophilous, because in our experience, self-pollination (isolated) did not occur.

The degree of seed's fertility of pollinated by bees inflorescences, varied depending on the distance between experimental inflorescences to the beehives, from 85.7% to 90.3%.

The average total mass of the seeds from free pollinated inflorescence was higher compared

to that of the self-pollinated inflorescences with 36.6 to 54.0 g or 260-740% (td = 7.5 to 16.3; P < 0.001), hence, pollinating by bees of parental varieties of sunflower "Express" and "NS-X 6006" is quite effective and increases the harvest of seeds from 3.6 to 8.4 times.

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# THE INFLUENCE OF THE BREEDING SYSTEM ON THE HATCHABILITY OF HEN MEAT RACES' REPRODUCTION

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***Abstract***

*During the present study, we have determined the values associated with the quantity and quality of the seminal material, influencing the hatchability and finally the biological and economic efficiency of the reproduction activity.*
*The conducted research was organized on a period of two years, the material being represented by commercially available hybrids of ROSS 308, 25 roosters and 250 hens. The study took place for three different control ages (25, 35 and 45 weeks) along the production cycle (week 19 - week 64).*
*The work took place in three different units, each corresponding to a different experiment (A - with analyzed parameters under the standard and using bedding made of chopped straws, B - with parameters that have been raised over the standard limits and using rice hulls as bedding, and C - with parameters at the producer's recommended level, and bedding made of wood shavings).*
*It was observed that the highest hatchability appeared associated with 25$^{th}$ week (67.39 %) and 45th week (87.89%) of experiment C and 35th week (89.89%) of experiment B.*
*The obtained results, although not statistically significant, appear to favor the use of classic bedding made of wood shavings, and of the technologically standard microclimate parameters, a situation in which the hatchability rate recorded the highest values.*

***Key words:*** *reproduction, hatchability, fertility.*

## INTRODUCTION

According to Henk Vaarkamp, 2010 (Breeding and Reproductive Technologies), the reproduction is defined as the selection and mating of animals by humans, with the main purpose of changing the traits of the next generation in such a manner as to correspond to the initial purpose of the process.
The reproduction traits are especially important for the efficiency of the domestic animals' breeding, at least from two points of view: first because a part of them influences the biological efficiency of the reproduction process, through the production of offspring in high numbers, creating the premises for the artificial selection (and touching the objective of improvement the genetic traits of a certain population); and the second is that this group of traits influences directly the economic efficiency though a high number of animals, for exploitation. These two aspects of efficiency are interdependent in the poultry sector, because these species have a series of reproduction represent at the same time the main product.

## MATERIALS AND METHODS

The reproduction ability of a male is appreciated by the number of females that have been recorded as being impregnated following the insemination, or after the necessary number of copulations has been achieved, in order to obtain a fecundation. *For poultry*, the roosters' capacity for reproduction can be quantified through the number of hatched eggs.
The technological factors (temperature, humidity, density, light intensity and extent) can affect the roosters' fecundity. Similar to a decrease in the fecundity of hens, in roosters a significant decrease of semen parameters could be observed, when certain conditions of microclimate-related stress are achieved.
Taking into account the latter, the *research in this study* have had as the main objective the investigation of the reproduction's efficiency

for ROSS 308 roosters, under the influence of several microclimate factors, such as light intensity and stocking density, as well as of other traits, which, when corroborated, would determine the reproductive capacity of roosters, which would further influence the hatchability and finally the biological and economic efficiency of the reproductive process.
For these reasons, the team designed three types of experimental series:

- Series A, which has as a main purpose the investigation of the influence of several microclimate factors set to parameters which would be under the standard limits, as well as using chopped straws as bedding material, on roosters' reproductive capacity;
- Series B, which investigated the effect of the parameters for microclimate factors such as light intensity and stocking density, set above the standard limits as well as using rice hulls as bedding material, on the traits which would determine the reproductive capacity of roosters;
- Series C, which investigated the influence of setting the light intensity and stocking density parameters within the standard limits, as well as using a classic bedding material of wood shavings, on the reproductive capacity of roosters.

The experiments took place for a period of two years, in three different poultry breeding units, each unit corresponding to a different set of experimental series: Avicola Călărași, S.C. Agrafood S.A. and Avicola Focșani.
The experimental groups were set to 25 roosters and 250 hens.
The investigation took place for three weeks (week 25, week 35 and week 45) during the production cycle (19-64 weeks).
In order to study the variation of hatchability, which would present a *binomial repartition*, the team used the following statistical methods:

- a comparison between the frequencies, based on a normal approximation;
- Fisher test for a comparison between the binomial proportions, known as the "Fisher's exact test";
- "Chi" square test, with Yates' correction for continuity applied on binary contingency tables (Dragomirescu, 1999).

## RESULTS AND DISCUSSIONS

The reproductive capacity of an animal, called *fertility*, is sometimes expressed through the use of *fecundity*, which in turn is used when related to the coupling of gametes; or using the word *prolificity*, which denotes the number of offspring, or *natality* (birthrate), which designates the process at population's level (the average number of offspring for each female or 100 females). In poultry, the use of *natality* is improper, in turn the *hatchability* being preferred.
The birthrate is a population's trait which refers to the degree at which this is adapted to the environment, more precisely the degree in which the population is adapted to the exploitation technology.
It is, next to the descendants' survival, the point to which it can be observed the effect of natural selection, which would eliminate the unsuitable ones, especially considering the reproductive capacity, through this decreasing the natality (Drăgănescu, 1984).
In table 1 and figure 1, there were included the values of the hatchability recorded through the three experimental series, for the 25$^{th}$ week.

Table 1. Hatching ability in the 25$^{th}$ week

| Specification | Fertile eggs | Total viable chicken | Hatching ability % |
|---|---|---|---|
| A | 37 | 18 | 48.65 |
| B | 70 | 41 | 58.57 |
| C | 46 | 31 | 67.39 |

By analyzing the data included in Table 1 and Figure 1, it can be observed that the highest value for hatchability for the 25$^{th}$ week was recorded for the experimental series C (67.39%). Thus, it seems that choosing the standard values for technological parameters and the use of a classic bedding material of wood shavings would have a favorable influence on hatchability.
In order to validate this observation and to test the statistical significance of the differences between the hatchability parameters recorded for the 25$^{th}$ week on the three experimental series, table 2 includes the results of "Chi square" test, with Yates' correction.

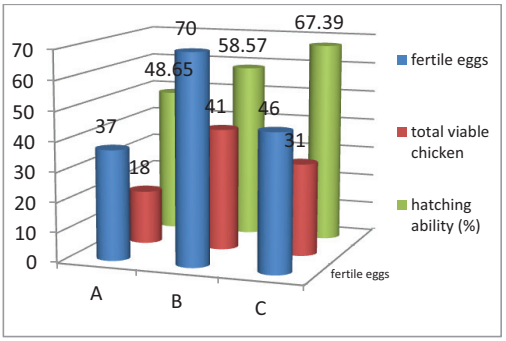


Figure 1. Hatching ability in the 25th week

Table 2. Comparison of hatching ability using $\chi^2$ test with Yates correction between experimental series, 25th week

| Specification | Fertile eggs | Viable chicken | Total | $\chi^2$ |
|---|---|---|---|---|
| A | a=37 | b=18 | a+b=55 | 0.13$^{NS}$ |
| B | c=70 | d=41 | c+d=111 | |
| Total | a+c=107 | b+d=59 | a+b+c+d=166 | |
| A | a=37 | b=18 | a+b=55 | 0.49$^{NS}$ |
| C | c=46 | d=31 | c+d=77 | |
| Total | a+c=83 | b+d=49 | a+b+c+d=132 | |
| B | a=70 | b=41 | a+b=111 | 0.09$^{NS}$ |
| C | c=46 | d=31 | c+d=77 | |
| Total | a+c=116 | b+d=72 | a+b+c+d=188 | |

By analyzing the results included in Table 2 and reading the table value using a degree of freedom and a significance level of 0.05, the conclusion is that there are no statistically significant differences between the hatchability values for the three experimental series for the 25th week.

In Table 3 and Figure 2 there were included the recorded hatchability values for the three experimental series on the 35th week.

Table 3. Hatching ability in the 35th week

| Specification | Fertile eggs | Total viable chicken | Hatching ability % |
|---|---|---|---|
| A | 170 | 138 | 81.17 |
| B | 178 | 160 | 89.89 |
| C | 194 | 171 | 88.14 |

By analyzing the data included in table 3 and figure 2 it can be observed that the highest value for the hatchability in the 35th week was recorded for experimental series B (89.89%).

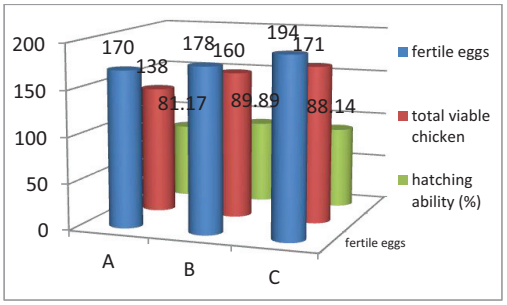


Figure 2. Hatching ability in the 35th week

It appears that setting the values for the technological parameters above the standard limits, as well as using rice hulls as bedding material would have a positive influence on hatchability.

In order to test the statistical significance between the values of hatchability parameter for the three experimental series in the 35th week, the results of "Chi square" test were included in Table 4, after applying the Yates' correction.

Table 4. Comparison of hatching ability using $\chi^2$ test with Yates correction between experimental series, 35th week

| Specification | Fertile eggs | Viable chicken | Total | $\chi^2$ |
|---|---|---|---|---|
| A | a=170 | b=138 | a+b=308 | 0.32$^{NS}$ |
| B | c=178 | d=160 | c+d=338 | |
| Total | a+c=348 | b+d=298 | a+b+c+d=646 | |
| A | a=170 | b=138 | a+b=308 | 0.20$^{NS}$ |
| C | c=194 | d=171 | c+d=365 | |
| Total | a+c=364 | b+d=309 | a+b+c+d=673 | |
| B | a=178 | b=160 | a+b=338 | 0.003$^{NS}$ |
| C | c=194 | d=171 | c+d=365 | |
| Total | a+c=372 | b+d=331 | a+b+c+d=703 | |

The result shows that the influence of technological parameters and the bedding material type does not present any statistical significance, the observed differences being caused by other factors, especially the sampling error, which does not influence the results.

Table 5 and figure 3 show the hatchability values recorded for the 45th week, on all three experimental series.

Table 5. Hatching ability in 45th week

| Specification | Fertile eggs | Total viable chicken | Hatching ability % |
|---|---|---|---|
| A | 125 | 93 | 74.4 |
| B | 140 | 120 | 85.71 |
| C | 157 | 138 | 87.89 |

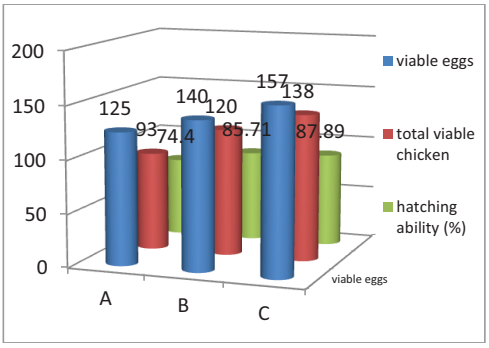


Figure 3. Hatching ability in the 45th week

After a closer analysis of the data included in these, it was observed that the highest value for the hatchability parameters during the 45th week was recorded for the experimental series C (87.89%). Thus, for the 45th week, the standard values of the technological parameters and using a classic bedding material of wood shavings leads to a positive outcome for the hatchability.

When analyzing the results included in Table 6, reading the table value with a degree of freedom and a statistical significance level of 0.05, it is concluded that there are no statistically significant differences between the three experimental series, during the 45th week.

The lack of statistically significant differences between the three designed experiments concerning the hatchability would reveal the fact that the technological parameters and the type of bedding material would only influence the traits until a certain point.

Table 6. Comparison of hatching ability using $\chi^2$ test with Yates correction between experimental series, 45th week

| Specification | Fertile eggs | Viable chicken | Total | $\chi^2$ |
|---|---|---|---|---|
| A | a=125 | b=93 | a+b=218 | $0.45^{NS}$ |
| B | c=140 | d=120 | c+d=260 | |
| Total | a+c=265 | b+d=213 | a+b+c+d=478 | |
| A | a=125 | b=93 | a+b=218 | $0.70^{NS}$ |
| C | c=157 | d=138 | c+d=295 | |
| Total | a+c=282 | b+d=231 | a+b+c+d=513 | |
| B | a=140 | b=120 | a+b=260 | $0.004^{NS}$ |
| C | c=157 | d=138 | c+d=295 | |
| Total | a+c=297 | b+d=258 | a+b+c+d=555 | |

## CONCLUSIONS

For the hatchability, the obtained results, although lacking significance from a statistical point of view, seem to favor the use of a classic wood shavings bedding material as well as standard values for the technological parameters, thus facilitating an increase in the hatchability.

During this study, the results show the excellent traits of the ROSS 308 hybrid, its great adaptability, offering very food results concerning the reproduction, no matter the type of bedding material or the values of the technological parameters. Most probably, the limitation for a certain values of reproduction traits would be represented by the nutrition.

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# Retracted article: DETERMINATION OF GRASSLAND AREAS BY USING REMOTE SENSING AND GEOGRAPHIC INFORMATION SYSTEMS, WITH SPECIAL REFERENCE TO ISPARTA, TURKEY*

**Cihan DOGAN, Yalçın BOZKURT**

***Abstract***

*This study was carried to determine the size and quality and potential of pastures by using Remote Sensing (RS) and Geographic Information Systems (GIS),in the province of Isparta located on the West Mediterranean Region of Turkey. In this study, Merkez, Şarkikarağaç and Yalvaç districts were chosen as test areas which comprise of 80% of whole grassland area of Isparta Province. A digital map showing areas of pasture belonging to the district of Şarkikaraağaç was prepared and the grassland area was estimated. Sowing times for grass samples was identified as May\ July and September in 2011 to determine dry matter (DM) in the amount of biomass, botanical composition and ADF, NDF and crude protein (CP) values. The amounts of biomass on DM basis per hectare in the districts of Merkez, Şarkikaraağaç and Yalvac 3.11, 2.71 and 2.69 ton/ha, respectively. Botanical compositions for Merkezleguminosae 21%, Gramineae 37% and 42% others respectively; Şarkikaraağaç 36%, 40% and 25%; Yalvaç 36% and 33% was 31%. Average ADF and NDF contents were increased during the vegetation period; CP rates were decreased. Both DM yields and protein contents towards the end of vegetation period were reduced. Therefore, the most appropriate animal grazing period was determined as between the beginning of May and the early September, and also in relation to the botanical composition of grasses in grassland areas, Gramineae was found to be greater than other species. Therefore, cattle grazing would be a more appropriate grassland management system in the study area. When setting up an inventory that is required for animal pasture and the results of similar studies related to Isparta province, the realization of an effective pasture management and exploitation of RS and GIS technologies a*

.

***Key words:*** *Geographic Information System, Remote Sensing, Grassland, Isparta Province, Botanical composition.*

## INTRODUCTION

Remote sensing in general is often described as acquiring information on physical and spatial characteristics of objects without physically touching them by using satellite images, and their objects are defined as spatial and qualitative perception (Lillesand and Kiefer, 1994; Eastman, 2003, Jensen, 2005).

Geographic Information Systems (GIS) is the whole set of tools that collect, store, make query, transfer and display earth-referenced data for a specific purpose. It is also an information system that references spatial coordinates or geographic coordinates and designs to work with these data (Aronoff, 1989; Hummer, 1991; Burrough, 1992).

Remote Sensing (RS) technology, when combined with Geographic Information Systems (GIS) technology, provides up-to-date information about the Earth's resources and techniques compared to conventional methods for agricultural applications (Derenyi, 1991; Alparslan and Divan, 2002).

RS and GIS were used in a research conducted in the eastern part of Turkey, the boundaries of grassland areas were determined and the rangeland quality classes were determined. In the same study, the grazing potentials of the grassland and the grazing capacities were also determined by grazing the animals (Bozkurt et al., 2010).

Therefore, in this study it was aimed to determine the rangeland boundaries and the suitable grazing times and the grazing systems for animal production by RS and GIS in the province of Isparta, Şarkikaraağaç region.

## MATERIALS AND METHODS

*Geographical location*

Isparta has an average altitude of 1050 m and an area of 8.933 $km^2$. It is located between $30^0$20' and $31^0$33' East longitudes and

$37^0$18'and $38^0$30' Northern latitudes Geographical Coordinate System (UTM). It is surrounded by Burdur province in the West and the South-West; Afyon province in the North and West; Northeast, Konya in the east and south-east and Antalya in the South.

*Topographic structure and climate*

Isparta and the area around its vicinity is quite mountainous and rugged. 68.4% of the Isparta Province's surface area is composed of mountains. In addition, 40.9% of the province's surface area is very steep (Anonymous, 1994).

According to meteorological parameters and natural vegetation cover, Isparta province is located in the transition zone between the Mediterranean climate and the continental climate prevailing in Central Anatolia. As averages for many years, the total annual rainfall in Isparta is 501.5 mm. The distribution of rainfall within the year, the minimum precipitation is 11.6 mm between August and December with the highest rainfall of 71.5 mm.

*Study Area and Sample Collection*

In this study, Merkez, Şarkikarağaç and Yalvaç districts were chosen as test areas which comprise of 80% of whole grassland area of Isparta Province.

This research was carried out for a total of 11 months in 2011 and the botanical composition and the vegetation measurements were conducted in the pasture areas which were presserved as non-grazing areas for 5 months before the experiment started. The weight and quadrat methods were used to determine the quantitative characteristics of grassland vegetation.

A total of 27 samples were taken by using a 0.5 $m^2$ quadrat at 3 different time periods (May-July-September) from 3 test areas and for each 3 regions so-called Merkez, Şarkikaraağaç and Yalvaç respectively.

Five replicates of grass heights were taken from the quadrat before the sowings were performed in each area, and the grass samples were cut at a height of 3 cm above the ground level in the quadrat. The fresh weights of the samples were determined using a scale with 2 g sensitivity (TEM-30 kg capacity) immediately after sowing.

The botanical composition was determined on weight basis and samples were taken from plant cover, species were separated and weighed separately. This method is the most reliable in critical studies. For this reason, it is the most appropriate method for determining the species in grasslands (Avcıoğlu, 1983).

*Chemical and Statistical Analysis*

The sampled materials were dried in a laboratory for 24 hours at 70°C, then weighed to obtain dry weights and the results were calculated in ton/ha. Kjehdahl method was used for CP analysis and Ankom 2000 fiber analyzer device was used for ADF and NDF (AOAC, 1995).

Statistical analysis were performed using Minitab 10 statistical software program and one way analysis of variance was used for significance of probabilities at 5% significance level. Tukey pairwise multiple comparison test was used to determine the differences between means.

*Image processing and Geographic correction*

Aster 2006 satellite data was obtained from Süleyman Demirel University Remote Sensing and GIS Centre. The satellite image with a 15 × 15 resolution taken in May was used due to the suitable temporal resolution as well as the obviousness of the green parts of vegetation in the areas outside the agricultural area in the spring months. Therefore, this satellite image was used to coordinate the digital topographic maps of Şarkikaraağaç district as reference by the Erdas 9.2 software program.

*Specifying and filtering study area boundaries*

The boundaries of the Şarkikaraağaç district were determined and filtering processes were performed using the boundary layer on the image to obtain an image of the Şarkikaraağaç district.

*Supervised Classification*

Coordinates were determined at the controlled points in the field studies where the supervised classification process is performed and the reflected values of these points and the histograms of the images generated from the satellite images are combined according to the supervised classification method.

## RESULTS AND DISCUSSIONS

The average fresh and dry weight of herbage biomass, and chemical composition of vegetation by test areas are shown in Table 1.

There were no statistically significant differences in average grass height between test areas (P>0.05). Grass height means were 20.64, 21.93 and 21.36 cm in Merkez, Şarkikaraağaç and Yalvaç respectively. There were no statistically significant differences in average fresh weight of herbage biomass between test areas (P>0.05). Fresh weight of herbage biomass means were 9.4, 7.59 and 7.79 ton/ha in Merkez, Şarkikaraağaç and Yalvaç respectively.

Table 1. Average fresh and dry weight of herbage mass and percentage of crude protein, ADF and NDF means by test areas

| Test Areas | [(1)]Grass Height Means (cm) | Herbage Mass Fresh Weight Means (ton/ha) | Herbage Mass Dry Weight Means (ton/ha) | Crude Protein Means (%) | ADF Means (%) | NDF Means (%) |
|---|---|---|---|---|---|---|
| Merkez | 20.64 | 9.4 | 3.11 | 10.13 | 31.62 | 51.21 |
| Şarkikaraağaç | 21.93 | 7.59 | 2.71 | 11.26 | 31.41 | 51.65 |
| Yalvaç | 21.36 | 7.79 | 2.69 | 10.43 | 32.10 | 51.39 |

(1) Average of 3 sampling time May, July, September

There were no statistically significant differences in dry weight between test region (P>0.05). Dry weight means were 3.11, 2.71 and 2.69 ton/ha in Merkez, Şarkikaraağaç and Yalvaç respectively. However, there was a tendency for fresh and dry herbage biomass values to be higher for Merkez test area.

Average yields of dry weights were found to be 3.11ton/ha in Merkez district, 2.71 ton/ha in the Şarkikaraağaç district and 2.69 ton/ha in the Yalvaç district. However, Babalık (2008) found that dry matter yield in non-grazed areas was 1582 kg/ha in Isparta Merkez district. This value was almost 2 times less than the value obtained in this study. The difference between these two studies is thought to be due to the differences in elevation and botanical composition of both study areas.

Crude protein contents were 10.13, 11.26 and 10.43% in Merkez, Şarkikaraağaç and Yalvaç respectively and the differences were not statistically significant between test areas (P>0.05).

In line with these results, the content of the average crude protein was very close to each other in the test districts. However, the highest crude protein content was found for Şarkikaraağaç test area with 11.26%.

There were statistically significant differences in ADF and NDF values of vegetation between test regions (P>0.05). ADF values were31.62, 31.41 and 31.1% in Merkez, Şarkikaraağaç and Yalvaç respectively. NDF were 51.21, 51.65 and 51.39% in Merkez, Şarkikaraağaç and Yalvaç respectively.

It was observed that ADF and NDF contents increased in all the districts as the vegetation season progresses. For this reason, as the vegetation time progresses, it is obvious that it is caused by the increase of the fibrous structure within the plant

Distribution of botanical composition by species is shown by test areas in Table 2.

According to the results obtained from the test areas, the botanical compositions on dry basis were 21, 37 and 42% for Leguminosae, Gramineae and others as respectively in Merkez district; 35, 40 and 25% in the Şarkikaraağaç, and 36, 31 and 33% respectively in Yalvaç.

Table 2. Distribution of botanical composition by species are shown by test areas

| Botanical Composition | Test Area | | | |
|---|---|---|---|---|
| | Merkez (%) | Şarkikaraağaç (%) | Yalvaç (%) | Means (%) |
| Leguminosae | 21 | 36 | 36 | 31 |
| Gramineae | 37 | 40 | 31 | 36 |
| Others | 42 | 24 | 33 | 33 |

[#]Only none significant regression coefficients had superscripts (ns), the rest were significant at P<0.05.

In a study carried out in 2008 in Merkez by Babalık (2008), it was found that distribution of species of botanical composition in protected areas was 58.89% for Gramineae, 11.36% for Leguminosae and 29.75% for others. The botanical composition values obtained in this study in Merkez district, Leguminosae were found to be 3 times more; Gramineae 2 times less; and others 1.5 times more than those found in the study by Babalık (2008).

As a matter of fact, Gökkuş (2001) stated that in a grassland with a lot of Gramineae is more suitable for grazing cattle. In this study, it was determined that Gramineae were greater in test areas especially in Şarkikaraağaç district and therefore it can be emphasised that it is more suitable for cattle grazing than other animal species in terms of botanical composition in this test area.

According to a study carried out by Babalık (2007) on Isparta Davraz mountain Kozağacı plateau, the area covered with grasslands was found to be 23.1%, while in the botanical composition, 67.4% of Gramineae and 12.1% of Leguminosae and 20.5% of other families.

As a result of application of satellite image processing by RS and GIS and the vegetation studies performed within the scope of determination of the grassland areas of Şarkikaraağaç district, the following map of the study area was produced (Figure 1).

The fields are colored according to the situation of land use on the prediction map obtained as a result of image processing applications. Each color represents the use of land in that area. In the study, the 13 points were determined on the map as control points and these points were determined as grasslands in the field studies and 10 of them were classified as rangelands and 3 of them as rangeland+cultivated lands.

In Figure 1, irrigable agricultural fields of the province are shown in red, irrigated agricultural fields in yellow, rare vegetation covered in brown, and the estimated grassland areas, which mainly constitute our study area are symbolised in purple colour.

Estimated grassland for the Şarkikaraağaç district is 2325.4 hectare and the yield of dried grass from Şarkikaağaç is 2.71 ton/ha. After necessary calculations are made, the amount of dry biomass that can be obtained from these estimated pasture areas is calculated as approximately 6.5 ton.

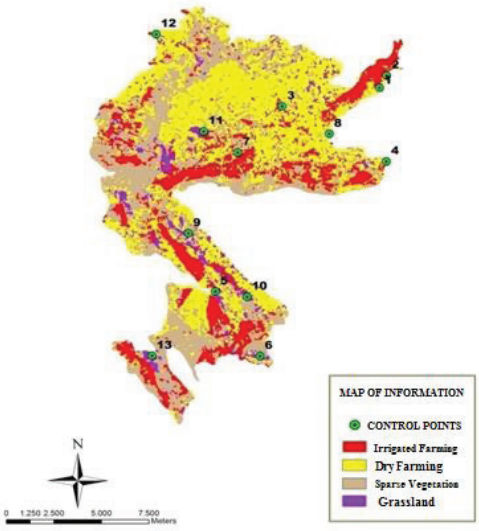


Figure 1. Grassland area map obtained from Aster 2006 satellite data in Şarkikaraağaç

The similar results were obtained in a study conducted by Babalık (2008) in which it was investigated the changes in the amount of biomass yield in grazing areas during summer and autumn periods and to provide information to be used by farmers to improve animal production in the region.

In a research carried out by Bozkurt et al. (2010), using RS and GIS, they prepared the maps showing the rangeland quality classes by determining the boundaries of Kars province metropolitan areas. In this study, the grazing potentials of the grassland and the grazing capacities were determined by grazing the animals.

## CONCLUSIONS

It was found that the most suitable grazing time can be started at the beginning of May and terminated at the beginning of September since both dry biomass yield and protein ratios are reduced at the end of the vegetation period, and also that considering the vegetation cover and botanical composition the area is more suitable for both sheep and cattle production and the leader-and follower grazing system can be recommended for farmers in the region.

In terms of animal production potential, knowing the boundaries of pasture areas will guide farmers and decision makers in terms of

animal production and will help all the sectors involved to use the available resources correctly. Determination of pasture areas with their locations determined by digital maps and determination of suitable grazing times and grazing systems in pasture areas are considered to provide great contribution to livestock potentiality of Şarkikaraağaç district and Isparta province as a whole.

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# Retracted article: IMPROVEMENT OF MEAT LAMB PRODUCTION IN MURES COUNTY BY CROSSBREEDING OF LOCAL TSIGAI BREED WITH GERMAN BLACKHEADED BREED

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# THE IMPORTANCE OF CONTROLLING INCUBATION FACTORS IN DUCK BREEDERS

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***Abstract***

*The aim of this paper is to highlight the importance of having a good control of incubation factors of ducks because they are the key in obtaining high technological and economical results in duck husbandry. Incubation factors can be divided in two great categories: 1. reproduction factors (mating system, the technologies used in duck husbandry genetic quality of the birds, sex ratio in the flock, the age of the birds, etc.); 2. incubation factors (egg collection, egg storage, preheating, incubation temperature, humidity, ventilation and movements of the eggs). Having a very firm control of this factors, one can achieve good incubation results which, later, can be translated in to bigger production of meat (and secondary productions) and bigger income for the farmer.*

***Key words****: egg production, incubation, incubation factors, meat production, reproduction factors.*

## INTRODUCTION

In duck husbandry, incubation is a must for obtaining good technological and economical results and is a very important step for the industrialisation of this sector of animal science, having in regard the higher and higher request of food that is accessible, of quality and diverse.

The incubation can be defined as being a set of operations which allow that, from a certain number of eggs, to be obtained a maximum number of ducklings at a minimum cost.

By knowing the interactions between reproduction and incubation factors, one can optimise the quantitative and qualitative production (meat, eggs, fatty liver (foie gras) and feathers).

The main factors that influence the outcome of incubation for ducks and were evaluated for this paper are shortly presented here.

***Factors influencing reproduction:***

*The quality of ducks* refers to the genetic potential of the individuals that are used for reproduction. Knowing the genetical potential of each individual is a must, because genetic improvement is the only way for getting economical quality of the production.

*Mating system and breeding method*. In the case of ducks, breeding is made by hausing together the male with females. It shuld be given a special attention to the risc of consangvinisation which determines a bad incubation index by greater embrion mortality. Opposite to that, the hibridation determines an increasing of hatching rates due to low embryonic mortality (Popescu-Miclosanu, 2007).

*The age of individuals*. Studies have revealed that eggs obtained from young females that are at their first cycle of laying are smaller and the fecundity is decreased. It is recommended that the individuals from the reproductive group to be as homogeneous as possible (Popescu-Micloșanu, 2007).

Table 1. The variability of eggs weight depending on breed and selection for incubation (Popescu-Micloșanu, 2007)

| Breed | Weight variability (g) | Weight variability of incubation eggs (g) |
|---|---|---|
| Barbarie | 80-90 | 85-90 |
| Pekin | 60-80 | 70-80 |
| Indian Runner | 65-80 | 70-80 |
| Campbell | 65-80 | 70-80 |

Another paper that reveles the importance of age in duck reproduction was publicated in Poultry Journal by Applagate et al. (1988). The results had shown that birds with a bigger age have produced eggs with higher weith and,

comparative with eggs from younger birds, have a better incubation index.

*The date of the lot formation.* For obtain a large number of effective matings and high fecundity, the lot is recommended to be done 4 to 6 weeks before the laying period starts, (Popescu-Miclosanu, 2004).
*Sex ratio* is recommended to be, for light breeds, 1 male for 5 females, and for heavy ones, 1 male for 4 females. Controlling this sex ratio determines a high fecundity (Tipurită, 1986).

***Factors influencing egg incubation:***
*Egg gathering.* In the case of palmipeds, egg gathering must be done several times a day, due to the fact that they can lay eggs not only in the nest but also on the floor. Eggs intended for incubation must not be cracked and have a clean shell.
*Egg depositation.* The storing of eggs for incubation must be done in a special room where the optimal temperature for egg storage is as in Table 2.

Table 2. Duration of storage of eggs depending on temperature (Popescu-Micloșanu, 2008)

| Temperature (°C) | 0 | 1 | 10 |
|---|---|---|---|
| Duration of storage (days) | 4 | 9 | 3 |

Humidity in the storage room is also very important and should be between 75-80%.
Care should be taken when the storage period exceeds one week. If this is the case, the stored eggs must be turned 2 to 6 times a day (Popescu-Miclosanu, 2007).
*Preheating.* It is known that embryonic development starts from 21°C, so preincubation involves heating the eggs at a temperature of 24 - 32°C with about 6 - 12 hours before being introduced into the incubator.
*The incubation temperature* should be between 37.5-38.0°C, and the optimal temperature is 37.7°C (Dinea, 2008). Decreasing the temperature under 35.5°C or raising it to more than 39.0°C leads to a high percentage of embryonic mortality. A study Harum et al. (2001) on Barbarie showed that the optimal temperature used in incubation that gave the most satisfying results was 37.5°C, when combined with daily spraying of eggs, and a 30-minute cooling per day.
*Humidity.* The recommended value in the incubator for duck eggs is between 55 and 60% and in the hatcher, even higher than 95%. When the humidity is too high, the weight of the ducklings grows, and when it is too low, the duckling may stick to the membranes and so, the hatch became impossible.
A study from 2012 on Pekin, presented in the journal Poultry Science by El-Hanoun et al. (2012), shows that the best incubation results were obtained at a relative humidity of 60%.
*Ventilation* is very important, fulfilling two functions: a) ventilation regulates the amount of fresh air introduced into the incubator by removing carbon dioxide and providing an adequate amount of oxygen; b) and the second function of ventilation is that it maintains the internal air circulation, preventing the temperature from rising and the accumulation of harmful gases in the incubator (Dinea, 2008).
*The position and the turn of the eggs*. The eggs may be placed in the incubator in a horizontal or vertical position according to the requirements of the incubator.
The turn of the eggs prevents sticking the yolk of the shell membrane. The turn can be done every two hours.

## MATERIALS AND METHODS

In 2016, from the egg production of Barbarie, Pekin, Indian Runner and Campbell breeds from the Moara Domneasca Didactic Farm, after sorting, six series of 100 or 150 eggs per breed were incubated.
The data obtained in 2016, when all the incubation indices were respected (Table 3), were compared with data from 2015, when no special attention was paid to incubation technology (Table 4). Systematization and statistical analysis of data were done in Excel.

## RESULTS AND DISCUSSIONS

For the Barbarie and Pekin breeds, 6 series of 100 eggs were introduced to the incubation, and 6 series of 150 eggs were introduced for the Indiana Runner and Campbell breeds.

Table 3. Data obtained in 2016 at the Moara Domneasca Didactic Farm

| Breed | Eggs | Ducklings | Clear | Dead |
|---|---|---|---|---|
| Barbarie | 600 | 306 | 168 | 126 |
| Pekin | 600 | 450 | 92 | 58 |
| Indian Runner | 900 | 530 | 226 | 144 |
| Campbell | 900 | 649 | 150 | 101 |

Table 4. Data obtained in 2015 at the Moara Domneasca Didactic Farm

| Breed | Eggs | Ducklings | Clear | Dead |
|---|---|---|---|---|
| Barbarie | 600 | 235 | 200 | 165 |
| Pekin | 600 | 388 | 126 | 86 |
| Indian Runner | 900 | 484 | 264 | 152 |
| Campbell | 900 | 600 | 155 | 145 |

In order to achieve these results, both factors influencing reproduction and that influencing egg incubation have been respected.

***Factors influencing reproduction:***

*The quality of individuals*. The groups of ducks (Table 5) were homogenous made from females that were at the second and third cycle of laying.

The birds from which hatching eggs were recolted were healthy, not presenting diseases or breeding problems.

Table 5. The breeding groups (heads)

| Breed | Females | Males |
|---|---|---|
| Barbarie | 40 | 10 |
| Pekin | 50 | 15 |
| Indian Runner | 50 | 15 |
| Campbell | 50 | 15 |

*Mating system and breeding method*. Birds were raised in pure lines, on the ground, in collective boxes.

*The age.* Groups were made from birds being at the second and third cycle of laying and a percent of 10 to 15% of birds being at the first cycle of laying that were selected having a optimum weight and being able to lay good quality eggs.

*The date of lot formation.* For the formation of the lot, a method suggested by Popescu Miclosanu in 2007, was used.

So, lot formation was made with about 5 weeks before the laying began, to produce intense mating and thus a high index of fecundity.

Sex ratio was as in Table 6.

Table 6. Sex ratio

| Breed | Males / Females |
|---|---|
| Barbarie | 1:4 |
| Pekin | 1:3 |
| Indian Runner | 1:3 |
| Campbell | 1:3 |

***Factors influencing egg incubation:***

*Eggs collection* was done 2-3 times a day, as follows: at 8 o'clock, at 10 o'clock and at 12 o'clock

*Eggs storage.* It was made in the storage room. The eggs were stored for 3 to 7 days depending on the laying season, the number of eggs or the formation of the series required for the incubation. The storage room fulfills the conditions presented in table 7.

Table 7. Parameters in the storage room (Dinea, 2008)

| Duration | Temperature (°C) | Humidity (%) |
|---|---|---|
| 1-3 days | 18-21 | 75 |
| 3-7 days | 15-17 | 75-80 |
| > 7 days | 12-14 | 80 |

*Preheating.* In the experiment conducted at the Didactic Farm, the preheating of each series of eggs was done with great care for 12 hours until they reached the temperature of 26°C.

*The incubation temperature* was 37.7-37.9°C, with no variation in the incubation period of each series.

*Humidity* in the incubator ranged between 65% and 70%, periodically checked, to intervene if necessary, using a complex device called thermo-anemo-lux-meter (Figure 1).

Figure 1. Thermo-anemo-lux-meter (http://www.intratechengineers.com)

In the hatchery, the humidity was 95%. The weight of the ducklings obtained was the normal one encountered in each of the four breeds.

*Ventilation* was appropriate, with an adequate gas exchange, not to endanger embryonic development.

*The position and turn of the eggs.* The incubator was a vertical one, and the turning of

the eggs was done by tilting. The turn was done every two hours.

*Data analysis.* After processing the data, we found the following:

- The greatest positive impact due to control of incubation factors was recorded in eggs from the Barbarie breed, which produced 30.20% more ducklings. In the Pekin breed, the increase was 16.00%, the Indian Runner 9.50%, and Campbell of only 8.20%.
- The most significant reduction in the percentage of clear eggs was recorded in the Pekin breed (-27.00%), and the least significant (-3.20%) in the Campbell breed. In the other two breeds, the percentage of clear eggs decreased by about 15% (Table 8).
- Percentage of embryo mortality was the lowest in Pekin (-32.60%) and Campbell (-30.30%), and in Barbarie and Indian Runner breeds was reduced with 23.60% and 5.30%, respectively.

Table 8. Synthesis of duck eggs incubation data obtained in the years 2015 and 2016 at the Didactic Farm

| | Eggs | | Ducklings | | | Clear | | | Dead | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | 2015 | 2016 | 2015 | 2016 | 2016/2015 | 2015 | 2016 | 2016/2015 | 2015 | 2016 | 2016/2015 |
| Barbarie | 600 | 600 | 235 | 306 | 30.2% | 200 | 168 | -16.0% | 165 | 126 | -23.6% |
| Pekin | 600 | 600 | 388 | 450 | 16.0% | 126 | 92 | -27.0% | 86 | 58 | -32.6% |
| Indian Runner | 900 | 900 | 484 | 530 | 9.5% | 264 | 226 | -14.4% | 152 | 144 | -5.3% |
| Campbell | 900 | 900 | 600 | 649 | 8.2% | 155 | 150 | -3.2% | 145 | 101 | -30.3% |
| Total | 3000 | 3000 | 1707 | 1935 | 13.4% | 745 | 636 | -14.6% | 548 | 429 | -21.7% |

For the precision of the interpretation of these data, three ANOVA single factor tests were performed: for the number of ducklings, number of clear eggs and number of eggs with embryonic death. The results showed that there was no significant difference between the two years of production for any of the categories.

The value of P was: for the number of ducklings of 0.609, for the number of clear eggs of 0.529, and for the number of embryonic death eggs of 0.289.

## CONCLUSIONS

The analysis of the results presented in Tables 3 and 4 shows that in all four breeds, much higher performances were achieved in 2016 when the factors influencing the production and those influencing the incubation were strictly respected.

By respecting the factors that influence reproduction, a larger number of eggs can be obtained, with a high degree of fecundity, and by respecting the influential factors of hatching, the number of ducklings that are obtained is much higher.

In figures, by controlling reproductive and incubation technology, the percentage of hatching increases by approx. 13.40%, and the percentages of clear eggs, respectively of mortality decreases by 14.60% and 21.70%.

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# PRELIMINARY DATA ON GROSS MARGIN COMPARISON OF DAIRY FARMS IN TWO REGIONS OF ALBANIA

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***Abstract***

*The purpose of the study was to analyze and compare the economic efficiency of dairy farms in two regions of Albania. This was a descriptive and quantitative survey and the target population was the dairy farmers collaborating with Agriculture Regional Directories of Elbasan and Shkoder. The farmers and expert panel was used to calculate the gross margin. The annual farm incomeand the cost of milk production were studied. The milk cost ranged from 0,271to 0,30 Euro/kg, while milk price ranged from 0,310 to 0,356 Euro/kg, while the meat price ranged from 2,2 Euro/Kg to 2,5 Euro/Kg. The farms in Shkodra have a negative result only from the milk production, but the Gross Margin is improved from the meat selling. In Elbasan region the results are better; and incomes from milk are higher than incomes from meat while in Shkoder is vice-versa. The market price of one kilogram of meat is equal to 6.75 kg of milk. In variable cost, feed took the highest share by 81.2 percent. Feeding keeps the highest share within variable cost: 78.1% for Elbasan region and 75.6 % for Shkodra region. The Gross Margin per Cow (GMpC), for all the farms monitored, have positive values. The GMpCmilk+meatranged from 145.1 to 480.2 Euro/Cow in Shkoder and from 380.9 to 1480.8 Euro/Cow in Elabasan, while the GMpC milk in Elbasan had positive value (160.1 to 886.8 Euro/cow) in Shkodrathree farms hada negative value (-1.3 to 7.1 Euro/Cow) and the rest had positive value but lower than of Elbasan (47.8 to 136.8 Euro/Cow). This is one of the reasons, emphasized by MARDWA, that the analysis of the competitiveness of agriculture of Albania shows that currently only a small share of farms can compete in the regional market, EU and international level.*

***Key words:*** *dairy farm, gross margin, farm income, income per cow.*

## INTRODUCTION

Albania continues to be a predominantly rural economy with about 20 percent of GDP generated by agriculture, and about 51% of it is provided by animal production (INSTAT, 2015). In addition,agriculture is the largest employing sector, accounting for approximately 52% of total employmentof which 60 percent are involved in herding and rearing livestock (MAFCP, 2007). Only 6,5 % of the farm holders are women even thoughwomen are the main labor force in the farms (MARDWA, 2014).

Due to the favorable natural resources, animal husbandry in general and especially cattle (milk and meat) activities have a long tradition in the country, and the value of livestock production is almost 50 percent of the total value of agricultural production (MAFCP, 2012).The dairy sector plays a significant role in the economy of Albania. Milk is one of the most important agricultural products and accounts 22 percent of gross agricultural output (42% by volume of the livestock sector output), and cow milk represents85 percent of it(Foy Reed and Skreli, 2013; INSTAT, 2015; and author's calculations).

Most of the dairy farms are relatively small-scale producers and a small percentage of large producers,which handle a large share of the total dairy herd.The structure of the dairy farms is dominated by the category of holdings with 1-5 cows. This group includes farms with 1.9 heads of cattle on average (only regarding farms that keep cattle), of this the number of milking cows is 1.66 (Gjeçi and Biçoku, 2015). Such farms are producing exclusively for own consumption or limited direct sales.Only about 3372 farms have more than five dairy cows (MAFCP, 2008 and 2012).

According to the statistics provided bythe Ministry of Agriculture Rural Development and Water Administration (MARDWA) and Albanian Institute of Statistics (INSTAT), milk produced by cows increased from 421 000 tons

in 1990 to 1 131 000 tons in 2015. In addition to home production, about 7-10 percent of milk and milk products consumed (calculated as raw milk equivalent) in recent years has been imported.

The average milk yield in 1990 was 1482 litres/cow/year and recorded 2712 litresin 2013 (INSTAT 1991 and 2015), which is considered very low compared to the average of the EU-27, which is slightly higher than 6500 litres per cow per year. The low capital intensity of production has resulted in low productivity, relatively high production costs and low profitability, which in turn prevent the accumulation of capital for financial investment, thus perpetuating the low production and productivity levels on many dairy farms.

The Albanian Government started in 2007 the subsidy/support scheme program, but livestock sector was not part of it. The 2008 program comprised some direct support measures (premium per cow) for dairy/livestock farming and subsidized interest rates of loans for agro-food processing companies. Since 2010 the program has changed from the support related to the number of cows to the milk delivered to the milk processors. In the last eight years livestock has received 1.02 billion ALL (7.5 million EURO), or about 16.3% of total budget expenditures. Within livestock support 45.6% of the expenditures were given to the cattle sector (Musabelliu et al., 2014).

The present study was undertaken to evaluate the gross margin of dairy farms, in two regions (Shkoder and Elbasan) as few studies are conducted in Albania on the milk production profitability. According to several authors (Delgado et al., 2003; Dhuyvetter, 2010) larger producers may survive with low unit profit because of the large volume of business; and returns in dairy farming are deeply determined by variable cost, production cost and the correlations existing between farm size, milk yield, variable cost, total cost and milk price are important to be studied and keep under control by farmers

The information about this survey may help dairy farmers and other stakeholders in the dairy industry to try and improve economic inefficiency.

## MATERIALS AND METHODS

This study was conducted to collect farm data pertaining to revenue and expenses on medium sized dairy farms (in Albania, the farms breeding 11-50 Livestock Unit are considered medium sized ones)and make an economic analysis based on gross margin.The gross margin is calculated as the difference between total income and the total expenses (variable cost). Variable cost includes the cost of: feed (from farm fodder production and feed bought in the market), labor (from a family member), veterinary service, water, electricity, transportation, and miscellaneous.

Data analyzed in this paper were collected through the questionnaire from the representative dairy producers.Twenty-six farms were monitored and interviewed in two regions of Albania (Shkoder and Elbasan). In addition, in each region was conducted a panel discussion with the participation of dairy farmers and livestock experts.

***Data collection*:** A structured questionnaire was used and thefollowing data were recorded:

Income and expenses: (i) Milk yield per cow; (ii) Milk production per farm; (iii) Quantity and price of milk sold; (iv) Quantity and price of meat sold (slaughterweight); (v) Expenses for the fodder production; (vi) Expenses for the animal feed bought in the market; (vii) Expenses for veterinary service and cow's insemination; (viii) Expenses for fuel, electricity, water, trips, lease on the land, and the land tax; (ix) Gross Margin per Cow from sales of milk and meat (GMpCmilk+meat);

(x) Gross Margin per Cow from sales of milk (GMpC milk).

***Data analysis*:** A model in Microsoft Excel programwas developed for data analysis,while the statistical data processing was done with Statgraphics Centurion XVII.

## RESULTS AND DISCUSSIONS

Data on number of cattle and cows per farm, milk yield, Gross Margin per Cow (GMpC milk + meat and GMpC milk), milk price, meat price, and milk cost**,** are summarized in Table 1, as shown below.

Table 1. Technical data

| District | Number of cows | No of Farms | No. of Cows per farm (average) | Milk yield (litre) | GMpC (milk + meat) Euro | GMpC (milk) Euro | GMpC (meat) Euro | Milk cost (Euro/ kg) | Milk price sold (Euro/ kg) | Meat price (slaughter weight) sold (Euro/kg) |
|---|---|---|---|---|---|---|---|---|---|---|
| Elbasan | 15-32 cows | 14 | 21.5 | 5440 | 877.3 | 463.6 | 413.7 | 0.271 | 0.356 | 2.5 |
| Shkoder | 15-25 cows (medium size farms) | 12 | 21.0 | 6000 | 247.1 | 60.0 | 187.1 | 0.30 | 0.31 | 2.2 |

Source: Data from the farm visits and interviews. 1 Euro = 136 Leke

The number of cows of dairy farms for both regions is equal (Elbasan ranged 11-32 cows and Shkodra 11-34 cows). The milk yield of dairy farms in Elbasan (ranged from 4400 to7480 kg/cow) is 9.3 percent lower than the Shkodra farms (ranged from 5100 to 7100 kg/cow). However the milk cost of dairy farms of Elbasan region is 9.7 percent lower than the milk cost of dairy farms of Shkodra region. In addition the milk price in Elbasan region is 14.8% higher than in Shkodra, because in Elbasan several farms are breeding cows of NRF breed, which has a higher milk fat content.

Feeding keeps the highest share within variable cost: 78.1% for Elbasan region and 75.6 % for Shkodra region. This figure is much higher of 58.3 percent published by INSTAT (2012), however the data of INSTAT includes variable and fixed cost.

The returns of the dairy farms came from the sale of milk and meat, however,in Shkoder 2/3 of the incomes is from meat. In Elbasan the income ratio milk:meat is 52.8/47.2 percent.

The Gross Margin per Cow (GMpC), for all the farms monitored, have positive values. The GMpC(milk+meat) ranged from 145.1 to 480.2 Euro/Cow in Shkoder and from 380.9 to 1480.8 Euro/Cow in Elabasan, while the GMpC(milk) in Elbasan had positive value (160.1 to 886.8 Euro/cow) in Shkodra three farms hada negative value (-1.3 to 7.1 Euro/ Cow) and the rest had positive value but lower than of Elbasan (47.8 to 136.8 Euro/Cow).

The income per farm (IpF) in Elbasan is 18862 Euro (ranged from 7332 to 50350) while in Shkoder about three times lower, 5189 Euro (ranged from 2002 to 12485).These significant differences are coming as the result of the price of milk and meat sold, which is higher in Elbasan region (Table 1) and higher daily gain of calves in Elbasan as farmers are inseminating the old cows with beef breed. In addition, several farms in Shkodraare losing money from milk production, as the cost of production is very high. Several studies have found a negative relationship between expenditures for purchase feed per cow and measures of financial profitability (Gloy et al., 2001).A higher milk yield requires a higher production cost, an aspect that farmers should take into consideration and handle in the most efficient way (Popescu, 2014).

This is one of the reasons, emphasized by MARDWA, that the analysis of the competetiveness of agriculture of Albania shows that currently only a small share of farms can compete in the regional market, EU and international level.

Comparing Shkodra and Elbasam dairy farms forIpF(milk+meat) vs. Number of cows/year; and MilkCost (cent/Euro) vs. Milk Yield was used Statgraphics Centurion XVII (Figure 1 and Figure 2).

Shkodra dairy farms: IpF (milk+meat) = -802.259 + 292.52 * Number of Cows. The correlation coefficient equals 0.716304, indicating a moderately strong relationship between the variables.

Elbasan dairy farms: IpF milk +meat = -3195.69 + 921.818 * Number of Cows. The correlation coefficient equals 0.925841, indicating a relatively strong relationship between the variables.

Since the P-value in the ANOVA table is less than 0.05, for both groups of farms, there is a statistically significant relationship between IpFmilk+meat and Number of cows at the 95.0% confidence level.

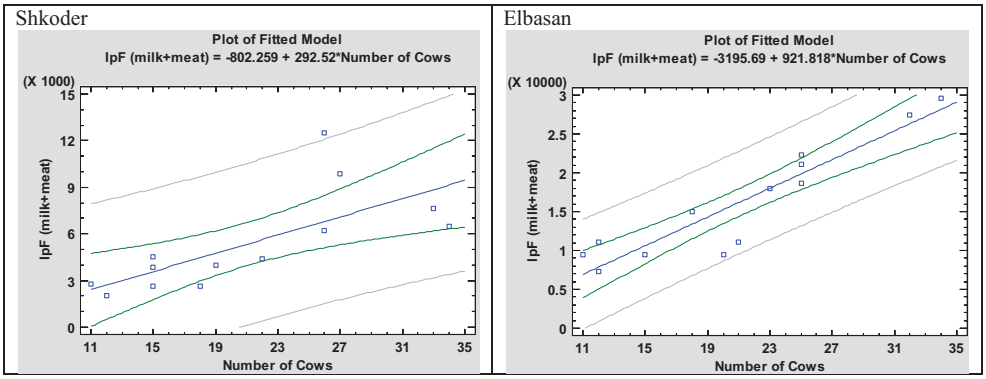


Figure 1. IpF (milk+meat) vs. Number of cows/year

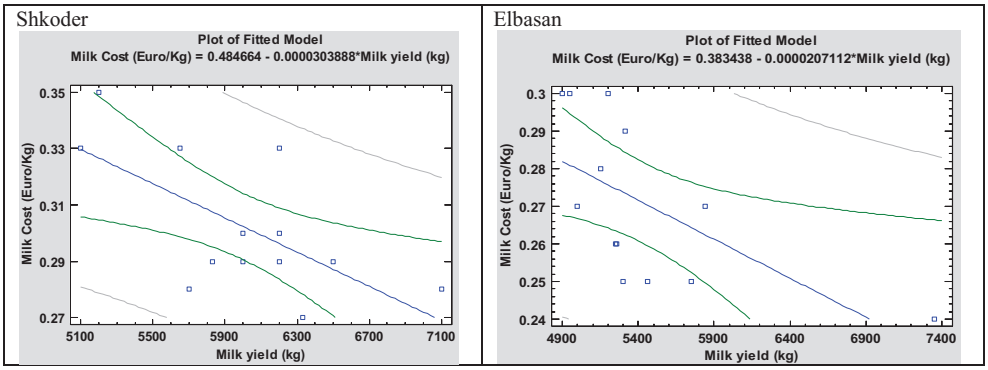


Figure 2. Milk Cost (euro cents/kg) vs. Milk Yield (kg)

Shkodra dairy farms: Milk Cost (Euro/Kg) = 0.484664 - 0.0000303888*Milk yield (kg). The correlation coefficient equals -0.65705, indicating a moderately strong relationship between the variables.

Elbasan dairy farms: Milk Cost (Euro/Kg) = 0.383438 - 0.0000207112*Milk yield (kg)The correlation coefficient equals -0.617142, indicating a moderately strong relationship between the variables. Since the P-value in the ANOVA table is less than 0.05, for both groups of farms, there is a statistically significant relationship between Milk Cost (euro cents/kg) and Milk Yield at the 95.0% confidence level.

These data of our study show that Farms of Elbasan had better results than those of Shkoder for production cost, incomes per farm, incomes per cow, gross margin per cow, milk and meat price.

## CONCLUSIONS

The production and economic results of our study are much better for the dairy farms of Elbasan region than for those of Shkodra region. The milk cost of the dairy farms of Elbasan regions is 10 percent lower and the milk price 13 percent higher than those of dairy farms in Shkodra region.

The Gross Margin per Cow (GMpC), for all the farms monitored, have positive values, but the farms of Elbasan region had 3,5 times higher the GMpC (milk+meat) compare with Shkodra ones.

The returns of the dairy farms came from the sale of milk and meat, however, in Shkoder 2/3 of the incomes is from meat. In Elbasan the income ratio milk:meat is 52.8/47.2 percent.

These significant differences are coming as the result of the price of milk and meat sold, which is higher in Elbasan region. In addition, the cost of production of dairy farms is higher in Shkodra region and as result, several dairy farms are losing money from milk production. Maybe farmers of such category are careless as they are getting money from the grant schemes and other businesses.

The extension service of Shkodra should train farmers to keep the financial record separate for milk, meat, and other crops/businesses. In addition, the extensionists should assist farmers in improving the profitability and long-term viability of their operations.

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# EFFECTS OF VITAMIN ADDITIVE DIETS ON COLONY FOUNDATION SUCCESS IN BUMBLEBEE, *Bombus terrestris*

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***Abstract***

*The effects of vitamin additive diets on colony foundation success in Bombus terrestris were investigated in this experiment. A total of 120 artificially hibernated queens were used. Queens were randomly divided to four groups (30 queens for each group). Queens and their colonies were fed with different diets: standard sugar syrup and normal pollen (group 1), vitamin additive sugar syrup and normal pollen (group 2), standard sugar syrup and vitamin additive pollen (group 3) vitamin additive sugar syrup and vitamin additive pollen (group 4). No significant differences were found in egg laying and colony foundation ratio of queens among the experimental groups. However, vitamin addition to pollen or sugar syrup negatively affected the marketable colony production ratio. Marketable colony production ratios of queens were found 60.00%, 26.66%, 53.33%and 45.00% in four groups, respectively. While feeding with vitamin additive diet affected colony initiation time, other traits such as timing of first worker emergence, timing of gyne (young queen) production, timing of switch point and timing of competition point was not affected. Total numbers of individuals produced in colonies were also determined. Significant differences were found only in terms of total number of young queens (gynes) among the groups, but not total number of workers and males. Results showed that feeding with vitamin additive diet has not positive effect on colony development traits in B. terrestris.*

***Key words****: Bombus terrestris, colony development, feeding, vitamin.*

## INTRODUCTION

Bumble bees which have economic and ecological importance also play a crucial role in the pollination of a wide variety of field, forage and fruit crops, particularly greenhouse crops. Bumblebee pollination reduces pollination labor costs and improves the quality and quantity of crops (Velthuis and van Doorn, 2006). There are about 250 species of bumble bees (Williams, 1998). Currently five species of bumble bees are reared commercially. The main commercially reared species is *Bombus terrestris*. The large-scale laboratory rearing of *B. terrestris* has been promoted around the world since 1985. Annually, more than one million *B. terrestris* colonies are commercially produced by over 30 producers worldwide (Gosterit and Gurel, 2016). The mass rearing of bumblebees includes some stages such as colony initiation, queen and male rearing, mating, and breaking of diapause. These stages are realized in controlled conditions for sustainable rearing. Knowledge and technological possibilities are needed for provide the required conditions and achieve mass rearing (Beekman and van Stratum, 2000; Kwon et al., 2003; Amin et al., 2007).

*B. terrestris* colonies show much variation in the number of workers, males, and gynes (young queens) produced (Duchateau and Velthuis, 1988). Significant variations are also seen in the colony production ratio and colony initiation time. These traits are important criteria in laboratory rearing of *B. terrestris.* Colony development characteristics are affected by different factors such as the genetic structure, environmental conditions, diseasesandparasites, and food quality (Riberio et al., 1996; Cnaani et al., 2000).

*B. terrestris* queens and their colonies are fed ad libitum with freshly thawed pollen which collected by honeybees and sugar solutions (50 Brix) in mass rearing (Riberio et al., 1996; Gosterit, 2016).

It is known that pollen quality affects the colony development. Pollen which containing high content of protein, amino acid and vitamin is preferred for successful rearing (Genissel et al., 2002; Baloğlu and Gurel, 2015).

In honeybees, *Apis mellifera* colonies are fed with supplementary food containing vitamin premix to stimulate the egg laying of queens, improve the brood rearing, obtain more yield, and prevent the diseases and stress (Herbert and Shimanuki, 1978; Kumova, 2000). This study was carried out to determine the effects of feeding with vitamin additive diets on colony foundation success in *B. terrestris.*

## MATERIALS AND METHODS

A total of 120 laboratory reared which mated and hibernated *B. terrestris* queens were used in the study. Four experimental groups of queens (30 queens for each group) were established and queens of each group were fed ad libitum with different diets: standard sugar syrup and normal pollen (group 1), vitamin additive sugar syrup and normal pollen (group 2), standard sugar syrup and vitamin additive pollen (group 3) vitamin additive sugar syrup and vitamin additive pollen (group 4). Vitamin premix which used for supplementary feeding for honeybees (containing 500.000 IU Vitamin A, 50.000 IU Vitamin D3, 500 mg Vitamin E, 1.000 mg Vitamin C, 200 mg Vitamin B1, 250 mg Vitamin B2, 100 mg Vitamin B6, 0,5 mg Vitamin B12, 500 mg, 150 mg Vitamin K for its 100 g) was added to sugar syrup and pollen cake. The proportion of vitamin premix was 5‰ in pollen cake and sugar syrup.

Standard rearing procedure was followed for rear colonies (Gosterit and Gurel, 2016). All queens were placed in starting boxes and allowed to found colonies in a climate-controlled room (27–28 °C and 50 % RH). One callow *B. terrestris* worker was placed next to each queen to stimulate their egg laying. The nests were checked every day and the syrup and pollen were replaced or added when necessary. After the first worker emergence (beginning of social phase), the nests were transferred to the larger rearing boxes and colony development was controlled by daily observation.

Egg laying ratio, colony production ratio and marketable colony production ratio of queens were calculated. Queens that produced more than 10 workers were considered to produce colony, and colonies that reached 50 or more workers were considered to marketable. Developmental traits such as colony initiation time, timing of first worker emergence, timing of gyne (young queen) production, timing of switch point, timing of competition point, and total number of individuals were also determined.

Descriptive statistics of colony development traits were calculated. Data were square-root transformed and tested for normality before analysis. One-way analyses of variance were run to determine the effects of vitamin additive diets on colony development traits (Minitab Statistical Software, Version 16.2.4). Two-proportion *z*-tests were used to compare the percentages of the queens that laid eggs and produced 10 and 50 workers.

## RESULTS AND DISCUSSIONS

In *B. terrestris*, egg laying and colony production ratios of queens are varied within a wide range (Velthuis and van Doorn, 2006; Baloglu and Gurel, 2015). In the present study, effects of feeding with pollen and sugar syrup containing vitamin premix on egg laying ratio, colony production ratio and marketable colony production ratio are shown in Table 1.

Table 1. Egg laying, colony production and marketable colony production ratios of queens (%) (a, b: P<0.01)

| Experimental groups | N | Egg laying ratio | Colony production ratio | Marketable colony production ratio |
|---|---|---|---|---|
| Group 1 | 30 | 93.33 | 73.33 | 60.00[b] |
| Group 2 | 30 | 96.66 | 66.66 | 26.66[a] |
| Group 3 | 30 | 96.66 | 73.33 | 53.33[b] |
| Group 4 | 30 | 86.66 | 66.66 | 45.00[ab] |

Feeding of queens with vitamin additive pollen and sugar syrup affected their marketable colony production ratio (P<0.01) but not egg laying and colony production ratios. In the study, 28 of 30 queens in group 1, 29 of 30 queens in group 2 and group 3, and 26 of 30

queens in group 4 laid eggs. While the queens fed with normal sugar syrup and normal pollen founded more marketable colony (60.00%), queens fed with vitamin additive sugar syrup and normal pollen founded less marketable colony (26.66%). On the other hand, marketable colony production ratio of queens fed with vitamin additive sugar syrup and vitamin additive pollen was found as 45.00%.

In *B. terrestris*, there are three main stage of colony development: colony initiation, switch point and competition point. The hibernated queen lays diploid eggs and produces the first workers in the first stage. The second stage is when the queen switches to laying haploid eggs concurrently with diploid eggs. The third stage (competition point) is characterized by oophagy by the founder queen, egg-robbing and attacks on the founder queen by workers (Duchateau and Velthuis, 1988). Egg-laying by workers also takes place in the third phase (Cnaani et al., 2000). Switch and competition points are determinative for colony life cycle. In the present experiment, no significant differences were determined between the groups in term of production time of sexuals (males and young queens), switch point and competition point (Table 2). Feeding with vitamin additive diets only affected the colony initiation time ($P<0.01$).

Table 2. Developmental characteristics of colonies founded by queens fed with vitamin additive diets (a, b: $P<0.01$)

| Characteristics | Experimental groups | N | $\bar{x}$ ±s.e | min. | max. |
|---|---|---|---|---|---|
| Colonyinitiation time (days) | Group 1 | 28 | 12.64 ± 0.63 [b] | 10 | 24 |
| | Group 2 | 29 | 16.31 ± 1.17 [a] | 10 | 37 |
| | Group 3 | 29 | 12.44 ± 0.62 [b] | 10 | 24 |
| | Group 4 | 26 | 12.81 ± 0.44 [b] | 10 | 17 |
| First workeremergence (days) | Group 1 | 23 | 35.26 ± 1.04 | 24 | 48 |
| | Group 2 | 21 | 36.86 ± 0.92 | 31 | 48 |
| | Group 3 | 23 | 36.10 ± 0.81 | 31 | 45 |
| | Group 4 | 21 | 36.47 ± 0.73 | 31 | 45 |
| Timing of gyneproduction (days) | Group 1 | 16 | 12.78 ± 1.99 | -5 | 22 |
| | Group 2 | 6 | 8.67 ± 3.19 | -5 | 19 |
| | Group 3 | 13 | 11.46 ± 2.15 | -5 | 22 |
| | Group 4 | 12 | 11.50 ± 1.36 | 2 | 22 |
| Switch point (days) | Group 1 | 14 | 0.00 ± 4.24 | -32 | 21 |
| | Group 2 | 10 | -3.30 ± 3.79 | -22 | 11 |
| | Group 3 | 14 | -8.07 ± 3.88 | -36 | 21 |
| | Group 4 | 15 | -3.00 ± 2.94 | -17 | 21 |
| Competitionpoint (days) | Group 1 | 22 | 26.55 ± 1.12 | 18 | 42 |
| | Group 2 | 13 | 27.54 ± 1.58 | 21 | 35 |
| | Group 3 | 21 | 27.10 ± 1.10 | 18 | 35 |
| | Group 4 | 20 | 27.40 ± 1.04 | 22 | 39 |

Table 3. Total number of individual produced in colonies founded by queens fed with vitamin additive diets

| Diets | Colony traits | N | $\bar{x}$ ±s.e | min. | max. |
|---|---|---|---|---|---|
| Standard sugar syrup and normal pollen | Total number of workers | 22 | 108.73 ± 6.51 | 58 | 160 |
| | Total number of males | 16 | 34.94 ± 7.57 | 2 | 110 |
| | Total number of gynes | 18 | 29.44 ± 4.55 | 3 | 60 |
| Vitamin additive sugar syrup and normal pollen | Total number of workers | 20 | 74.90 ± 11.10 | 13 | 183 |
| | Total number of males | 13 | 22.23 ± 5.34 | 6 | 70 |
| | Total number of gynes | 9 | 30.89 ± 5.95 | 8 | 60 |
| Standard sugar syrup and vitamin additive pollen | Total number of workers | 23 | 96.80 ± 11.10 | 8 | 230 |
| | Total number of males | 16 | 30.13 ± 6.24 | 3 | 87 |
| | Total number of gynes | 14 | 63.50 ± 9.59 | 11 | 126 |
| Vitamin additive sugar syrup and vitamin additive pollen | Total number of workers | 20 | 100.00 ± 13.00 | 30 | 225 |
| | Total number of males | 19 | 40.58 ± 7.17 | 3 | 111 |
| | Total number of gynes | 12 | 49.60 ± 11.00 | 2 | 123 |

Different factors such as split sex ratios, worker/larva ratios, food quality and quantity, and the diapause history of the founder queen affect the individual production (Duchateau and Velthuis, 1988; Duchateau et al., 2004; Gosterit and Gurel, 2009; Holland et al., 2013). The high number of workers, males and young queens (gynes) were produced in group 1 (108.73 ± 6.51), group 4 (40.58 ± 7.17) and group 3 (63.50 ± 9.59), respectively (Table 3).

## CONCLUSIONS

Balanced nutrition is the most important dietary factor for effective growth in insects. Previous studies have shown that the origin of the pollen diet might influence the development and the reproductive capacities bumblebee colonies. Pollen which contains the some amino-acids, lipids, or vitamins affects the reproductive capacities of *B. terrestris* queens. It is known that supplementary feeding of honeybees with diets containing vitamin premix increases the some physiological properties. However, our results showed that standard diet is more effective than vitamin additive diets for colony development of *B. terrestris.*

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# THE IMPACT OF A LIGHTING PROGRAM WITH ASYMMETRIC TIME INTERVALS AND THE DENSITY PER UNIT AREA DURING FINISHING AND RESULTS IN SLAUGHTERING YOUTH MALE QUAIL OF BALOTEȘTI POPULATION

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***Abstract***

*In order to study the impact of a lighting program with asymmetric time intervals and different densities during the finishing period of youth quail for meat and slaughter results of youth male quail from population of Balotești in the period 28 – 49 days of growth was organized an experiment on a total of 300 male. At the age of 28 days were divided into cages for youth quail in two batches: the first batch consisting of 150 males has undergone a program of continuous photoperiod lighting with 16 hours per day (lots B and D), and the second batch has undergone a program of lighting with asymmetric time intervals with duration of 16 hours per day (10 hours light + 2 hours dark + 6 hours light + 6 hours dark) (lots A and C). Also, in the two batches was tested the effect of an experimental density of 250 cmp /head comparative with a control density of 125 cmp/head (lots B and D). From researches it is established that the best performances were recorded at the males from the batch A, who were subjected to a lighting program of 16 hours with asymmetric time intervals and was applied the experimental density of 250 cmp/head. The average live weight at the age of 49 days was with 6.47 % higher at batch B comparative with the batch A, with 9.21 % at batch C and with 13.71 % at batch D. Also in the batch A was registered the largest average carcass weight at age of 49 days (with 6.25 % from the batch B, with 11.02 % from the batch C, with 15.85 % from the batch D).*
*Considering that have been registered superior performance at batch A is advisable when quail males are raised in the direction of meat production to be used a lighting program of 16 hours with asymmetric time intervals and a lower density of males in the cage because it significantly influence growth and slaughter performance.*

***Key words :*** *quail, growth, light, density, carcass.*

## INTRODUCTION

In general, many breeders use continuous light in raising youth quail, but it was found that the continuous light may exert a negative effect on growth.
For example, at chickens raised with continuous light has determined a severe physiological stress (Campo and Davila, 2002; Kliger et al., 2005).
In general, the lighting programs asymmetrical fractionated presents an interesting potential for birds whereas help applying food restrictions and allow saving of electricity (Popescu-Micloșanu., 2007).
Density per unit area commonly practiced during the growth of youth of quail is 100 – 150 cmp/head (Velcea M., 2001).

## MATERIALS AND METHODS

Research was conducted on a number of 300 males quail from Balotesti population in the period 42 – 49 days of finishing. From the age of 28 days, males have been divided into four batches according to the following experimental scheme (table 1).
The lighting program with asymmetric time intervals with duration of 16 hours a day had the following structure: 10 hours light + 2 hours dark + 6 hours light + 6 hours dark. The lighting program with continuous photoperiod refers to the cycle of 16 hours light with 8 hours darkness.
The environmental conditions were falling within the limits laid down by the specialty literature.

Table 1. Experimental scheme of the research

| Specification | Density | |
|---|---|---|
| | 250 cmp/head | 125 cmp/head |
| Lighting program with asymmetric time intervals | Batch A | Batch B |
| Lighting program with continuous photoperiod | Batch C | Batch D |

The research was carried out in the framework of the quail holding of Ionita T. Lucian individual enterprise located in the village of Gherghita, Prahova County, Romania.
The data have been processed using Microsoft Excell 2010, and the significance of the differences between the averages has been tested using Student test.

## RESULTS AND DISCUSSIONS

### Evolution of the average growth performance at males from the 4 batches in the period 42 – 49 days

The average live weight at 42 days the highest was recorded at males from the batch A (181.40 ± 2.26), with 4.37 % higher as the males from the batch B, with 10.29 % higher as the males from the batch C and with 11.72 % higher as the males from the batch D. The same situation occurred at the age of 49 days, differences in favour for batch A being something higher. Average gain growth was higher by almost 50 % in the case of batches which has been applied to the density of 250 cmp/head (A and C). The average consumption of compounds feed in the period of 42 – 49 days was higher in the case of batch A (185.00 ± 3.18 g) comparative with batches B, C and D. Specific consumption was almost 50 % lower at batches A and C comparative with batches B and D.

### Slaughter results obtained at the males from the 4 batches during finishing period at 42 days and 49 days

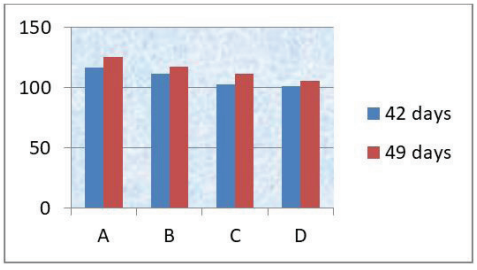


Figure 1. The average weight of the carcase at the 4 batches analysed at ages 42 and 49 days

In general, slaughter results at the age of 42 days (average carcass weight of 116.60 ± 1.80 g and 64.77 % ± 0.56 slaughtering efficiency), and at the age of 49 days (average carcass weight of 125.73 ± 1.73 g and 65.46 % ± 0.66 slaughtering efficiency) were higher in the case of batch A.
If slaughtering efficiency has not experienced any significant differences between the 4 batches, in terms of the average carcass weight were very significant differences at the age of 42 days, and at the age of 49 days (lowest average weight was recorded at the batch D, respectively 101.53 ± 0.78 g at the age of 42 days and 105.80 ± 1.52 g at the age of 49 days).
So at the age of 42 days, and of 49 days were very significant differences in favour of the batch A and the batch B comparative with batches C and D.
The average proportion of blood recorded insignificant variations at all 4 batches and at both ages analysed (at the age of 49 days between 1.85% ± 0.12 at batch B and 2.61 % to batch C and D.
Average proportion of flocs the lowest was recorded in the case of batch C at the age of 49 days (11.84 % ± 0.33), while the highest was recorded in the case of batch B at the age of 42 days (13.02 % ± 0.97), differences between the 4 batches being so insignificant to 42 days and 49 days.
The average proportion of organs and intestines recorded variations between 18.27 % ± 0.34 in the case of batch A at the age of 49 days and 20.82 % ± 0.26 in the case of batch C at the age of 42 days, the differences between the 4 batches being insignificant in both ages analysed.
The average weight of the chest was higher in the case of batch A at the 49 days of age (60.27 g ± 0.81, when was recorded the highest average carcass weight) comparative to the average weight of the chest to the other batches at the two analysed age.

Table 2. Average growth performance over the period 42 – 49 days and the average slaughter results at 42 days and at 49 days at quails from the 4 analysed batches

| Specification | Batch A | | Batch B | | Batch C | | Batch D | |
|---|---|---|---|---|---|---|---|---|
| | 42 days | 49 days | 42 days | 49 days | 42 days | 49 days | 42 days | 49 days |
| Live weight (g) | 181.40 ± 2.26 | 192.60 ± 3.83 aaa | 173.47 ± 2.00 | 180.13 ± 2.19 aaa | 162.73 ± 1.18 | 174.87 ± 2.57 bbb | 160.13 ± 1.14 | 166.20 ± 1.93 bbb |
| Average gain - 42 – 49 days (g) | 11.20 ± 0.85 | | 6.67± 0.35 | | 12.94 ± 0.68 | | 6.07 ± 0.23 | |
| Average consumption of compounds feed (g/head) | 185.00 ± 3.18 | | 175.15 ± 3.44 | | 173. 65 ± 3.75 | | 169.25 ± 2.88 | |
| Specific consumption (g c.f./g gain) | 16.52 ± 0.96 | | 26.25 ± 1.85 | | 13.42 ± 1.15 | | 27.83 ± 1..55 | |
| Weight of the carcase after bleeding (g) | 177.40 ± 2.24 | 188.40 ± 3.47 aaa | 169.00 ± 2.07 | 176.80 ± 2.14 aaa | 158.47 ± 1.20 | 170.33 ± 2.70 bbb | 156.33 ± 1.11 | 161.87 ± 1.96 bbb |
| Weight of the carcase after plucking (g) | 155.33 ± 2.10 | 162.33 ± 3.41 aaa | 147.00 ± 2.51 | 158.73 ± 2.18 aaa | 139.79 ± 1.42 | 149.45 ± 2.84 bbb | 137.07 ± 1.33 | 142.20 ± 1.77 bbb |
| Weight of the carcase after evisceration (g) | 116.60 ± 1.80 | 125.73 ± 1.73 aaa | 111.60 ± 1.74 | 117.87 ± 3.07 aaa | 103.13 ± 0.78 | 111.87 ± 1.89 bbb | 101.53 ± 0.78 | 105.80 ± 1.42 bbb |
| Cutting efficiency (eviscerated shell/live weight) (%) | 64.77 ± 0.56 | 65.46 ± 0.66 ns | 64.51 ± 0.52 | 65.42 ± 0.59 ns | 63.27 ± 0.27 | 63.96 ± 0.41 ns | 63.41 ± 0.33 | 63.66 ± 0.46 ns |
| Weight of the blood (g) | 4.00 ± 0.29 | 4.20 ± 0.29 ns | 4.47 ± 0.26 | 3.33 ± 0.23 ns | 4.27 ± 0.21 | 4.53 ± 0.34 ns | 3.80 ± 0.24 | 4.33 ± 0.25 ns |
| Weight of the flocs (g) | 22.07 ± 1.14 | 21.07 ± 0.40 ns | 22.00 ± 1.62 | 18.07 ± 0.54 ns | 18.73 ± 0.46 | 20.87 ± 0.68 ns | 19.27 ± 0.56 | 19.67 ± 0.57 ns |
| Weight of the organs and the intestines (g) | 29.80 ± 0.52 | 30.47 ± 0.45 ns | 29.00 ± 0.37 | 30.07 ± 0.36 ns | 29.07 ± 0.34 | 29.13 ± 0.79 ns | 27.80 ± 0.52 | 28.33 ± 0.66 ns |
| Proportion of the blood (%) | 2.21 ± 0.17 | 2.18 ± 0.15 ns | 2.58 ± 0.16 | 1.85 ± 0.12 ns | 2.61 ± 0.13 | 2.61 ± 0.21 ns | 2.37 ± 0.14 | 2.61 ± 0.15 ns |
| Proportion of flocs (%) | 12.42 ± 0.59 | 11.19 ± 0.10 ns | 13.02 ± 0.97 | 12.22 ± 0.28 ns | 11.84 ± 0.33 | 12.30 ± 0.45 ns | 12.33 ± 0.38 | 12.15 ± 0.31 ns |
| Proportion of the organs and the intestines (%) | 19.21 ± 0.31 | 18.27 ± 0.34 ns | 19.80 ± 0.38 | 18.96 ± 0.23 ns | 20.82 ± 0.26 | 19.64 ± 0.48 ns | 20.29 ± 0.38 | 19.94 ± 0.45 ns |

Table 3. The weight and proportion of the component parts of the carcase to the males from the 4 batches during finishing period at 42 days and 49 days

| **Specification** | **Batch A** | | **Batch B** | | **Batch C** | | **Batch D** | |
|---|---|---|---|---|---|---|---|---|
| | 42 days | 49 days | 42 days | 49 days | 42 days | 49 days | 42 days | 49 days |
| Weight of the carcase (g) | 116.60 ± 1.80 | 125.73 ± 1.73 aaa | 111.60 ± 1.74 | 117.87 ± 1.07 aaa | 103.13 ± 0.76 | 111.87 ± 1.89 bbb | 101.53 ± 0.78 | 105.80 ± 1.42 bbb |
| Weight of the chest (g) | 53.87 ± 0.79 | 60.27 ± 0.81 aaa | 50.40 ± 0.94 | 53.13 ± 0.13 aaa | 48.67 ± 0.56 | 51.80 ± 1.05 bbb | 46.93 ± 0.63 | 49.40 ± 0.96 bbb |
| Weight of the thighs (g) | 28.20 ± 0.48 | 28.73 ± 0.69 ns | 27.67 ± 0.62 | 27.20 ± 0.66 ns | 26.93 ± 0.66 | 26.80 ± 0.52 ns | 26.47 ± 0.48 | 27.07 ± 0.55 ns |
| Weight of the cord (g) | 23.60 ± 0.64 | 23.92 ± 0.63 ns | 23.06 ± 0.46 | 23.87 ± 0.82 ns | 21.67 ± 0.51 | 22.00 ± 0.36 ns | 23.13 ± 0.69 | 22.47 ± 0.47 ns |
| Weight of the wings (g) | 8.43 ± 0.26 | 9.27 ± 0.25 ns | 8.07 ± 0.12 | 8.20 ± 0.30 ns | 7.67 ± 0.23 | 8.40 ± 0.19 ns | 8.00 ± 0.24 | 7.8 ± 0.17 ns |
| Proportion of the chest (%) | 46.24 ± 0.43 | 47.95 ± 0.67 ns | 45.18 ± 0.55 | 45.13 ± 0.65 ns | 45.19 ± 0.44 | 46.33 ± 0.61 ns | 46.21 ± 0.39 | 46.73 ± 0.85 ns |
| Proportion of the thighs (%) | 24.20 ± 0.26 | 22.92 ± 0.64 ns | 24.89 ± 0.72 | 23.06 ± 0.36 ns | 26.11 ± 0.42 | 24.00 ± 0.43 ns | 26.05 ± 0.39 | 25.65 ± 0.62 ns |
| Proportion of the cord (%) | 20.26 ± 0.49 | 19.11 ± 0.61 ns | 20.70 ± 0.71 | 20.31 ± 0.78 ns | 21.02 ± 0.29 | 19.70 ± 0.32 ns | 22.79 ± 0.37 | 21.29 ± 0.52 ns |
| Proportion of the wings (%) | 7.24 ± 0.20 | 7.41 ± 0.28 ns | 7.24 ± 0.10 | 6.94 ± 0.19 ns | 7.44 ± 0.23 | 7.52 ± 0.14 ns | 7.88 ± 0.24 | 7.34 ± 0.21 ns |

The average proportion of the chest in total carcass was the highest in the case of the batch A at the 49 days of age (47.95 % ± 0.67), and the lowest was recorded in the case of the batch B at the age of 49 days (45.13 % ± 0.65), differences between the 4 batches at the age of 42 days, and at the age of 49 days being insignificant.

The average weight of the thighs was higher in the case of the batch A at the 49 days of age

(28.73 g ± 0.69) comparative to the average weight of the thighs to the other batches on the two analysed age. The average proportion of the thighs in total carcass was the highest in the case of the batch C at the age of 42 days (26.11 % ± 0.42), and the lowest was recorded in the case of the batch A at the 49 days of age (22.92 % ± 0.64), differences between the 4 batches at the age of 42 days, and at the age of 49 days being insignificant.

The average weight of the cord was higher in the case of the batch A at the 49 days of age (23.92 g ± 0.63) comparative to the average weight of the cord to other batches on the two analysed age. The average proportion of the cord in total carcass was the most reduced in the case of the batch A at the 49 days of age (19.11 % ± 0.61), while the highest was recorded in the case of the batch D at the age of 42 days (22.79 % ± 0.37), differences between the 4 batches at the age of 42 days, and at the age of 49 days being insignificant.

The average weight of the wings was higher in the case of the batch A at the 49 days of age (9.27 g ± 0.25 g) comparative to the average weight of the wings to other batches on the two analysed age. The average proportion of the wings in total carcass was the highest in the case of the batch D at the age of 42 days (7.88 % ± 0.24), and the lowest was recorded in the case of the batch B at the age of 49 days (6.94 % ± 0.19), the differences between the 4 batches at the age of 42 days, and at the age of 49 days being insignificant.

In a study carried out in Romania (Elena Popescu-Micloşanu et al., 2008), about slaughter results of males quail at the age of 42 days, maintained at 24 hours light per day, were set the following parameters of carcass quail: 70.86 % carcass efficiency (with skin), the chest of 41.04 % and thighs of 24.3 % from live weight.

In a study conducted in Nigeria on a number of Japanese quail males with age of 10 weeks has been set an carcass efficiency of 67.82%, a proportion of the chest of 34.41% and a proportion of the thighs of 24.02%, which, as a percentage, are similar to those recorded in the "Balotesti" population in this study.

In a study conducted in Iran (Vali et al., 2005) on a number of youth quails aged of 49 days is mentioned an average weight of the carcass of 121.70 g, a carcass yield of 66.24%, an average chest weight of 49.64 g, corresponding to a proportion of 40.84% and a weight of thighs of 27.67 g, corresponding to a proportion of 23.03%.

In a study conducted in Bulgaria (Genchev et al., 2004) on an number of youth quails with age of 35 days has been established a carcass efficiency of 64.5%, a proportion of the chest of 25.38% and a proportion of the thighs of 16.3%, characteristics that are lower than those determined at quails from the population of this experiment at the age of 6 weeks.

## CONCLUSIONS

Live weight at 49 days was higher with 5.82% at batch A comparative with batch B (to which it was applied to a lighting program with asymmetric time intervals lasting 16 hours and a density of 250 cmp/head) and with 4.96% (batches at which has been applied a continuous lighting program with duration of 16 hours and a density of 125 cmp), differences being very significant. Also, in the same direction were recorded differences as regards the gain growth, consumption of compound feed and specific consumption.

Eviscerated carcass weight was with 7.86% higher to the batch A comparative to the batch B and with 6.07% at batch C comparative to the batch D. The weight of the chest was with 11.85% higher to the batch A comparative to the batch B and with 4.63% at batch C comparative to the batch D.

Considering the higher performance recorded at batches A and C is advisable that when quail males are raised in the direction of meat production to be used a lighting program of 16 hours with asymmetric time intervals and a lower density of males in the cage, because they significantly influence the growth and slaughter performance of males.

In order to establish the light impact on the growth at youth quails should be still conducting detailed research.

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# THE EVOLUTION AND PROSPECTS OF THE SHEEP SECTOR IN ROMANIA IN 2000 – 2014 PERIOD

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***Abstract***

*This scientific paper aims to present a detailed analysis of sheep breeding sector in Romania for the period 2000-2014. The data presented here were collected from various official sources and specialized publications and they revealed a decrease in the sheep livestock in the first period analysed(2000 – 2006), followed by a sustained growth until now (2007 – 2014).Meat production was relatively constant because the behaviourof the Romanian consumers towards for this aliment is determined by the Orthodox tradition during the Easter. Milk production had a course inversely with the evolution of the sheep livestock. The wool production, in this period, recorded an almost total loss of economic value.*

***Key words****: livestock, meat production, milk production, sheep, wool production.*

## INTRODUCTION

The sheep, next to cattle and goats, are one of the most important ruminants species exploited for the production of meat and milk. Besides these productions, the sheep also provide the main source of wool worldwide.

Quantitatively, in the year of 2014, the sheep occupied the 4th place in the ranking of the main species producing meat, both worldwide, providing 2.95% of meat production, and in the European Union and Romania with a share of 1.97% respectively 6.45% (Figure 1).

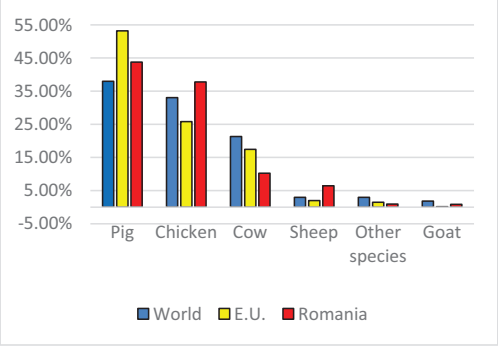


Figure 1. Meat production structure
(FAOSTAT 2016)

In terms of milk production (Figure 2), in 2014, sheep milk accounted for 1.30% of the total milk produced worldwide (4th place), 1.75% of EU production (2nd place) and 12.93% of milk produced in Romania (2nd place).

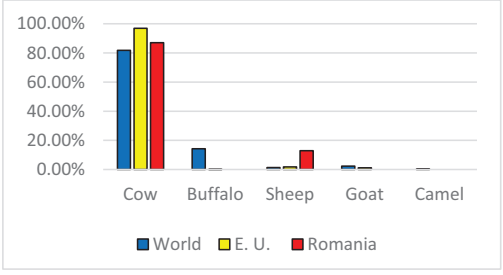


Figure 2. Milk whole fresh production structure
(FAOSTAT 2016)

Both,sheep milk and meat, have outstanding nutritional and organoleptic qualities, and represents an important source of energy, protein with high biological value, and also vitamins and macro minerals, superior to other species of ruminants. In summary, in terms of quality, sheep milk is distinguished by a high content in minerals and vitamins (Ashworth, 2000) and the meat, by taste, aroma and

texture, and also, through a higher digestibility than beef meat (Manole, 2008).
From the point of view of economic efficiency, the sheep have satisfactory levels of production without having special needs regarding the housing or nutrition technologies.

## MATERIALS AND METHODS

To characterize the evolution of sheep husbandry sector in Romania during 2000 - 2014 were collected and processed official data provided by various official sources such as FAOSTAT, Ministry of Agriculture and Rural Development, Statistic National Institute, and publications in the field. The raw data were processed statistic and graphic using Excel, in order to interpret and issue assumptions about the prospects of this sector in Romania to facilitate the elaboration of development strategies.

## RESULTS AND DISCUSSIONS

***Population number evolution.*** For the analysed period, the sheep livestock from Romania had registered an upward trend, being in 2014 by approximately 12.50% higher than in 2000 (Figure 3).

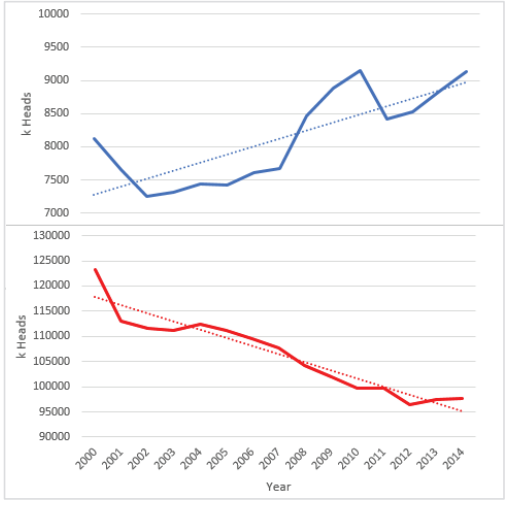


Figure 3. Sheep population evolution in Romania (blue) and in E.U. (red) in 2000 – 2014 period (FAOSTAT, 2016)

The annual growth rate ranged around 1.00%. However, it should be noted that, compared to the maximum livestock registered in Romania, the average livestock of analysed period is only 43.61% (8,128,149 heads towards 18.6 million heads in 1985).
The historically minimum level for sheep population was recorded in 2002, when the livestock was only 7.2 million heads.
In the EU, the evolution of the number of sheep for the same period had a downward trend, decreasing by approximately 26.11% from 123.2 million to 97.6 million heads.

***Milk production.*** It has registered, both in the analysed period, and in general, a relatively constant growth (Figure 4). Thus, the production obtained in 2014 was 673,477 tons, 110% higher than in 2000.

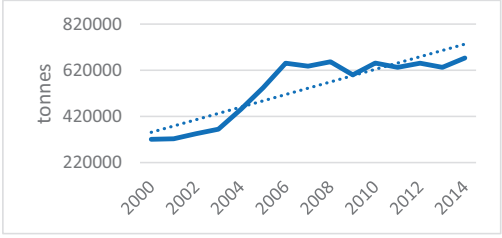


Figure 4. Milk production evolution in Romania (FAOSTAT, 2016)

It is noteworthy that both the maximum and minimum historically levels of production have been recorded in the period under review as follows: historical low was 320,800 tonnes in 2000, and the historical maximum was 673.477 tonnes and it was registered in 2014. Given how the evolution of production correlates with the sheep population dynamics, it can be said that the increase in milk production, with approximately 110.0%, recorded in the period 2000 to 2014, is the effect of genetic improving of the population, which increased during the same period with only 12.50%. Basically, the numerical growth of the population is 8.80 times slower than that of production.

***Meat production.*** In terms of meat production, the Romanian sheep livestock could occupy an important place alongside swine, broiler and beef because, although it capitalize less efficient the feeds, this species can consume some forages, which can't be used by other

species, that are very inexpensive and may graze in inaccessible areas for cattle. At the same time, meat occupies the largest share (approximately 50.00% of the total) in the economy of sheep husbandry (Saghin, 1955, quoted by Drăgănescu, 2006).

Given all this, the national strategy for boosting this sector has the following objectives: the organization of lamb fattening units, increasing sheep meat consumption per capita, which currently stands at approximately 3.30 kg, facilitation of the commercialization of productions and the orientation of the genetic improuvement programs to meat – milk morpho-productiv type sheep (MARD, 2016).

Sheep meat is an important source of protein (18.32 to 20.37%, depending on theage at slaughter) which contains all of the essential amino acids (Taftă, 2008).

Quantitative evolution of the meat production has upward trend (Figure 5) but, for the analysed period, it is not so spectacular, compared to the situation of milk production.

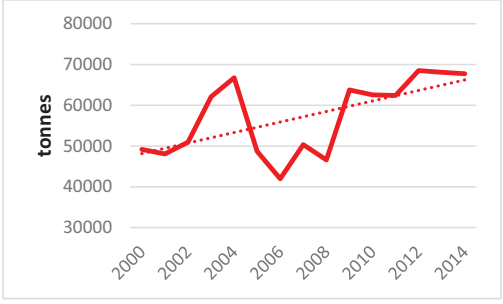


Figure 5. Meat production in Romania (FAOSTAT, 2016)

Compared to the beginning of the period, in 2014, sheep meat production was 37.76% higher, and the average production was 57,183.13 tons (FAOSTAT 2016), which represents only 53.34% of the maximum production, registered in 1990.

The historical minimum was recorded in 2006 and was around the value of 41,993 tonnes (FAOSTAT 2016). The average weight at slaughter was 20.70 kg for the analysed period.

***Wool production.*** Wool is the raw material that stays at the basis of some materials with high economic value but, because of artificial textile industry development and the lack of effective national policies for the efficient capitalisation of this production, the market value was significantly diminished lately.

For the analysed period, this production recorded a slightly upward trend (Figure 6), the amount in 2014 reaching the value of 18,600 tons, with 3.35% more than in 2000. Average production in the period was 19,087 tons.

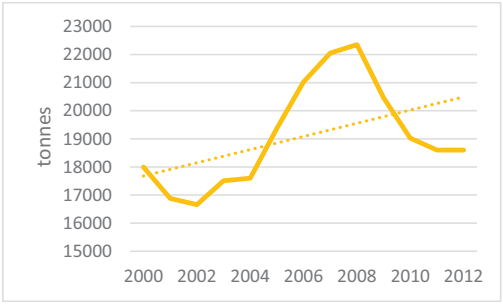


Figure 6. Wool production evolution in Romania (FAOSTAT, 2016)

***The import and export.*** From the sheep productions, Romania has imported and exported live animals, meat and wool.

For the 2013 year, the value of imports was at value of $ 4,024,000 and that of exports at $ 28,094,000 (Table 1).

Table 1. Sheep production import and export (FAOSTAT, 2016)

| SPECIFICATION | IMPORT | | EXPORT | |
|---|---|---|---|---|
| | QUANTITY | VALUE (1000 $) | QUANTITY | VALUE (1000 $) |
| MEAT (tonnes) | 765 | 3,191 | 2,106 | 12,635 |
| WOOL (tonnes) | 183 | 833 | 16,221 | 15,459 |
| Total | - | 4,024 | - | 28,094 |

In the structure of imports, the value of meat occupies the main share (79.30%), followed by the value of wool (20.70%).

Romania exported mainly wool, the value accounting for 55.03% of total exports, while meat occupied a share of 44.97%. The value of exports was about 7 times higher than that of imports.

***Perspectives.*** Given the trends registered for sheep sector in Romania, it can be expected the following:

- Romania will occupy a more important place among big sheep breeder countries such as the

UK, France and Spain. This statement is sustained by the way the sheep populations have evolved in Romania and the European Union in recent years (Figure 3);

- The sheep breeders will specialisation in two main directions: production of meat for the internally and externally consumption, and milk - meat production obtained in traditional systems;
- Increasing the efficiency of meat export by replacing live animals exports with carcasses obtained from genetic improved animals;
- Realization of traditional products, such as cheese specific to some geographical areas, that can be sold at very convenient prices for the sheep breeders;
- The reorientation of farmers to this species.

## CONCLUSIONS

Sheep are one of the most important species that produce milk and meat from Romania.

In the recent years, the evolution of sheep population in Romania registered a significant increase, while in the European Union, herds are declining.

Both, milk and meat production have increased in the analysed period.

Wool production lost in economic importance in recent years.

The value of exports is about 7 times higher than that of imports.

In the future, there are some positive aspects about the sheep sector in Romania by having an appropriate context for the development of sheep farms.

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# THE EEFECT OF COMBINATION OF CRUDE SALIVE GLAND EXTRACTE OF STOMOXYS CALCITRANS (DIPTERA: MUSCIDAE) WITH COLOSTRUM IMMUNOGLOBULIN-G ON IGG SERUM LEVEL OF YOUNG HORSES

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***Abstract***

*This experiment was conducted to evaluate the influence of crude salive gland extracted from Stomoxys calcitrans applied to twelve young horses traditionally maintained. The treatment in study realized by injecting 0.5 ml of SGE to the foals compared to the control group of animals without SGE treatment. Each group was divided into two sub groups received colostrum and the other did notreceived any IgG colostrum. The IgG level was 60 gr/L colostrum and distributed to the foals with 9.6 gr IgG$^{-1}$J. The injection of SGE realized at the first day of experiment. The volume of blood collection was 3 ml through vena jugulaire at the 14th days after SGE injection. As soon as possible after collection, the blood was centrifuged and then its serum was placed in micro-tube to be observed. We did not found interaction of SGE and IgG on imunoglobuline-G serum (P>0.05) while the IgG serum level increased very significantly (P<0.001) as the influence of single treatment of SGE as described in data of A2B1 and A2B2, compared to the control without SGE.In other side this study showed an important relation between entomology and animal husbandry especially to the health care improvement for the young animals by using the antigen substance of the insect (Stomoxys calcitrans).*

*Key words: Entomology, Immunoglobuline,Stomoxys calcitrans.*

## INTRODUCTION

In entomology *Stomoxys calcitrans* (Diptera: Muscidae) flies are well known as stable flies, cosmopolite, economically are pests and able to affect the health of livestock. This insect can transmit infectious diseases in livestock and humans (Graczyk et al., 2001). Such insect can be a serious pest to livestock production, but in other side, this insect has antigen-5 accumulated in salivary gland that able to stimulate the synthesis of IgG antibodies of mammals (Ameri et al., 2008; Campbell et al., 2001).
Today, immunization studies using salivary protein from horn fly (*Haematobia irritans*) demonstrated the ability to reduce the size and to slow the progression of the eggs if this flies itself in immunized animal (Cuop et al., 2004). Ameri et al. (2008) showed that the content of the crude extract of salivary glands (SGE), *Stomoxys calcitrans* dominated by immunoglobulin binding protein or proteins called antigen 5 (AG5) with BM 27 kDa that give immuno-reactive response in cattle.
The problem underlying in this study was, whether SGE *Stomoxys calcitrans* provided immuno-reactive response or not in young horses having placenta of epitheliochorial.
In fact, horses which categorized as animal with epitheliochorial placenta, caused a high risk of failure of passive transfer of immunoglobulins. Therefore the use of crude of SGE could be an alternative solution for immunity enhancement of horses.

## MATERIALS AND METHODS

The collection of *Stomoxys calcitrans* were carried out at the farm of 'Sentrum Agraris Lotta' (SAL). Twelve foals were used which were maintained in traditional farms in Minahasa region in North Sulawesi Indonesia. Identification of antigen proteins realized in Laboratoryof Immunology and Parasitology at the University of Salamanca, Spain.

**Preparation of crude SGE**

Salivary gland extract (SGE) of *Stomoxys calcitrans* obtained according to the procedure Swist et al. (2002). The dissection of *Stomoxys calcitrans* was placed in a Petri dish and placed nice. This dissection was realized under photonic microscope model Meiji EMZ-TR. We removed the head and the abdomen segment then we took carefully the part of salivary gland site in upper of the front legs in thorax, then transferred the salivary glands in the glass vessel filled with 1M Phosphate buffer solution with pH 6 and centrifuged at 5000 rpm for 10 min, then the supernatant obtained as saliva gland extract (SGE). After getting SGE extract, followed by identification with ND-100 spectrophotometer and separation by SDS-PAGE.

**Research procedures**

Each group divided into sub-groups: those who received the colostrums and other group did not received. The IgG content of colostrum utilised was $\pm$60g IgG/L of fresh colostrum. The treatment of colostrum IgG was distributed to animal with a consumption of 160 ml colostrum per hours(≈ 9.6g ofIgG-1J) in the first day after foaling. The treatment of SGE was delivered by injection subcutaneous on first hour of the experiment. Then blood samples were taken approximately 3 ml of venous jugular after 14 days of treatment. The blood was centrifuged immediately and then serum was collected in Eppendorf tube to prepare for the IgG analysis. Data were subjected to two ways ANOVA in completely randomized design in which groups were arranged in 2 X 2 factorial model. Factor A= crude of SGE (A1=0 ml SGE; A2=0.5mlSGE) which 0.5 ml equivalent to 100 µg. Factor B=Colostrum IgG (B1=0 IgG; B2=9.6 g IgG) each with three replication.

**Statistical Analysis**

To evaluate the effect of SGE crude and IgG colostrum on foal serum IgG level, the variance of data obtained statistically analysed according to Zar (1996).

Table 1. Group Treatment of SGE and Colostrum IgG

| Repetition | SGE | | Colostrum IgG | |
|---|---|---|---|---|
| R1 | 0 ml SGE | 0.5 ml SGE | 0 g IgG | 9.6 g IgG |
| R2 | 0 ml SGE | 0.5 ml SGE | 0 g IgG | 9.6 g IgG |
| R3 | 0 ml SGE | 0.5 ml SGE | 0 g IgG | 9.6 g IgG |
| Total | (A1B1) | (A1B2) | (A2B1) | (A2B2) |

**Analysis of Serum IgG**

The blood serum IgG level analysed by using *Single Radial ImmunoDiffusion* method, started with the following procedures: filling well 4 with 15 µl of standard, and then filled the trench (well) gel 15 µl sample of blood plasma. Then moved the plate into the incubator box at a temperature of 30-40$^{o}$C left for about 16 hours so that antibodies diffused in a gel containing anti-IgG antigen, after which the plate was filled with a solution of 2% acetic acid and incubated for one minute. The following stage was the drain plate and the gel rinsed twice using deionized water.

After that for the last time, the plate is filled with deionized water or distilled water and incubated for approximately ten to fifteen minutes. The next step was measurement the IgG content base on the radius precipitation according to the IDBiotech (2009).

**RESULTS AND DISCUSSIONS**

The identification of antigens protein of SGE showed a highest value of antigens proteins in the SGE substance collected from *Stomoxys calcitrans*. Through protein analysis using SDS-PAGE we identified several proteins belonged to SGE of stable fly which were similar results as reported Wang et al. (2009).

The effect of stable fly SGE and IgG colostrum treatment on serum IgG of young horses presented in Table 2.

Table 2. SGE and IgG Colostrum Treatment on Young Horses Serum IgG Secretion

| Factor A (SGE ) | | Factor B (Colostrum IgG) | | | IgG serum level |
|---|---|---|---|---|---|
| | | B1 | 0 g | | |
| | | | $A1B1_1$ | 1 | 2.84 $g.L^{-1}$ |
| | | | $A1B1_2$ | 2 | 3.51 $g.L^{-1}$ |
| | | | $A1B1_3$ | 3 | 2.10 $g.L^{-1}$ |
| A1 | 0µgAg5 | | | | |
| | | B2 | 9.6 g | | |
| | | | $A1B2_1$ | 1 | 4.72 $g.L^{-1}$ |
| | | | $A1B2_1$ | 2 | 4.38 $g.L^{-1}$ |
| | | | $A1B2_1$ | 3 | 3.26 $g.L^{-1}$ |
| | | B1 | 0 g | | |
| | | | $A2B1_1$ | 1 | 6.08 $g.L^{-1}$ |
| | | | $A2B1_2$ | 2 | 5.54 $g.L^{-1}$ |
| | | | $A2B1_3$ | 3 | 6.24 $g.L^{-1}$ |
| A2 | 100µg5 | | | | |
| | | B2 | 9.6 g | | |
| | | | $A2B2_1$ | 1 | 5.66 $g.L^{-1}$ |
| | | | $A2B2_2$ | 2 | 6.92 $g.L^{-1}$ |
| | | | $A2B2_3$ | 3 | 6.77 $g.L^{-1}$ |

The data showed that there had no interaction between treatment factors (P>0.05), although the serum IgG levels tended to be increased after treating with crude of SGE.

This performance linked to Swist et al. (2002) where substances dominated by proteins of 27 kDal, which played an important role as immune-reactive in cattle (Torrand Mangwiro, 2000).

The influence of IgG antibodies for passive transfer of antibodies was not significant (P>0.05) on IgG production in the body of foal. Lowest levels of serum IgG concentrations are obtained in animals without receiving IgGn or SGE injection.

All animals treated with SGE and colostrum IgG tended to have a higher concentration of IgG in the blood serum. This probably caused by the domination of antigen 5 protein as immunogen in SGE (Ueti et al., 2007).

Local foal breed with the traditional maintenance systems provided a very significant effect (P<0.001) on IgG antibodies in foal blood serum.

Antigen 5 treatment according Kresno (1996) classified the primary immune response in serum IgG which would peak 10-14 days after antigen exposure, which in the absence of antigen which cannot happen primary immune.

## CONCLUSIONS

The role of salivary gland extracts, obtained from insect *Stomoxys calcitrans* can be used to improve the IgG antibodies circulated in young horses. However it will be important to continue the research to evaluate the role of the combination of colostrum IgG and SGE on specific IgG antibodies production in young horses.

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# HEAT STRESS IN RUMINANTS

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***Abstract***

*Heat stress is one of the most important environmental stressors that reduce productivity in animal breeding. The productivity of farm animals is significantly reduced due to temperature increases. As a result, significant economic losses occur during the summer months. Temperature humidity index developed according to air temperature and relative humidity relation is a widely used method for determining the effect of heat stress for domesticated animals. The ruminants entering the heat stress have lower feed consumption and consequently lower yields. This review was conducted to determine the effects of negative stressors on ruminants, to show how to reduce the effects of these factors, and to determine what the physiological changes due to temperature stress are.*

***Key words:*** *ruminant, heat stress, yield.*

## INTRODUCTION

High air temperature is one of the important environmental influences that affect ruminant animals as well as to all living organism. Accordingly, environmental conditions include all external factors that affect the growth, development and overall efficiency of the animal. Heat stress is defined as vital functions in animals' bodies and the inability to remove body heat caused by digestion of feeds from the body with increased air temperature. In this case, there are many metabolic problems in the body and loss of yields as well. Heat stress causes crucial economic loss and health problems in all animal species. The air temperatures in which the ruminant animals are most comfortable, depending on the relative humidity in the air are between 5°C and 25°C (Akman, 1998; Yousef, 1985; Mellado et al., 2013; Schüller et al., 2014).

When high relative humidity is added to high ambient temperature, the stress effects become even worse. The temperature humidity index THI (Temperature Humidity Index) is used in predicting the stress intensity that the temperature and the nematum are formed together (Hansen 2007). Thus, during the summer and winter months, the physiological responses of the animals to the environmental stress showed that seasonal hot and cold stress had an impact on the change of blood parameters. Reductions in dry matter consumption by 6-30%, loss of milk yield 15-20%, loss of fertility 40-50%, compulsory slaughter rate is 7-8% due to fertility loss, increase in death rate by 2%, respectively, in the heat stress of ruminants. It is known that this loss is about 325 EUR annual loss per animal (St Pierre et al., 2003).

During the hot stress, feed consumption decreases and therefore all yields decrease. In general, the response to warm stress of animals can be expressed as an increase in respiratory rate, a decrease in heart rate and an increase in serum (Dinçel and Dikmen, 2013).

As a result, optimum environmental conditions must be ensured in order to obtain the highest yield from a healthy animal.

### Factors that Cause Heat Stress in Ruminants

Ruminants are on the one hand trying to reduce the ambient temperature of the environment they are on, while at the same time reducing the heat they have abzorbed. This indicates that the animals are devoted to metabolic body heat at a certain time. Ruminants have a tendency to heat-up or to lose heat depending on some factors. These factors are following;

- The amount of solar radiation,
- The grade of cooling in the night,
- Ventilation and air flow,
- Duration of hot conditions

As a result of these factors above mentioned the air temperatures remain at high levels and many problems arise due to heat stress if the animals are not able to move heat away.

In ruminants, due to heat stress, all yields, especially milk yield, will come down. This is why animals exposed to heat stress will reduce feed consumption and consequently reduce the amount of dry matter consumed. High-yielding animals also have more natural metabolic activity and will be more affected by high temperature stress as they produce more heat (Jones and Stallings, 1999).

Due to the decrease in dry matter consumption in animals causes the weakening of the immune system of the animals, growth retardation in young animals, while the high environmental temperature will also have negative effects on reproduction. These effects are respectively; Premature embryonic deaths and low birth weight; Failure of the oestrus cycle, decline in reproductive efficiency; Service period, prolongation of the calving interval, prolongation of time between first insemination and calving, failure in uterine and hormonal functions, decrease in semen quality and quantity (Özkütük, 1990; Smith et al., 1998; West, 2003).

**Determination of Heat Stress in Ruminants**

Stress determination in domestic animals is rather difficult. Because it is influenced by many factors.

The parameters used to determine the temperature stress are the yield, behavior and health of the animals.

The biggest challenge in measuring stress is variation among animals.

Because the response of each animal to the strase varies according to age, social relations, human-animal relationships, genetic factors. Animals have developed a number of defense mechanisms against changes in internal and external environments.

Abnormal conditions cause stress in animals and try to harmonize with the various responses they have shown. Different stress factors cause the yield to decrease by changing the metabolism in animals (Yorulmaz, 2014).

Behavioral and physiological changes occur in animals during heat stress. These changes are reported below respectively.

Behavioral Changes

- They minimize their movements,
- They prefer cool and shadow places,
- shift feeding behavior to cool times,
- Reduce feed consumption,
- If rough and concentrated feeds are given as alternatives, they prefer concentrate feeds with lower heat increase value,
- They increase water consumption.

**Physiological Changes**

- They try to remove excess heat from the body by evaporation by raising the respiration. In the meantime, removing excess $CO_2$ from the body means that $H_2CO_3$ is removed and the pH of the blood increases.
- H + excretion in kidneys responding to elevation in blood pH is reduced, more HCO- and cations are increased, especially Na excretion.
- In heat stress, animals lose 2/3 of water loss by evaporation, 1/3 by breathing from the body. Sweating increases in hot conditions. However, cattle have up to 10% sweating capacity. In an overwhelming amount of K removes from the body.
- The rate of reticulo-rumen movements and the abandonment of the digestive system of consumed feeds decreases. At the same time the total volatile fatty acid production in the rumen is reduced. The molar ratio of acetic acid in volatile fatty acids increases.
- Blood flow to digestion and other internal organs is reduced and blood flow to the skin surface increases.
- Urine discharge increases.

**Detection Methods of Heat Stress in Ruminants**

Determination of heat stress in animals can be determined by physiological and biochemical methods.

Physiological methods can be determined by various measuring instruments generally while biochemical methods can be determined by detecting the level of hormone in the blood.

**Body temperature regulation**

Sweating and breathing are less important to regulate body temperature so that evaporation

becomes more important in the excretion of excess heat in the body. In animals exposed to prolonged hot weather, they have developed some mechanisms to reduce body temperature. An example of these mechanisms is the reduction of heat production and feed intake. When the ambient temperature rises above 36°C, body temperature is distributed to the ears and feet which constitute approximately 23% of the body surface (Young 1983). Body temperature can be monitored by some physiological measures such as respiratory rate and rectal temperature. The effects of the environmental temperature on the respiratory, pulse rate and rectal temperature is given (West, 2003).

**Pulse Rate**

The number of pulses generally indicates the balance of blood circulation with metabolic status. The heat loss caused by the diffusion is realized by the blood flow. However, the number of pulses is increased due to the increase in blood flow under the skin when the temperature is high according to the studies carried out. Some researchers reported that there is a difference between races in terms of skin cooling rate. At very high temperatures the pulse rate may be reduced due to a decrease in the rate of metabolism. As a matter of fact, the ambient temperature is reported to be higher when the temperature is increased from 20°C to 35°C (Fuquay, 1981). Acid and propionate absorption is greater than 20 ° C, as well as $O_2$ carried at high temperature (at 35°C). The increase in the amount of $O_2$ taken through the blood can result heat increase in hot regions in ruminants. Hyperthermia, in other words, a significant increase in heart rate was observed when the rectal temperature rose above 42°C (Fuquay, 1981; Marai et al., 2007).

**Respiratory Rate**

Respiratory rate is an important indicator of stress. In domestic mammals, breathing is achieved by removing $CO_2$ from body tissues by replacing the body tissues with $O_2$ in order to remove the moisture from the body under normal conditions and to prevent hypothermia at high ambient temperature. Sheep lose about 20% of the body's heat produced by the respiratory tract at neutral ambient temperature (12°C). Moisture loss increases at high ambient temperature (35°C) and this loss accounts for 60% of total heat loss. In ruminants, respiratory rate is higher in summer than in winter (Srikandakumar et al. 2003).

**Rectal Temperature**

Rectal temperature is a measure taken in the rectal area to measure the internal temperature in animals. For example, in sheep, they try to keep it in a fairly narrow range under unfavorable climatic conditions and try to keep it stable to constant body temperature. Rectal temperature varies from 38.3 to 39.9°C under normal conditions. It is important that the ambient temperature rise from 18° C to 35°C in terms of rectal temperature in sheep. Adverse effects occured when the rectal temperature rises above 42°C. Exposure to elevated temperature is handled by the Temperature Moisture Index (SNI) at which temperature and humidity are assessed together. Changes in the rectal temperature during the year can be observed (Srikandakumar et al., 2003).

**Thyroid Hormones (T3, T4) and Cortisol Levels in Blood**

Stressors cause changes in the physiology of animals. In Stressed animals, cortisol release, increased body heat and pulse rate as well as the influence of many hormones (Roussel et al., 2006).

In addition to cortisone, thyroid hormones are sometimes used to identify stress. Thyroid hormones (Triiodothyronine = T3, Thyroxine = T4 and TSH) act on target tissues to stimulate oxygen production and heat production in every cell of the body. They change basal metabolism levels by providing more glucose to the cells, stimulating protein synthesis, increasing fat metabolism, circulating and activating the nervous system. Releasing of thyroid hormones is reduced in stress situations such as very Hot and humid weather, lightness, pain, excitement, bleeding, trauma. (Polat and Dellal, 2008).

According to SNI (Temperature Moisture Index) values, T3 and T4 hormone levels decreased with the increase of SNI values. This decline in temperature stress conditions is mainly due to the slowing of carbohydrate metabolism and thus a reduction in energy

(heat) production in order to keep animals' body temperatures (Koluman-Darcan et al., 2013).

**Precautions to Reduce Heat Stress**

Precautions that can be taken to reduce heat stress must be practical and economical before anything else.

- Selecting an appropriate breed
- Providing shelter space in Animal barn
- Control of water quality and temperature
- Addition of vitamins and minerals to foods
- Restrictions on carriage
- Rectal temperature monitoring
- Feeding (Proper Ration Preparation)
- Ventilation and shower effect (Evaporative cooling)
- Change of feeding hours
- To take care of the cleanliness of the barns
- Wetting of roughage
- Cold Water Supply
- Use of Some Feed Additives

## CONCLUSIONS

The most important climatic stress source in animals is temperature and proportional humidity, and these two factors cause different effects on the ruminants. According to this, when the temperature is high, the humidity of the air is high, which makes it very difficult for the animals to have balanced body heat. The adverse effects of environmental conditions are more important in intensive production conditions, especially in high yielded animals.

The highest yield expected those animals may be possible if appropriate environmental conditions are provided. In the summer months when temperature stress is observed, careful regulation of rations will increase the profitability of the enterprise because it prevents less damage to the farm animals from the heat as well as prevents losses in production.During these periods, some arrangements should be made to prevent the negativity of the factors that cause the heat stress.

As a result, optimum environmental conditions must be met in order to obtain the highest yield from ruminants

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# PHYSICAL CHARACTERISTICS OF THE MUSCLE *Biceps femoris* AND *Longissimus dorsi* OF MALE AND FEMALE NEW ZEALAND WHITE CROSSBREED RABBITS

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***Abstract***

*In general muscles of male rabbit is more active than the female one, the function in each carcass also more active, suspected there is a different characteristic of muscles in each of male and female rabbit carcass. The purpose of this research is to know the physical characteristics of muscle Longissimus dorsi and Biceps femoris of New Zealand White Crossbreed Rabbit. Research using the Design Pattern of nested (nested), muscle Longissimus dorsi and Biceps femoris is nested within the sex of males and females. Data were analyzed with analysis of variance followed by Duncan's multiple range test. Variables observed were: muscle acidity (pH), the water holding capacity, and the color, i.e. the brightness (L), reddish (a) and yellowish (b). The results showed that gender has no effecy on pH, Water Holding Capacity and color of muscle of New Zealand White crossbreed rabbit. Acidity (pH), Water Holding Capacity and color of Biceps femoris and Longissimus dorsi of male rabbit had no different, similarly on female rabbit.*

***Key words****: rabbit, sex, muscular, physical.*

## INTRODUCTION

The quality of meat is a broad term which includes the characteristics of chemical, physical and sensory. Some physical characteristics that can be measured are color, pH and water holding capacity. These characteristics are interconnected, so that the investigations needs to be conducted simultaneously to evaluate the meat quality (Hernandez et al., 1998).

Acidity (pH) of the rabbit meat after 24 hours of cutting on *Longissimus dorsi* mucle was ranged from 5.6-5.7, and bisep femoris ranged from 5.7 (Pla et al., 1998). Acidity (pH) of *Longissimus dorsi* muscle on male animal (5.64) higher than female animal, which is 5.54 (Pla et al., 1998). While the pH of *Biceps femoris* muscle is not much different, which is 5.74 in female rabbit and 5.72 in male rabbit (Yalçin et al., 2006). Result of the research of Barron et al. (2004), that the pH of *Longissimus dorsi* muscle of male rabbit is 6.3 and female rabbit is 6.1, then the pH of *Biceps femoris* of male rabbit is 6.1 and female rabbit is 6.0.

The meat color is an important visual characteristic of it first impression. The meat color varies according to carcass part, and influenced by many factors, including feed, species, race, age, sex, stress (activity level and muscle type), pH and oxygen (Bizkova and Tumova, 2010; Soeparno, 2009).

Water Binding Capacity by meat protein or Water Holding Capacity (WHC or WBC) is ability of meat to bind its water or the water which is added during external force influence, for example meat cutting, heating, grinding, and pressure on acidity (pH) is higher, from the isoelectric point of meat proteins 95.0-5.1) but below pH 7-10, has better water holding capacity and less drip (Soeparno, 2009).

## MATERIALS AND METHODS

Sample used *Longissimus dorsi* (LD) and *Biceps femoris* (BF) muscle from rabbit meat which was refrigrated in 6$^{o}$C for 22-24 hours, age cut 12 weeks from 24 female rabbits and 24 male rabbits.

Measured variable:

Meat Acidity (pH)

Measurement of pH meat is done by using pH-meter.

a. Meat color
   Measurement of meat color is done by using tool Chromameter Minolta CR-

400. The measurements result are displayed in scale L* (brightness), a* and b* (redness and yellowness).

b. Water Holding Capacity (WHC)
Water Holding Capacity is determined by the method of Hamm (Soeparno, 2009)

$$\text{Water Holding Capacity} = \text{KA\%} - \frac{\text{mg } H_2O}{300 \text{ mg}} \text{ x } 100\% \quad (10)$$

mg $H_2O$: amount of free water that comes out of the meat after pressing in miligrams.
KA%: the percentage of water content.
300 mg: the number of samples of raw meat in milligrams.

Experimental Design:
The design used nested, muscle type (*Longissimus dorsi* (LD) and *Biceps femoris* (BF) nested in gender (female and male), with 4 groups of weaning weight (400-700) as replication.

## RESULTS AND DISCUSSIONS

The result of the research of physical characteristic of rabbit meat (pH, water holding capacity, and color) on male and female rabbit is listed in Table 1.

Table 1. Influence of sex toward physical characteristic of meat rabbit.

| Measurament Variabel | Sex | |
|---|---|---|
| | Male | Female |
| Ph | 5.24 a | 5.32 a |
| Water Holding Capacity | 58.5 a | 59.77 a |
| Colour | | |
| A | 14.56 a | 13.35 a |
| L | 62.82 a | 63.26 a |
| B | 36.04 a | 37.02 a |

Means for the same item in the same column with the same letter was not significantly different.

The data in Table 1 indicates that pH meat of male rabbit higher that the female. This is due to male rabbit more active than the females, so the glycogen reserves in male rabbit is lower. Decrease in pH causes the water which is associated with muscle protein is running out leaving the muscle fibers and lower water holding capacity. Water Holding Capacity will be even better if the pH value which is generated further away from the isoelectric point of meat proteins ie 5.0 to 5.1, but still below pH 7 to 10 (Soeparno, 2009).

Water Holding Capacity of meat of female rabbit is higher than the male one. This indicates that the meat of female rabbit has the ability to bind water better than the male rabbit. Brightness of the meat of male rabbit is better than the female's, but the color of redness and yellowness of female rabbit meat is higher than the male's.

Statistical analysis showed that the acidity (pH), water holding capacity and meat color of male and female rabbit was not significantly different. This is due to the treatments which was given to the rabbit before slaughtering in each treatment were the same, so the glycogen reserves in the muscle remain the same, so that in the process postmortem glycolysis produce the same ultimate pH. The same pH will cause the same water holding capacity. The color difference in male and female muscles are not significant, because the rabbit was slaughtered in young age (12 weeks). Meat with good quality must be brighness in color, red or pink but not brown, purple or greyish (Wariss, 2000).

The physical characteristics of muscles (pH, water holding capacity, dan meat color) male and female rabbit are listed in Table 2.

A decrease in pH muscle after withering largely determined by the rate of postmortem glycolysis and glycogen reserves of muscle and a normal ulimate meat pH is between 5.4-5.8 (Soeparno, 2009). pHu range ( pH ultimate) in rabbits were based on the muscle's location according to Hullot and Ouhayoun (1999) ranged between 5.4-6.4. While Kowalska et al.(2011) obtained pH 24 hours after cutting the group of easily stressed rabbit by 5.22 while pH value of a group of rabbits which can cope with stress is 5.70. Value of ultimate pH (pH 24 hours after slaughtering) obtained in this study can be categorized as low, ranging from 5.22 to 5.31.

Table 2. Effect of muscle type in gender against physical characteristics of meat rabbits

| Measurement Variabel | Muscle Type | | | |
|---|---|---|---|---|
| | Male | | Female | |
| | BF | LD | BF | LD |
| pH | 5.22 a | 5.26 a | 5.33 A | 5.31 A |
| Water Holding Capacity | 57.51 a | 58.76 a | 61.24 A | 58.30 A |
| Colour | | | | |
| A | 14.75 a | 14.37 a | 13.21 A | 13.49 A |
| L | 62.80 a | 62.69 a | 64.08 A | 62.08 A |
| B | 8.87 a | 9.28 a | 8.45 A | 8.56 A |

Means for the same item in the same column with the same letter was not significantly different.
BF: *Biceps femoris*
LD: *Longissimus dorsi*

Meat color is a key determinant, because it is the only criteria that can be used by consumers to purchase meat.
The characteristic color of the meat depends on the species and type of muscle.
Color of rabbit meat can be affected indirectly by environmental factors associated with the production conditions (Dal Bosco et al., 2002), stress before slaughtering (Maria et al., 2004) and muscle activity in living animals (Dalle Zotte et al., 2009).

## CONCLUSIONS

Sex had no effect on pH, water holding capacity and muscle color of breed of New Zealand White rabbits. Acidity (pH), Water Holding Capacity and color of *Biceps femoris* and *Longissimus dorsi* of rabbits male were no different, similarly on the female rabbit.

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# STUDY REGARDING ANIMAL ORGANIC FARMING IN ROMANIA – CURRENT STATUS AND TRENDS

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***Abstract***

*Organic farming is a sector of great perspective for Romania, due to the fact that it benefits appropriate conditions for the development of such a system of agriculture in comparison with economically developed countries, where super intensive agricultural technologies are used extensively, based largely on chemical fertilizers and pesticides. In the present study, based on an analysis about organic animal production, performed for 2015-2016 there are apparent large possibilities of having profit from this type of agriculture in the enlarged European Community. Talking about economic purpose animals, the principal objective that organic farming has in view is their genetic foundation, improvement and preservation. At the same time, this system aims to improve the condition of life of the animals, by providing not only their physiological needs but also the principles of human care, excluding obviously excessive concentration and permanent indoor keeping (Ilie, 2016). In the past two years, in Romania, organically certified farms have seen a genuine territorial expansion in relation to the previous years and the conventional farms.*

***Key words****: animal, organic, farming, Romania, trends.*

## INTRODUCTION

Human nutrition is the basis of its construction. Wellbeing, balance and health of each human being are in close touch with food. Developing appropriate feeding brings together with other correct behaviour items a healthier life for everyone. Although organic farming was considered forever the sole source for living food, man has been detached from the concept to the detriment of abundance and mostly profit. According to recent studies, Romania has made substantial progress with regard to cultivation in agricultural system, the number of agri-environmentalists operators, domestic agricultural products, but also in respect of the export of agricultural products and organic food (I.F.O.A.M, 2015).

This type of agriculture 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 (Ion, 2004; Ilie, 2013; Gonciarov, 2014). Animals used in organic animal farms must meet a number of features such as: being of indigenous breeds with high resistance to environmental factors, with robust constitution, able to be grown in extensively or semi intensively system, having very good reproductive indices, high fertility and fecundity (Tapaloaga, 2014; Ghimpeteanu, 2015).

## MATERIALS AND METHODS

Organic agriculture is based on observing the laws of life which consists in not direct feed plants with soluble fertilizer, but with living beings in soil that develops and delivers all that plants need. (Aubert, 1970).

Most experts, relying on the provisions of Regulation EC 834/2007 of the Council and Regulation of the Commission of Agriculture 889/2008 argue that organic farming has the same definition with biological or organic farming (Alexandrescu, 2011; Chaoui, 2008). Also, some theorists believe agro ecology and agricultural ecology have the same meaning: agro ecology or agricultural ecology is the branch or discipline of ecology that deals with the multilateral study, particularly under a productive report of influences of environmental factors on plants and domestic animals (the so-called agricultural self ecology), as well as of structures and dynamics research on the of agro ecosystems (agricultural self ecology) (Bucata, 2004; Carvalho, 2006). In general, the objectives of organic agriculture are subject to the

sustainable development of agri-environmental systems (Davidson, 2005; Charlier, 2006; Cioruta, 2011).

When we relate to economic purpose animals, as the main objective on which organic farming has in view, it is the genetic fund, targeting respectively its improvement and preservation. At the same time, this system aims to improve the condition of life of the animals, by providing not only physiological needs but also the principles of human care, excluding obviously excessive concentration and permanent indoor keeping. In Romania, in the present study, based on a SWOT analysis about organic animal production, performed for the 2015-2016 period, there are apparently large possibilities of removing the profit from this type of agriculture in an enlarged European Community (CertRom, Time Foundation).

## RESULTS AND DISCUSSIONS

In Romania, organic farming covers approximately 450 thousand hectares, just over 3% of the total agricultural area of 14.7 million hectares. Over 70% of this area is covered by the cereal crops and industrial plants for processing, especially in flat areas from Satu Mare, Arad, Timiș and Dobrudja (CertRom, 2014, MARD, 2015).


Figure 1. Most used areas in cattle organic farming in Romania, conformingly CERTROM/2016 study

According to some statistics of the Ministry of Agriculture, much of the organic production, 80% is exported. Main trading partners of the ecologic sector are Germany, Austria, Italy, Netherlands, Switzerland and Denmark, which buy raw materials from Romania and then devolved to sell processed products.

The number of companies operating in this sector has risen to about 3800 units in 2007 to approximately 27000 in 2012, increasing by 7 times, but of these only 200 do marketing deals and only 100 process organic products.

According to some data from the Ministry of Agriculture and Rural Development, ecological products market in Romania is in the full process of development and diversification. The production of organic processed or semi-processed products was small and poorly diversified: 480 tons of sheep cheese, 268 tons of Schweitzer, 330 tons of yellow cheese, 600 tons of honey, 100 tons of canned vegetables and fruits. During 2007-2012, the organic cultivated land increased by 3.5 times, and the number of operators in the organic farming has grown from 3834 to 26736. In Romania, organic farming increases from year to year in a weighted average rate of 23 percent, according to the data from the Ministry of Agriculture and Rural Development.

If the area under cultivation in the 2010 eco-system does vastly exceeded 200 thousand hectares, in 2015, the area under cultivation in organic farming in exceed 290 hectares, and according to official data, in the past 5 years the area under cultivation in organic farming increased by 37%. Interestingly, Romania has come in a short span of time among the first 20 organic exporters worldwide. We export grains, mushrooms, berries, nuts with a value of 75 million euro in 2006 (Agricultural News Magazine, 2015).

Livestock production farms in Romania represents an activity connected with the land, in which animals must have access to the areas in the open air, and their number per unit area must be limited to ensure the integrated system between livestock production and crop production (Dobre, 2009; Ilie, 2011; Nitu, 2012). To avoid soil erosion and excessive grazing, the number of animals depending on the area available is determined. In organic farming, animals must be reared according to the rules laid down in the detailed rules. In choosing species and breeds of livestock production, it is taken into account their ability to adapt to local conditions, resistance to disease and vitality (Tapaloaga, 2014).

In the context of an organic farm, pasture/arable land ratio is very important, and represents the basis for ruminants management properly, also very important is the ratio legumes and grasses for proper fertilization, cows and sheep are the species to be exploited most, because through their metabolism returns nutrients in the soil. Poultries and pigs consume cereals and their ratio must have a high energy and protein concentration, so it is difficult for such a formulated ration based only fodder cultivation to be done, being necessary to add supplements.

According to CertRom, if in 2015 it was registered a number of 15627 heads of animals reared in organic farming or in conversion, year 2016 comes with a significant increase of this number with about 5620. This means that Romania is still booming on this programme.

Table 1. Organic certified and in conversion animals in 2015, conformingly CertRom

| County | Species | $C_1$ | $C_2$ | $C_3$ | Organic | No of animals/ farm |
|---|---|---|---|---|---|---|
| Botoșani | bovine | | | | X | 5 heads |
| Botoșani | bovine | | | | X | 6 heads |
| Buzău | bovine | | | | X | 9 heads |
| Buzău | sheep | | | | X | 62 heads |
| Caraș-Severin | bovine | | X | | | 128 heads |
| Caraș-Severin | bovine | | | | X | 54 heads |
| Caraș-Severin | bovine | X | | | | 179 heads |
| Caraș-Severin | bovine | | | | X | 588 heads |
| Caraș-Severin | bovine | X | | | | 1 head |
| Caraș-Severin | bovine | | | | X | 15 heads |
| Caraș-Severin | goats | | | | X | 43 heads |
| Caraș-Severin | sheep | | | | X | 5896 heads |
| Caraș-Severin | sheep | | | X | | 354 heads |
| Caraș-Severin | sheep | | X | | | 3142 heads |
| Caraș-Severin | sheep | X | | | | 4223 heads |
| Caraș-Severin | sheep | X | | | X | 1870 heads |
| Caraș-Severin | sheep | | | | X | 112 heads |
| Cluj | goats | | | | X | 129 heads |
| Dolj | goats | | X | | | 160 heads |
| Dolj | sheep | | X | | | 190 heads |
| Gorj | bovine | | | | X | 4 heads |
| Hunedoara | bovine | X | | | | 2 heads |
| Hunedoara | bovine | | | | X | 41 heads |
| Hunedoara | sheep | X | | | | 10 heads |
| Iași | bovine | | | | X | 48 heads |
| Iași | goats | | | | X | 120 heads |
| Iași | sheep | X | | | | 6 heads |
| Iași | sheep | | | | X | 266 heads |
| Neamț | bovine | | | | X | 10 heads |
| Suceava | bovine | X | | | | 12 heads |
| Suceava | bovine | | | | X | 290 heads |
| Suceava | bovine | | X | | | 3 heads |
| Suceava | bovine | | | | X | 122 heads |
| Suceava | bovine | | X | | | 34 heads |
| Suceava | bovine | X | | | | 49 heads |
| Suceava | bovine | X | | | | 4 heads |
| Suceava | goats | | | | X | 155 heads |
| Suceava | sheep | | | | X | 49 heads |
| Suceava | sheep | | | | X | 148 heads |
| Suceava | sheep | | | | X | 126 heads |

Table 2. Organic certified and in conversion animals in 2016, conformingly CertRom

| County | Species | $C_1$ | $C_2$ | $C_3$ | Organic | No of animals/ farm |
|---|---|---|---|---|---|---|
| Botoșani | bovine | X | | | | 1 head |
| Botoșani | bovine | | | | X | 2 heads |
| Botoșani | bovine | | | | X | 4 heads |
| Botoșani | sheep | | | X | | 351 heads |
| Botoșani | sheep | | | | X | 627 heads |
| Buzău | bovine | | | | X | 8 heads |
| Buzău | sheep | | | | x | 62 heads |
| Caraș-Severin | bovine | X | | | | 47 heads |
| Caraș-Severin | bovine | | | | X | 484 heads |
| Caraș-Severin | bovine | | | | X | 5 heads |
| Caraș-Severin | bovine | X | | | | 52 heads |
| Caraș-Severin | bovine | | | | X | 12 heads |
| Caraș-Severin | bovine | X | | | | 287 heads |
| Caraș-Severin | bovine | X | | | | 4 heads |
| Caraș-Severin | bovine | | | | X | 5 heads |
| Caraș-Severin | bovine | | | | X | 3 heads |
| Caraș-Severin | bovine | X | | | | 156 heads |
| Caraș-Severin | goats | | | | X | 37 heads |
| Caraș-Severin | goats | | | | X | 67 heads |
| Caraș-Severin | sheep | X | | | | 6166 heads |
| Caraș-Severin | sheep | | X | | | 1226 heads |
| Caraș-Severin | sheep | | | X | | 5132 heads |
| Caraș-Severin | sheep | | | | X | 3861 heads |
| Cluj | goats | X | | | | 20 heads |
| Cluj | goats | | | | X | 97 heads |
| Dolj | bovine | | X | | | 4 heads |
| Dolj | goats | X | | | | 106 heads |
| Dolj | sheep | X | | | | 88 heads |
| Hunedoara | bovine | | X | | | 41 heads |
| Hunedoara | bovine | | | | X | 5 heads |
| Iași | bovine | | | | X | 22 heads |
| Iași | bovine | | | | X | 31 heads |
| Iași | bovine | | | | X | 4 heads |
| Iași | goats | X | | | | 248 heads |
| Iași | goats | | | | X | 41 heads |
| Iași | sheep | X | | | | 46 heads |
| Iași | sheep | | X | | | 11 heads |
| Iași | sheep | | | | X | 110 heads |
| Neamț | bovine | | | | X | 10 heads |
| Vaslui | bovine | X | | | | 15 heads |
| Vaslui | goats | X | | | | 27 heads |
| Vaslui | sheep | | | | X | 223 heads |
| Vaslui | sheep | X | | | | 80 heads |
| Suceava | bovine | | X | | | 9 heads |
| Suceava | bovine | X | | | | 61 heads |
| Suceava | bovine | | | | X | 425 heads |
| Suceava | bovine | X | | | | 34 heads |
| Suceava | bovine | | | | X | 239 heads |
| Suceava | bovine | | X | | | 21 heads |
| Suceava | bovine | | | | X | 3 heads |
| Suceava | goats | | | | X | 161 heads |
| Suceava | sheep | | | | X | 218 heads |
| Suceava | sheep | | | | X | 230 heads |
| Suceava | sheep | X | | | | 18 heads |

Table 3. Organic certified livestock, conformingly CertRom (Romania, 2015 )

| Nr. crt | Species | Number | UM |
|---|---|---|---|
| 1 | Bovine | 1133 | heads |
| 2 | Sheep | 6533 | heads |
| 3 | Goats | 407 | heads |
| | Total | 8073 | heads |

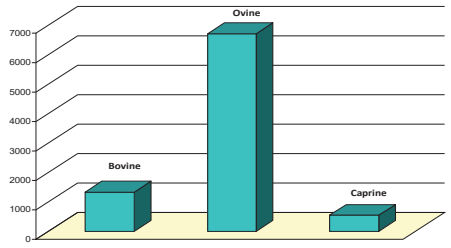


Chart 1. Organic certified livestock, conformingly CertRom (Romania, 2015 )

Table 4. Organic certified livestock, conformingly CertRom (Romania, 2016 )

| Nr. crt | Species | Number | UM |
|---|---|---|---|
| 1 | Bovine | 1334 | heads |
| 2 | Sheep | 6715 | heads |
| 3 | Goats | 543 | heads |
| | Total | 8592 | heads |

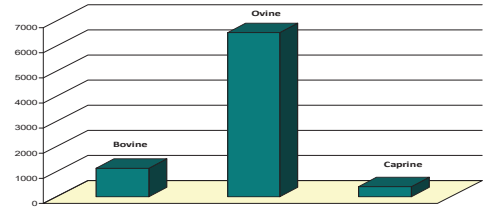


Chart 2. Organic certified livestock, conformingly CertRom (Romania, 2016 )

If between 2001 and 2010 the products obtained from animals reared in organic agriculture shared less than 1% Romanian people preferring traditional products because of the low prices in comparison with those of organic products, in recent years it has highlighted the emergence of increasingly more frequent small self-sustaining organic type farm (Development strategy for agriculture, food industry and forestry in the medium and long term, 2001-2005 and 2005-2010 (MARD).

Also, in regards to the animal sector, whether in 2005 a significant increase of certified organic livestock, especially sheep and goats, about 13 times more than in the previous years was registered, in the sector of processed products it was found out a significant increase, too, even doubling the organic honey production.

According to CertRom, Romania, in the past two years organically certified farms have seen a genuine territorial expansion in relation to the previous years and from conventional farms. In 2015, a total of 8261 heads animals raised in ecological system has registered (Table 5).

Table 5. Total number of animals reared in organic farming in 2015

| No. | County | Number | UM |
|---|---|---|---|
| 1 | Botoşani | 6 | heads |
| 2 | Buzău | 71 | heads |
| 3 | Buzău | 62 | heads |
| 4 | Caraş-Severin | 6654 | heads |
| 9 | Cluj | 129 | heads |
| 10 | Gorj | 4 | heads |
| 11 | Hunedoara | 41 | heads |
| 12 | Iaşi | 434 | heads |
| 15 | Neamţ | 10 | heads |
| 16 | Suceava | 850 | heads |
| | Total | 8261 | heads |

In 2016 it is noticed an increasing of the total number of animals reared in organic farming conformingly the table below (Table 6).

Table 6. Total number of animals reared in organic farming in 2016

| No. | County | Number | UM |
|---|---|---|---|
| 1 | Botoşani | 631 | heads |
| 2 | Buzău | 70 | heads |
| 3 | Caraş-Severin | 5742 | heads |
| 4 | Cluj | 97 | heads |
| 5 | Hunedoara | 41 | heads |
| 6 | Iaşi | 461 | heads |
| 7 | Neamţ | 10 | heads |
| 8 | Suceava | 1273 | heads |
| 9 | Vaslui | 223 | heads |
| | Total | 8548 | heads |

## CONCLUSIONS

1. Although in Romania still exists legislative and institutional concerns for the expansion of organic production and the formation of an internal market, we believe that we have a country that has the most of all the natural conditions of organic farming beneficial purposes, what is missing being the financial support but also the farmers encouragement through incentive measures so that they will think more increasingly to conversion.

2. Unfortunately, although relatively large quantities of organic products are produced compared to other countries, the Romanian consumer is a little more informed and interested in green products. In addition, an organic product is 20% more expensive than a conventional product, and consumers like the idea of buying cheaper or simply does not allow buying these products.
3. Among the socio-economic benefits generated by the organic farming can fit: development of multifunctional agricultural systems, decreasing to a level as low as negative impact of agriculture on the environment, diversification of production, reducing the consumption of non-renewable resources and improving the effectiveness of labour and the quality of life of farmers and as geographical area, in Romania, due to the varied and rich terrain, many species of animals could be reared.

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# THE EMPOWERMENT OF CRUDE EXTRACT ANTIGEN-GOF INSECT ON GOATS IMMUNITY ENHANCEMENT AN ENTOMOLOGY CONTRIBUTION IN ANIMAL HUSBANDRY

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***Abstract***

*The study was conducted to evaluate the proportion level of insect antigens and its effect on goat immuno-response by detecting the immunoglobulin serum level. Twenty-four young goats were used in this experimentmaintained in traditional farms without health control. The animals were divided in threegroups,respectively control group (0µ Ag-G/L) and the others were treated with 0.5 ml of antigen-G by subcutaneous injection which had a concentration of 100µ Ag-G/L). The parameter observed was the serum immunoglobulin level. The mean value of serum immunoglobulin level between treated and control groups were compared by t-test. There was a significance different of parameter between groups observed (P<0.05) which showed that corpus crude extract antigen-general of Bombyx mori was able to enhance the immune-response of goats.*

***Key words****: Antigen, insect,goats, immunoglobulin.*

## INTRODUCTION

Through entomology science many secrets of immunogen originated from insect could be discovered to contribute in the animal husbandry improvement. A fact that is in extensive farming with traditional maintenance, the animals exposed a high mortality level.

The local goats breed kept without special hygienic control caused health problems that led a difficulty to the farmer for getting profit on it. Consequently an alternative solution needed to overwhelm the problems, par example by studying the effect of corpus crude extract general antigen (CCE/Ag-G) to the young goat's immunity.

The insect antigens take an important role in immunogen enhancement substance in animal husbandry although this sciences information were still rarely publicized. Therefore empowerment of the insect antigens for mammalian livestock immunity has a good prospect to be revealed. In this line, our study used crude extract antigen total body liquid of *Bombyx mori* as general antigen (antigen-G).

The immunity improvement by using protein of saliva insect species of Haematobia irritans showed the ability to reduce the development of the flies which consumed blood of animal immunized with this type of proteins (Cuop et al., 2004).

Ameri et al., (2008) revealed that the saliva gland extract of stable flies dominated by immunoglobulin binding protein. This antigen has been studied for the immuno-reactive in cows.

In other side antigen in the venom of bees or *Vespidae* and a group of ubiquitous protein in other organisms included the snake venom be used by this organism to defend or to attack their preys and their enemies.

The function of this protein family presents in several ways as toxin and as *ion channel blockers* as exist in the snake venom (Yamazaki and Morita, 2004).The saliva of the flies consisted of immunogen which dominated by the antigen-5.

This immunogen protein produced in granular cells and accumulated in the saliva gland. Beside that this substances functioned for the ingestion process. The molecules categorized with this function also called *defensin* (Lehan et al., 1997).The antigen protein could move from epithelia cells to the surface of saliva

glandular through a process of exostosis which located in prothorax segment (Uetiet al., 2009).

## MATERIALS AND METHODS

The corpus crude extract general antigens (CCE/Ag-G) were extracted from insect of *Bombyx mori* caterpillars aging more than two weeks.

The larvae were selected to get the uniform of body comportments, and then euthanized to proceed for getting the crude extract antigen.

The characterization was done by simple procedure using spectrophotometry (N1E).

The level of antigen extract were categorized in three classes: LAg1, LAg2 and LAg3 which were respectively equaled to (LAg1> $23^{o}$→>$60g.L^{-1}$; $23^{o}$>LAg2>$21^{o}$→>$40g.L^{-1}$< 60. $.L^{-1}$; Ag2<$21^{o}$→<$60g.L^{-1}$).

This experiment used twenty-four goats of local breed traditionally maintained in open system environment without any special health control.

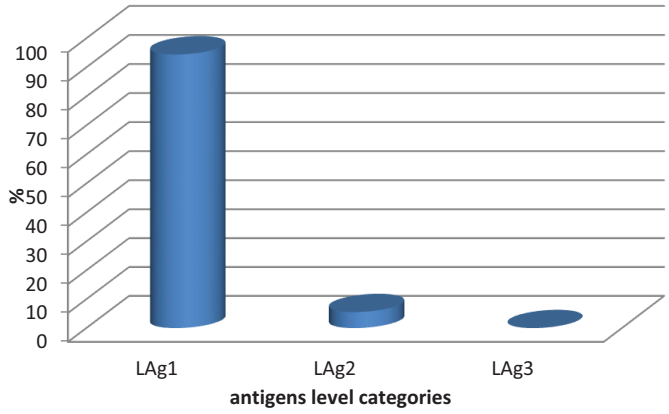


Figure 1. Proportion of CCE/Antigen-G Level Obtained

### Experimental Design

There were twelve samples of young goats as control (0µ CCE/Ag-$G.L^{-1}$) and twelve others were treated with 0.5 ml of antigen-G by subcutaneous injection which had a concentration of100µ CCE/Ag-$G.L^{-1}$).

The parameter measured in this study was the level of immunoglobulin protein in serum. Blood samples were obtained through vena jugular after 12 days of treatment.

### Statistical analysis

The mean value of serum immunoglobulin level between treated and control groups were compared by t-test according to the procedure of Zar (1996).

## RESULTS AND DISCUSSIONS

The figure 1 presented that almost of the samples were categorized as LAg1 which achieved 94.4% of total sample observed while decreased sharply in the level of LAg2 that reached only 5.5% and LAg3 level did not existed in the CCE of the *B. mori.*

This value indicated that the level content of corpus crude extract of antigen-G from this insect was relative qualified to be applied in the experiment to reveal its immune-response in mammalian animals.

The conversion of the containing of organic compound in the CCE of *B. mori* was 60 $g*L^{-1}$ of general antigen, even though there was not clear which specific antigen proteins took effect as primary immune-response.

Ma et al., (2010) reported that the antigen-5 proteins were the most important compound and immunogenic protein in the venom secretory duct of stinging insect.

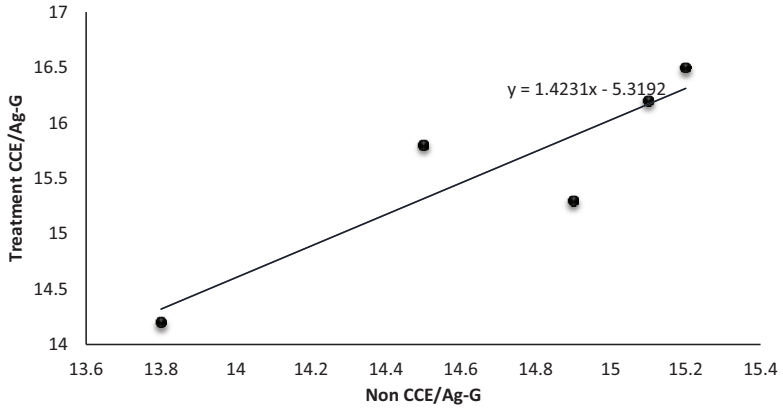


Figure 2. Comparison of Treatment and Control on Serum Immunoglobulin Level in Goats

Compared to the control, the general antigens extracted from *Bomby x mori* treated to goats resulted a significant response ($P<0.05$) on serum immunoglobulins level in goats.

This achieved value was 15.6 $\pm$ SEM 0.43 mg/ml while the mean value of the proteins serum observed in experiment animal without receiving antigen-G was lower than the others treated which obtained only 14.9 $\pm$ 0.25 mg/ml. In the level of serum immunoglobulin in young animals influenced by many factors included the colostrum consummation (Bulla et al., 2004).

The increasing of the total Ig up to 14.9 mg/ml could be caused by the reaction of antigen-G immunisation to the animal experiments. This response related to the report Ameri et al. (2008) that antigen-V originated from salivary gland extract of stable flies was able to stimulate the antibody level in cows.

This substance led to improve the mammalian individual immunity system. The ability of the mammalian individual to modulate the immunoglobulin production depended also on the presentation of antigens in the body (Pritchard et al., 2013) which facilitated by the T cell and CEF (*Chemoattractant Expression in Fibroblasts*) to activate the immunoglobulin.

## CONCLUSIONS

Corpus Crude Extract Ag-G could be an alternative agent for immunity enhancement in goats especially in an extensive farming where the hygienic control does not exist.

In the next future we need to continue this works to identify the most important immunogenic antigens in these extract substances.

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# THE INFLUENCE OF THE BREEDING TECHNOLOGY ON THE HEN MEAT RACES' REPRODUCTION

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***Abstract***

*The research conducted on this paper has had as main objective a study on the reproduction efficiency of ROSS 308 male line, under the influence of several microclimate factors such as the light intensity and the density of the flock, as well as other factors, which, if corroborated, would determine the fecundity capacity of the roosters.*
*Three experiments have been constructed (A - with analyzed parameters under the standard and using bedding made of chopped straws, B - with parameters that have been raised over the standard limits and using rice hulls as bedding, and C - with parameters at the producer's recommended level, and bedding made of wood shavings).*
*The observations have been made and the records have been taken during the production cycle (week 19 - week 64), over a period of 3 weeks (25, 35 and 45 weeks of age), for two consecutive years, on a group comprising 25 roosters and 250 hens, for each experimental series.*
*The results show the highest fertility rate for the experimental series C, over all the control weeks (50 %, 95, 1 %, 93, and 4 % respectively).*
*The differences, although not statistically significant, can turn the conditions of experimental series C very popular, making the wood shavings classic bedding a favorite, along with several technologically standard microclimate parameters. Overall, the fertility would register higher values (with 3-5 %), for which the economic efficiency would be making a difference (although not from a scientific point of view).*

***Key words:*** *reproduction, fecundity, fertility.*

## INTRODUCTION

Meat breeds reproduction farms represent one of the most important part of the poultry meat production chain. For this activity, the comprehension of the basic knowledge regarding the physiology of the reproduction process is extremely important in order to apply the management practices of feeding, maintenance, lighting programs and veterinary aspects.
The mating behaviour can contribute or reduce the stock fertility. In hens, the rooster will monopolize the mating process in a group of hens. The frequency of the mating was initially determined based on the rooster's libido, while the fertility was associated with the sex ratio (Craig et al., 1977). The mating behaviour is influenced not only by the roosters' aggressiveness, but also by the interactions between one rooster and another, and the sex ratio. In roosters, fertility is even harder to measure with precision, this being expressed by the fertile eggs' percentage after the mating (Drăgănescu, 1979; Drăgănescu and Grosu, 2003).

## MATERIALS AND METHODS

During the domestic breeding, through an optimal administration of the reproduction processes, the aim is to enlarge the stock, while being able to improve the stocks through genetic selection. The domestic animals' cyclogram for reproduction, a fundamental step is represented by the mating process itself or the artificial insemination, which is performed based on the reproduction process' proportion, on animals with a normal fertility level. The understanding of the issues on this matter would require a profound knowledge of all factors defining and influencing the fertility in animals, as a prerequisite for a biologically and

economically efficient insemination. Thus, through a correct evaluation of the characteristics which define the reproductive capacity of the roosters and the efficiency of the reproduction expressed as a point of view above, the team has designed three experiments:

- Series A had the main goal of evaluate the influence of certain microclimate factors, for which the values were set under the standard limits (stock density and light intensity) and the use of chopped straw as bedding material, on the quality of the semen as well as of other characteristics on which the roosters' reproductive capacity is based on.
- Series B had as a main objective the effect of breeding in an environment where all the parameters were set at values above the standard limits, while using rice hulls as bedding material, on the indicators of the quality of semen, as well as on other characteristics on which the roosters' reproductive capacity is based on.
- Series C was designed with the aim of establishing the measure in which maintaining a certain value of lighting intensity as well as of stock density while using wood shavings as bedding material, on the indicators of the quality of semen, as well as on other characteristics on which the roosters' reproductive capacity is based on.

The experiments were applied in three different units, each one corresponding to one of the described series: Avicola Călărași, S.C. Agrafood S.A. and Avicola Focșani, while the observations and the recording of the results were performed for a period of 3 weeks (25, 35 and 45) during the production cycle (19-64 weeks), for two years, on a group of 25 roosters and 250 hens, for each designed experiment.
In order to study the variation of fertility, which presents a binomial repartition, the team used:

- a comparison between the frequencies, based on a normal approximation;
- Fisher test for a comparison between the binomial proportions, known as the "Fisher's exact test";
- "Chi" square test, with Yates' correction for continuity applied on binary contingency tables (Dragomirescu, 1999).

## RESULTS AND DISCUSSIONS

For the poultry industry, roosters' fecundity is extremely important, any variation of this parameter having repercussions on the *biological and economic efficiency*.
Taking into consideration the high number of factors capable to influence the associated with the reproductive capacity of roosters, in order to verify the existence of any factor which would influence the values of the technological parameters and the type of bedding material, the team tested the significance of the observed differences between the fertility rates recorded on the three experiments, in control weeks.
In Table 1 and Figure 1 there were included the values of the recorded fertility rates for 25th week of study.

Table 1. Fertility rates and specific values for the 25th week

| Specification | Eggs placed | Clear eggs | Fertile eggs | Fertility % |
|---|---|---|---|---|
| A | 76 | 39 | 37 | 48.7 |
| B | 151 | 81 | 70 | 46.3 |
| C | 92 | 46 | 46 | 50.0 |

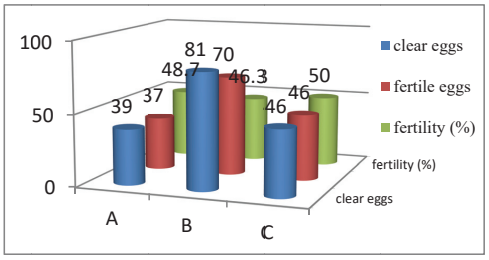


Figure 1. Fertility rates and specific values for the 25th week

Through the analysis of the data presented above, the conclusion is that the highest value of the fertility rate for the 25th week is recorded in series C. It appears, at a first glance, that the standard values of the technological parameters and the use of a classic bedding material (wood shavings) would have a considerably favorable influence on the reproduction efficiency.
By analyzing the data included in Table 2, the table value read while using 1 degree of freedom and a significance level of 0.05 would show the existence of differences which are not statistically significant between the fertility

rates recorded during the three series of experiments in the same week of study. Since these results are confirmed through two different tests, the team can infer pertinently on the influence of the technological parameters and the type of bedding material on fertility. Thus, it is shown that the inexistence of several significant differences between the three series, and as a consequence, the variation of the fertility rate among the three experimental series is determined by a different cause (individual variation, sampling error).

Table 2. Comparison of conception rates using $\chi^2$ test with Yates correction between experimental series, 25th week

| Specification | Fertile eggs | Clear eggs | Total | $\chi^2$ |
|---|---|---|---|---|
| A | a=37 | b=39 | a+b=76 | $0.04^{NS}$ |
| B | c=70 | d=81 | c+d=151 | |
| Total | a+c=107 | b+d=120 | a+b+c+d=227 | |
| A | a=37 | b=39 | a+b=76 | $0.002^{NS}$ |
| C | c=46 | d=46 | c+d=92 | |
| Total | a+c=83 | b+d=85 | a+b+c+d=168 | |
| B | a=70 | b=81 | a+b=151 | $0.18^{NS}$ |
| C | c=46 | d=46 | c+d=92 | |
| Total | a+c=116 | b+d=127 | a+b+c+d=243 | |

Table 3 and figure 2 show the recorded values of the fertility for the three experimental series, for the 35th week.

Table 3. Fertility rates and specific values for the 35th week

| Specification | Eggs placed | Clear eggs | Fertile eggs | Fertility % |
|---|---|---|---|---|
| A | 185 | 15 | 170 | 91.9 |
| B | 193 | 15 | 178 | 92.2 |
| C | 204 | 10 | 194 | 95.1 |

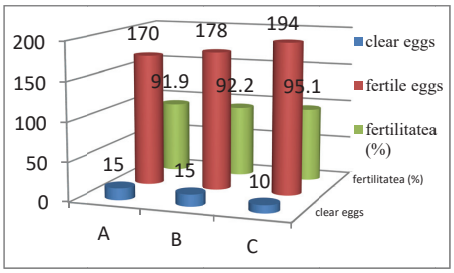


Figure 2. Fertility rates and specific values for the 35th week

Through an analysis of the data included in Table 3 and figure 2, it is observed that the highest value of the fertility rate for the 35th week was recorded for the same experimental series: C, which again leads to the conclusion that the standard values of the technological parameters and the use of a classic bedding material is favorable to an increase of the reproduction efficiency. In order to validate this observation, the results presented in table 4, the table value read at a degree of freedom as well as a significance of 0.05, would show the existence of certain differences without a statistical significance between the mating rates recorded between the three experimental series, for the 35th week (a null hypothesis would be accepted according to which all three experiments would not differ from on another from the efficacy point of view). Since these results show no significance, the team reached the conclusion that for the 35th week as well, the influence of the technological parameters as well as of the type of bedding material on the fertility rates would not present any statistical significance, the observed differences being caused by other factors, especially the sample error, which in return does not influence the obtained results.

Table 4. Comparison of conception rates using $\chi^2$ test with Yates correction between experimental series, 35th week

| Specification | Fertile eggs | Clear eggs | Total | $\chi^2$ |
|---|---|---|---|---|
| A | a=170 | b=15 | a+b=185 | $0.05^{NS}$ |
| B | c=178 | d=15 | c+d=193 | |
| Total | a+c=348 | b+d=30 | a+b+c+d=378 | |
| A | a=170 | b=15 | a+b=185 | $1.168^{NS}$ |
| C | c=194 | d=10 | c+d=204 | |
| Total | a+c=364 | b+d=25 | a+b+c+d=389 | |
| B | a=178 | b=15 | a+b=193 | $0.941^{NS}$ |
| C | c=194 | d=10 | c+d=204 | |
| Total | a+c=372 | b+d=25 | a+b+c+d=397 | |

In Table 5 and figure 3 there were included the fertility rate values for the three experimental series for the 45th week.

After analyzing the data included in table 5 and figure 3, it was concluded the highest value for the 45th week of study was again recorded for the experimental series C. This seems again to support the opinion that choosing the standard values of technological parameters and the use of a classic bedding material has a favorable influence on the reproduction efficacy.

Table 5. Fertility rates and specific values for the 45th week

| Specification | Eggs placed | Clear eggs | Fertile eggs | Fertility % |
|---|---|---|---|---|
| A | 144 | 19 | 125 | 86.8 |
| B | 158 | 18 | 140 | 88.6 |
| C | 168 | 11 | 157 | 93.4 |

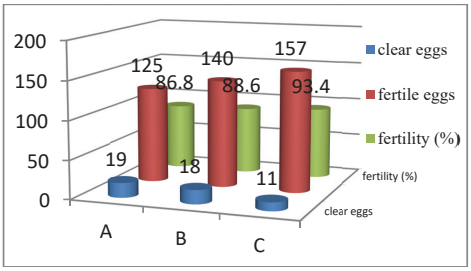


Figure 3. Fertility rates and specific values for the 45th week

After analyzing the data included in Table 5 and figure 3, it was concluded the highest value for the 45th week of study was again recorded for the experimental series C. This seems again to support the opinion that choosing the standard values of technological parameters and the use of a classic bedding material has a favorable influence on the reproduction efficacy.

Table 6. Comparison of fertility rates using $\chi^2$ test with Yates correction between experimental series, 45th week

| Specification | Fertile eggs | Clear eggs | Total | $\chi^2$ |
|---|---|---|---|---|
| A | a=125 | b=19 | a+b=144 | $0.09^{NS}$ |
| B | c=140 | d=18 | c+d=158 | |
| Total | a+c=265 | b+d=37 | a+b+c+d=302 | |
| A | a=125 | b=19 | a+b=144 | $3.21^{NS}$ |
| C | c=157 | d=11 | c+d=168 | |
| Total | a+c=282 | b+d=30 | a+b+c+d=312 | |
| B | a=140 | b=18 | a+b=158 | $1.80^{NS}$ |
| C | c=157 | d=11 | c+d=168 | |
| Total | a+c=297 | b+d=29 | a+b+c+d=326 | |

As observed below, by analyzing the results included in Table 6, the table value being read with a degree of freedom and a significance level of 0.05, it is concluded that there are no significant differences from a statistical point of view between the values of the fertility rate recorded during the three experimental series, in the 45th week (it would be accepted a null hypothesis according to which the conditions of the different experimental series would not differ from one another concerning the efficacy).

Thus, it can be concluded that for the 45th week as well, the influence of the technological parameters and the type of bedding material chosen to be used, on the fertility rate does not present any statistical significance, the observed differences being caused by various other factors, mainly the sample errors, which are known not to influence the obtained results.

## CONCLUSIONS

The results obtained during this study would suggest the fact that the different types of microclimate, the sex ratio and the type of bedding material do not influence significantly the characteristics associated to the reproduction efficiency. Most probably, these traits are controlled by far more complex mechanisms.

Also, the results of the present study would suggest that, at least for the team's chosen experimental conditions, the use of values set above the standard limits and the rice hulls as bedding material would have a certain negative effect, associated to stress, on the reproduction process and consequently on the fertility rate.

Although the results are not statistically significant, they could favor the conditions chosen for experimental series C, using a classic wood shavings bedding material as well as standard values for the technological parameters. As such, the fertility would record higher values, of over 3-5 %, in comparison to the rest, a very important success for the economic efficiency at the farm level (though not from a scientific point of view as well).

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# THE READ MEAT PRODUCTION IN TURKEY

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***Abstract***

*In Turkey, red meat production is mainly provided from cattle, buffalo, sheep and goats, and although there is a significant potential in terms of the number of animals, yields obtained those animals are low. Bovine fattening in Turkey is mostly done with dairy, combined and indigenous breeds which is carcass weights are lower than those of the beef breeds. It is noteworthy red meat per capita consumption in Turkey is fairly below the world average (19 kg / year) with 13 kg / year. In Turkey, a total of 1.15 m tons of meat produced in 2015 was obtained from bovine animals (87.8%) and from small ruminants (12.2%). In Turkey, 12.9% of the cattle and 96% of the sheep population are composed of low-yielding domestic races (Anonymus, 2015). In livestock production is very low. In order to improve red meat production and average carcass yield, besides animal breeding activities, it is important to develop management and feeding conditions, minimize breeding and early age animal slaughter, to develop and maintain support policies for the enterprises engaged in fattening activities*

*In this study, the present status of red meat production in Turkey and sitiuation of the last 10 years, the problems of red meat production and the recommendations for solution were presented.*

***Key words:*** *Red meat, cattle, buffalo, sheep and goat, carcass*

## INTRODUCTION

Animal husbandry, , has an important place in Turkey and as with the whole world in terms of the sufficient and balanced nutrition of the growing population and its use as a raw material for many fields. However, unlike other sectors, the livestock sector contributes to the economy of the country because it has many sectors and it also provides solution to the social problems of the country.

The importance of animal products in human nutrition is an indisputable fact for a country's population. At the same time, in the development of the country's economy, a high profit in terms of unit investment is an important sector that provides employment opportunities at the lowest cost. A significant portion of the country's population is engaged in agriculture (Demirbaş and Talim, 1999).

Turkey's red meat production resources are cattle, sheep, goats and buffalo. For many years sheep and goats have provided most of the red meat production. However, in recent years, both red meat production has not increased to the desired extent and the consumer has preferred to beef. In addition to the thought that the beef is relatively fat-free, the preference change resulting from the fact that more diversity can be provided in presenting it to the market, naturally, is also reflected in the production.

The vast majority of animal production in Turkey is performed by small-scale enterprises, who have no knowledge of quality and price formation, and often lack the traditional methods and economic consciousness of breeding. The traditional and irrational structure in these enterprises weakens the bargaining power of producers.

In Turkey, 12.9% of the cattle and 96% of the sheep population are composed of low-yielding domestic races (Anonymus, 2015). In livestock production is very low.

Vast majority of Turkey's red meat supply is provided by fattening enterprises. As a result of the researches and examinations carried out on this area, it is seen that the fattening enterprises in our country can not work with efficient capacity and struggle with various problems.

At the forefront of the problems to increase red meat production and consumption in Turkey is the introduction of input and supply. It can be seen that the fattening input costs are mainly

composed of the following three main items (Anonymus, 2015).
Animal Material (50-60% of input costs),
Feed costs (25-40% of input costs), and
Other expenses (5-15% of input cost such as labor, loan interest, drugs, veterinary, etc.)

## MATERIALS AND METHODS

In order to the evaluation of red meat production, the following indicators were used: number of bovine and small ruminant stock. The number of animal, red meat yield, meat production, meat consumption per head, the size of fattening enterprises. The data collected from Turkish Statistical Institute between the years 2005-2015 according to red meat were analysed.

## RESULTS AND DISCUSSIONS

Beef cattle holding size was shown in Table 1, There are total 374.951 beef cattle holding and about 76% of fattening enterprises capacity is between 1-10 head. Enterprises rate having 50 or more animal feeding capacities is only 3.5% (Kayhan, 2012).

Table 1. Distribution of Beef Cattle Holding- Size

| Holding-size (Head) | Holding Number | Rate % |
|---|---|---|
| 1-5 | 210,532 | 56.2 |
| 6-10 | 76,084 | 20.3 |
| 11-25 | 58,917 | 15.7 |
| 26-49 | 16,339 | 4.4 |
| 50-100 | 10,720 | 2.9 |
| 100-200 | 1,770 | 0.5 |
| 201+ | 589 | 0.2 |
| **Total** | 374,951 | 100.0 |

According to the data of the year 2015, there are 13,994,071 head cattle, 133,766 head buffalo, 31,507,934 head sheep and 10,416,166 head goats in Turkey. The number of cattle, 10.5 millions head in 2005, increased by 24% in 2015 to 13.9 mil. head. In the same year, the number of buffalo increased by 21.5%. The number of sheep from small cattle animals increased by 19.5% from the beginning of 25.3 mil. head in 2005 to 31.5 mil. head in 2015. The number of goats increased by 37.4% in the same year from 6.5 mil. head to 10.4 mil. head in 2015 (Anonymous, 2015). These increases are believed to have been the result of state support for livestock farming and cattle imports in recent years (Table 2).

Table 2. Bovine and Small Ruminant numbers by the years 2005-2015

| | Bovine | | | Small Ruminant | | |
|---|---|---|---|---|---|---|
| Years | Cattle | Buffalo | Total | Sheep | Goat | Total |
| 2005 | 10,526,440 | 104,965 | 10,631,405 | 25,304,325 | 6,517,464 | 31,821,789 |
| 2006 | 10,871,364 | 100,516 | 10,971,880 | 25,616,912 | 6,643,294 | 32,260,206 |
| 2007 | 11,036,753 | 84,705 | 11,121,458 | 25,462,293 | 6,286,358 | 31,748,651 |
| 2008 | 10,859,942 | 86,297 | 10,946,239 | 23,974,591 | 5,593,561 | 29,568,152 |
| 2009 | 10,723,958 | 87,207 | 10,811,165 | 21,749,508 | 5,128,285 | 26,877,793 |
| 2010 | 11,369,800 | 84,726 | 11,454,526 | 23,089,691 | 6,293,233 | 29,382,924 |
| 2011 | 12,386,337 | 97,632 | 12,483,969 | 25,031,565 | 7,277,953 | 32,309,518 |
| 2012 | 13,914,912 | 107,435 | 14,022,347 | 27,425,233 | 8,357,286 | 35,782,519 |
| 2013 | 14,415,257 | 117,591 | 14,532,848 | 29,284,247 | 9,225,548 | 38,509,795 |
| 2014 | 14,223,109 | 122,114 | 14,345,223 | 31,140,244 | 10,344,936 | 41,485,180 |
| 2015 | 13,994,071 | 133,766 | 14,323,941 | 31,507,934 | 10,416,166 | 41,924,100 |

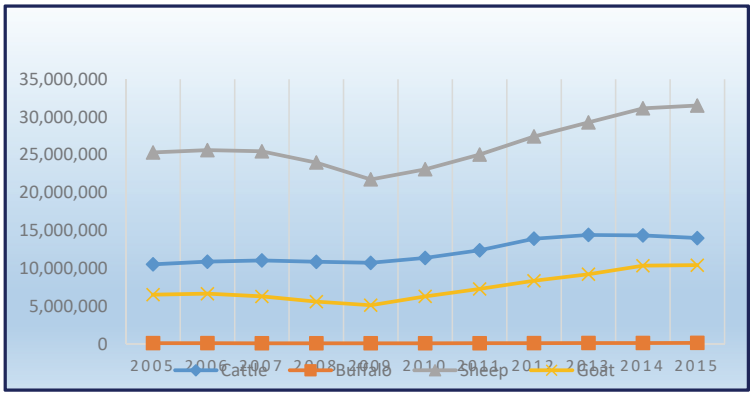


Figure 1. Bovine and Small Ruminant numbers

The number of slaughtered animals and quantity of meat by the years 2005-2015 was given in Table 3. Significant increases are observed in the last decade when the number of animals slaughtered and the amount of meat production per species are examined, While the bovine (cattle and buffalo) in meat production increased from 78.9% in 2005 to 87.8% in 2015, the ratio of small ruminants has decreased from 21% to 12.2% in the same years (Table 4).

Table 3. Number of slaughtered animals and quantity of meat by the years 2005-2015

| Years | Bovine | | | | Small Ruminant | | | |
|---|---|---|---|---|---|---|---|---|
| | Cattle | | Buffalo | | Sheep | | Goat | |
| | Number of slaughtered (Heads) | Quantity of meat (tons) | Number of slaughtered (Heads) | Quantity of meat (tons) | Number of slaughtered (Heads) | Quantity of meat (tons) | Number of slaughtered (Heads) | Quantity of meat production (tons) |
| 2005 | 1,630,471 | 321 681 | 8,920 | 1,577 | 4,145,343 | 73,743 | 688,704 | 12,390 |
| 2006 | 1,750,997 | 340 705 | 9,658 | 1,774 | 4,763,394 | 81,899 | 803,063 | 14,133 |
| 2007 | 2,003,991 | 431 963 | 9,532 | 1,988 | 6,428,866 | 117,524 | 1,256,348 | 24,136 |
| 2008 | 1,736,107 | 370 619 | 7,251 | 1,334 | 5,588,906 | 96,738 | 767,522 | 13,753 |
| 2009 | 1,502,073 | 325 286 | 4,857 | 1,005 | 3,997,348 | 74,633 | 606,042 | 11,675 |
| 2010 | 2,602,246 | 618,584 | 5,720 | 3,387 | 6,873,626 | 135,687 | 1,219,504 | 23,060 |
| 2011 | 2,571,765 | 644,906 | 7,255 | 1,615 | 5,479,546 | 107,076 | 1,254,092 | 23,318 |
| 2012 | 2,791,034 | 799,344 | 7,426 | 1,736 | 4,541,122 | 97,334 | 926,799 | 17,430 |
| 2013 | 3,430,723 | 869,292 | 2,403 | 366 | 4,958,226 | 102,943 | 1,340,909 | 23,554 |
| 2014 | 3,712,281 | 881,999 | 2,176 | 526 | 5,197,289 | 98,978 | 1,570,239 | 26,770 |
| 2015 | 3,765,077 | 1,014,926 | 1,391 | 326 | 5,008,411 | 100,021 | 1,999,241 | 39,990 |

Due to the relative increase in the average carcass weight of cattle slaughtered in recent years, the ratio of cattle in total meat production has increased (Table 5).

Table 4. Quantity of meat production and ratio by the years 2005-2015

| Years | Bovine Quantity of Meat (Tons) | Rate (%) | Small Ruminant Quantity of Meat (Tons) | Rate (%) | Amount of Red Meat (Tons) |
|---|---|---|---|---|---|
| 2005 | 323,258 | 78.96 | 86,133 | 21.04 | 409,391 |
| 2006 | 342,479 | 78.10 | 96,032 | 21.90 | 438,511 |
| 2007 | 433,951 | 75.39 | 141,660 | 24.61 | 575,611 |
| 2008 | 371,953 | 77.10 | 110,491 | 22.90 | 482,444 |
| 2009 | 326,291 | 79.08 | 86,308 | 20.92 | 412,599 |
| 2010 | 621,971 | 79.67 | 158,747 | 20.33 | 780,718 |
| 2011 | 646,521 | 83.22 | 130,394 | 16.78 | 776,915 |
| 2012 | 801,080 | 87.47 | 114,764 | 12.53 | 915,844 |
| 2013 | 869,658 | 87.30 | 126,497 | 12.70 | 996,155 |
| 2014 | 882,525 | 87.53 | 125,748 | 12.47 | 1,008,273 |
| 2015 | 1,015,252 | 87.88 | 140,011 | 12.12 | 1,155,263 |

Table 5. Carcass weight by the years (2005-2015)

| Year | Cattle | Buffalo | Sheep | Goat |
|---|---|---|---|---|
| 2005 | 197.29 | 176.79 | 17.79 | 17.99 |
| 2006 | 194.58 | 183.68 | 17.19 | 17.60 |
| 2007 | 215.55 | 208.56 | 18.28 | 19.21 |
| 2008 | 213.48 | 183.97 | 17.31 | 17.92 |
| 2009 | 216.56 | 206.92 | 18.67 | 19.26 |
| 2010 | 237.71 | 242.48 | 19.74 | 18.91 |
| 2011 | 250.76 | 222.61 | 19.54 | 18.59 |
| 2012 | 286.40 | 233.77 | 21.43 | 18.81 |
| 2013 | 253.38 | 152.31 | 20.76 | 17.57 |
| 2014 | 237.59 | 241.73 | 19.04 | 17.05 |
| 2015 | 269.56 | 234.36 | 19.97 | 20.00 |

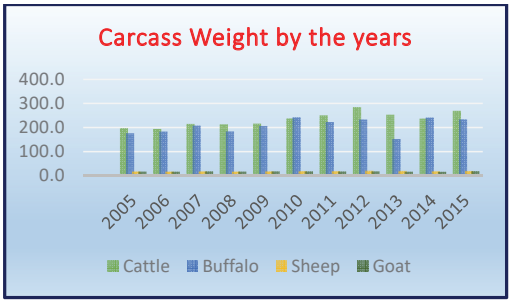


Figure 2. Carcass weight by the yeras 2005-2015

It was observed that meat prices did not increase much between 2005-2008 (Table 8). However, since the second half of 2009, meat prices have moved to a rapid increase. The meat prices which were 20.4 TL/kg in 2009 were 37.9 TL/kg in 2015. During the last decade between 2005 and 2015, meat prices increased by three times. In the same year, sheep prices also showed similar increases (Table 6).

Table 6. Red meat price change by the years 2005-2015

| Products | 2005 | 2006 | 2007 | 2008 | 2009 | 2010 | 2011 | 2012 | 2013 | 2014 | 2015 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Beef Meat (TL) | 12.1 | 13.2 | 14.2 | 15.3 | 20.4 | 24.1 | 24.3 | 23.8 | 25.9 | 28.4 | 37.9 |
| Mutton Meat (TL) | 10.8 | 11.5 | 11.6 | 13.6 | 20.8 | 26.3 | 26.8 | 24.0 | 25.8 | 26.8 | 30.8 |

Despite the high animal production potential in Turkey, population growth has increased by 16.2% over the last 10 years to 67,743,000 to 78,741,000 and domestic demand for meat products has led to an increase in meat prices.

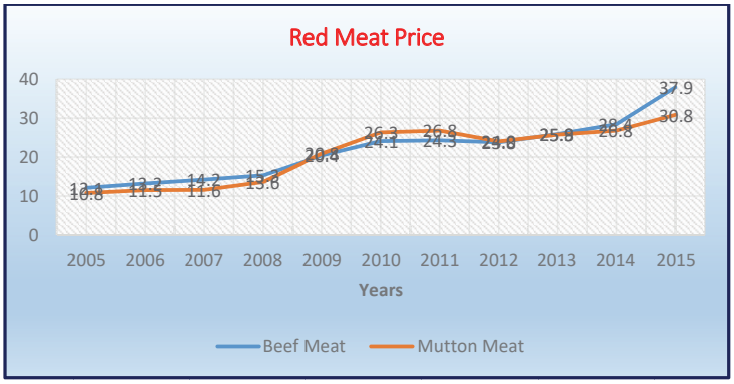


Figure 3. The Consumer red meat price change by the years 2005-2015

The greatest reason for the price increase is the inadequacy of animal material supply. However, there has been an increase in input prices due to the fact that feed and feedstocks are largely dependent on imports and the exchange rate is rising.

A decision was made on the necessity of intervening meat market with importation to regulate supply and demand balance in meat market and regulation of prices by reason of high meat prices. Turkey has been obliged to import duty-free meat and live animals from Europe within the framework of the Customs Union agreement signed by the European Union. Import value from different countries starting 2011 up to now was shown Table 7. As it seen, the imported meat quantity is much more than the exported read meat between the years 2001and 2015.

Table 7. Cattle Red Meat Supply and Use in Turkey (Tons)

| | 2011 | 2012 | 2013 | 2014 | 2015 |
|---|---|---|---|---|---|
| SUPPLY | | | | | |
| Production | 775.3 | 914.1 | 995.7 | 1007.7 | 1014.9 |
| Import | 110.7 | 25.5 | 6.2 | 648 | 9.4 |
| Total Supply | 886.0 | 939.6 | 1002.0 | 1008.3 | 1024.4 |
| USE | | | | | |
| Consumption | 885.8 | 939.3 | 1001.6 | 1008.0 | 1024.1 |
| Export | 216 | 324 | 390 | 386 | 263 |
| Per head Consumption | 11.9 | 12.4 | 13.1 | 13.0 | 13.1 |

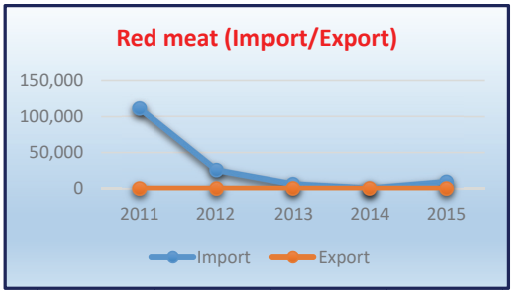


Figure 4. Foreign Trade of Red Meat

## CONCLUSIONS

There has been an increase in bovine and small ruminant numbers in Turkey over the years. This situation is not parallel to the yields per animal. The red meat sector is the livelihood sector. Problems such as high input costs caused excessive increase in meat prices in our country and imports made from countries where production costs were low in order to meet domestic demand victimized the beef producers.

In order to improve red meat production and average carcass yield, besides animal breeding activities, it is important to develop management and feeding conditions, minimize breeding and early age animal slaughter, to develop and maintain support policies for the enterprises engaged in fattening activities (Anonymus-a, 2011).

In animal production, the need for roughage and concentrated feed which plays an important role in profitable and efficient production must be provided in abundance,

quality and inexpensiveness according to needs. This is possible by improving and managing the meadow and pasture areas in terms of quantity and quality, giving the required seed to the production of feed crops and maintaining the support provided (Anonim-a, 2011).

The presence of infectious animal diseases in our country affects the red meat production negatively and causes economic loss. In this context, efforts to control and eradicate animal diseases should be accelerated.

One of the current and important problems of the sector is the need for loans and financing. In order to benefit from the economic development of the sector, appropriate loan and financing facilities should be created and priority should be given to especially for existing small and medium-scale fattening enterprises (Anonim-a, 2011).

Considering the geographical and ecological conditions in our country, importance should be attached to the breeding of suitable beef breeds in the regional basis. It is observed that only about 43% of Turkey's sheep and goats have declined in the last 20 years.

It is also possible for Turkey to take measures to increase the small ruminants number to reduce the pressure on beef in red meat production.

The increase in consumption demand due to developments such as the population increase and the level of social welfare in the world and Turkey in recent years have directly affected the red meat sector. Red meat prices have risen in the market, and as a result, interest in the red meat sector has increased. In order to meet the increasing demand, the producers have searched for a more productive production. As the statistics confirm, in recent years the most productive countries in the world in terms of meat yield per unit have been the European Union countries. The widespread culture in EU countries and the use of modern production techniques are seen as the most important reason for the EU's successful position in red meat production efficiency. Turkish red meat market has also developed in line with world trends in terms of production and consumption. However, these developments in Turkey have not been realized at the same level of developed countries due to their structural problems.

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# THE EFFECT OF DIFFERENT CAGE DENSITIES AND SEXES ON PELT QUANTITY OF NEW ZEALAND WHITE CROSBREED RABBIT

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***Abstract***

*New Zealand White crossbreed rabbit is a producer of meat and white pelt. Housing capacity and rabbitry area can affect production performance due to limited space allowance. The rate of growth of the male rabbit body weight tends to be faster than females, but is more easily experiencing stress due to its natural aggressivity, especially when kept in a cage with limited space that will ultimately have an effect on the quantity of pelt. The research aim was to study the influence of the density of cage and sex toward pelt quantity of New Zealand White Crossbred rabbit. The research used randomized complete block design with a 3x2 factorial pattern and four groups of rabbit at weaning weight as replicates. The first factor was stocking density (K1:1 rabbit/0,2 $m^2$, K2: 2 rabbits/0,2 $m^2$, K3: 3 rabbits/0,2 $m^2$), and the second factor was sex (S1: male, S2: female). A total of 48 crossbreed rabbits of New Zealand White aged 42 days were used in this research, consisted of 24 males and 24 females. Data were analyzed with analysis of variance followed by Duncan's multiple range test. Variables observed were quantity of pelt: pelt weight, pelt thickness, and pelt area. The results showed that there was no interaction between stocking density and sex in all parameters observed. Stocking density had an effect on pelt area, but not on pelt weight and thickness. Meanwhile, sex has an effect on pelt weight, but not on pelt thickness and pelt area. The conclusion is that the male rabbit pelt is better compared with females whilst density of 1 heads/0.2 $m^2$is better than 3 heads/0.2 $m^2$ and 2 head/0.2.*

***Key words****: cage, sex, production, pelt, rabbit.*

## INTRODUCTION

New Zealand White crossbreed rabbit is widely kept by farmers in Indonesia because it has potential as a provider of lean meat compared to ruminants, as well as fertilizer and pelt that still has economic value. Rabbit breeding is generally done individually or colonies in the cage. One cage ranges between 2-10 heads (Rommers et al*.,* 2007; Trocino and Xiccato, 2006). Optimal stocking density requirement in female and male rabbit need to be known in order to maximize the number of rabbits which are kept in cages in order to get a good performance of rabbit production, so it would obtain the quantity of good rabbit pelt.

The density of rabbit suspected of giving effect to the pelt's quantity because it will affect its body weight. Bigger body weight means big body volume, so that the pelt would be vastness. Better body weight at optimum density could maximizes the heaviness and vastness of the pelt. Pelt heaviness closely related to rabbit weight cut. In New Zealand White crossbreed rabbit with slaughter weight 1700-1900 grams will produce heavy pelt in the range of 122.73 g to 178.37 g of slaughter weight or 7.23% to 8.96% (Yurmiati, 2006). Cages with high density will cause the animal difficult to move so that there is a tendency to fat accumulation under the skin which will affect the thickness of pelt.

Local rabbits were cut at 15 weeks, weight 2274 gram, produced heavy pelt at 271 gram, and does not affected by sex (Lakabi et al., 2004), while pelt heaviness on New Zealand White crossbreed rabbit which was cut at 12 weeks and weight cut 2218 gram, namely 204±17.17 gram (Baiomy and Hassanein, 2011) higher on cage density more than 6 head/ $m^2$ and on male rabbit has heavier pelt (378.5 gram) compared to female rabbit (360.48 gram) (Vilalobos et al*.,* 2008).

Rabbit pelt has an area between 1.5 to 2.5 square feet or 0.14 – 0.23 $m^2$ (Sri Untari, 2005). Vastness of fresh pelt increased matching with pelt weight and cut weight because the bigger the rabbit means the vastness the pelt. The results of the research of Tao (1994) indicates pelt vastness of New Zealand White crossbreed rabbit by weight 3.43±0.36 kg is 1.197±94 $cm^2$

and Rex rabbit with weight 2.63±0.26 kg produces pelt vastness 972±96 $cm^2$. Sex affects rabbit weight and pelt vastness, which is pelt of male rabbit more vast than female rabbit, 1273±55 $cm^2$ and 1122±83 $cm^2$.

## MATERIALS AND METHODS

Experiments using 48 New Zealand White crossbreed rabbit age 6 weeks, consisting 24 male rabbits and 24 female rabbits, with weaned weight between 400-700 gram. Rabbit are placed randomly by sex in accordance with the treatment in 24 battery cage with a length of 50 cm, 40 cm wide, and 35 cm height (floor area 0.2 $m^2$) 24 pieces that include a single nipple drinking water and feed.
Rationing and drinking water are given twice a day in *ad libitum* at 07.00 and 16.00. Rations were used in this research is commercial ration with nutrition-shaped pellet feed as listed in Table 1.

Table1. Ration composition in the research

| Feed Ingredients | Rations* | Needs** |
|---|---|---|
| Protein (%) | 16 | 15 |
| Crude Lipid (%) | 5 | 3 |
| Crude Fiber (%) | 16 | 14 |
| Ca (%) | 1,36 | 0,5 |
| P (%) | 0,7 | 0,3 |
| Ash | 8 | - |
| Digestible Energy (Kcal/kg) | 2.576 | 2.500 |

*Commercial Ration Guyofeed
**Lebas, 1980 in McNitt et al., 2000
DE is calculated using a formula based on Fekete and Gilpert (1986) in Cheeke (1987): DE = 4253 – 32.6(%SK) – 144.4(%Ash)

Slaughtering is done at the 12 weeks rabbit after fasting 6-10 hours by cutting the throat to the esophagus, carotid artery and jugular vein severed, then the pelt is removed from the body.
Measurement Variable :

a. Pelt weight (gram)
   It obtained by weighing the rabbit's pelt shortly after debarking.
b. Pelt thickness (mm)
   Pelt thickness is obtained by measuring pelt thickness in some areas that is Croupon, shoulder, belly and tail using micrometer with accuracy of 0.001 mm. Sampel of pelt is shaved by using scapel to the base of fur.
c. Pelt area ($cm^2$)
   Pelt area is measuring length and width of Pelt and using Hegenaur method (1977).In Figure 1. Pelt are ($cm^2$) was obtained by using the formula of length(cm) x width of the pelt (cm).

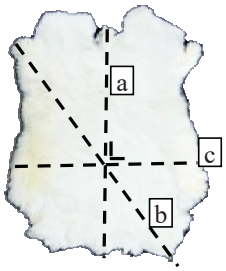


Figure 1. How to measure the length and width of pelt rabbit with Hegenaur method (1977).
a. lenght
b. diagonal help lines to gain pelt width
c. pelt width

The design is using Randomized Block Design factorial 3 x 2 with 4 groups of weaning weight (400-700 grams) as replicates. Factors treatment is given as follows:
1) Cage density factor (K), consist of three level :
   $K_1$ = 1 head/0.2 $m^2$
   $K_2$ = 2 head/0.2$m^2$
   $K_3$ = 3 head/0.2$m^2$
2) Sex Factor (S), i.e:
   $S_1$ = male
   $S_2$ = female

Based on the treatment, then obtained 6 treatment combination and each treatment was repeating 4 times, thus there are 24 units in this trial cage.

## RESULTS AND DISCUSSIONS

The result showed that cage density treatment and sex did not affect the weight and thick of pelt, but significantly affect ($P<0.05$)to pelt vastness. There were no interactions between cage density and sex against heaviness, thickness and vastness of pelt of New Zealand White crossbreed rabbit (Table 2).

Table 2. The effect of cage density and sex toward the quantity of pelt of New Zealand White crossbreed rabbit

| Variable | Density | | | Sex | | Interactions |
|---|---|---|---|---|---|---|
| | K1 | K2 | K3 | Male | Female | |
| Pelt Weight (g) | 163.43 A | 150.8 a | 155.68 a | 167.38 B | 145.74 A | Ns |
| Pelt Thick (mm) | 0.21 a | 0.18 a | 0.24 a | 0.23 A | 0.18 A | Ns |
| Pelt Area($cm^2$) | 937.28 b | 782.90 a | 843.55 ab | 840.18 A | 868.97 A | Ns |

K1 = 1 head/0.2 $m^2$, K2 = 2 head/0.2 $m^2$,K3 = 3 head/0.2 $m^2$
Means for the same item in the same row at the same treatment are significantly different ($P< 0.05$)
Ns = No Significant effect

**Influence of the cage density and sex on pelt weight.** Table 2 shows that the weight of the pelt siginificantly ($P<0.05$) influenced by sex, but it is not significantly affected by the density of the cage, and there was no interaction between the density of cages and sex to the weight of the fresh pelt. This shows that the density of cages and sex do not affect each weight cut of rabbit, so it does not affect the pelt. Slaughter weight is more influenced by hormonal factors contained in the treatment of sex, so there is no influence between the density of cages and sex.

Cage density factor did not significantly affect the weight of rabbit pelt. It shows that rabbit cut weight was not influenced by the cage density so that produce weight pelt which is not significantly different. Unlike sex, highly significantly affect ($P<0.01$) to the weight of the rabbit pelt. This is due to male rabbits heavier than the female rabbits. The results are consistent with Yurmiati (2006) that the increase in rabbit weight cut will be followed by the increase of fresh pelt weight.

Pelt weight which was produced in this research is 167.34 gram on male rabbit and 145.74 gram on female rabbit or ranged between 7.74 – 10.75% from range of the weight cut (1570.40 up to 2235.20 grams). Pelt weight in this research was in the range that obtained by Yurmiarti (2006) on male rabbit of New Zealand White crossbreed, which is 122.73 up to 178.37 gram from the weight cut (1700 gram - 1990 gram) or 7.23 – 8.96%, while Purnama (2006) gained the weight pelt percentage in the range 10-12% toward live weight (2256 – 2956 gram) at Rex rabbit showed that cage density and sex do not significantly affect pelt thickness, as well as the interaction of both of these factors.

**Influence of cage density and sex toward pelt thickness.** The result of observation of pelt thickness of each treatment (Table 2) showed that rabbit pelt thickness with cage density is highest i.e 3 head/0.2 $m^2$ but in statistic it is not significantly different. This is due to high density which makes rabbit has low activity which causing fat accumulation under the skin, showed with high pelt thickness, but rabbit is in growth period so that fat accumulation under the skin still low and cause pelt thickness not significantly different.

Male rabbit's pelt is bigger than female rabbit's pelt, but in statistic not significantly different. This is because the rabbit is on growth period, so that fatty under the skin is not optimum yet which causing pelt thickness of male rabbit is not different with female rabbit's pelt. The thickness of the pelt is associated with the accumulation of fat in the subcutaneous and korium layer. According to Tancous et al. (1981) in the subcutaneous layer has woven fat and a place of accumulation of fat, but fat is possibly in the middle of "corium" inside separates fat cells. The fat presence inside pelt later associated with weight cut and weight fat of carcass in this research. Tao (1994) gained same results which male rabbit is significantly has thicker pelt on every skin part which is observed respectively in male and female are shoulder (2.67±0.61 and 2.51±0.42), back (2.77±0.62 and 2.65±0.59) and tail (2.92±0.59 and 2.92±0.60).

**The effect of cage density and sex towards pelt area.** The observation toward pelt area showed that pelt area of rabbit is significantly influenced ($P<0.05$) by cage density but not significantly influenced by sex, and there was no interaction between both factors. It indicates that pelt area of the rabbit in all treatment of

cage density is same to male and female rabbit. The absence of interaction because the rabbit is still in growth period so that it pelt is not yet fully developed which led to vastness of the pelt is not affected by cage density and sex. Maynard and Loosli (1969) explained that the change in pelt is closely related to the growth that will result increasing on body volume so that the pelt which wrapped around the body surface will be more broadly follows body size of the rabbit.

The result obtained in this study are not in line with Maynard and Loosly (1969), that the increase in weight cut of the rabbits will be followed by increasing rabbit pelt's area. The results obtained in this study also inversely proportional to the statement of Tao (1994) that the sex effect on body weight and pelt area, where the male rabbit has more pelt area (1273 ± 55 $cm^2$) than the female one (1.122 ± 83 $cm^2$), both in Rex and New Zealand White crossbreed rabbits. It may be caused by differences in age and rabbit's type which are used in the maintenance to produce pelt. This research used New Zealand White crossbreed rabbit with final low body weight (1585.89 - 1909.05 gram) and was aged 12 weeks when cutting, while Tao (1994) used pure breeds rabbit with cut weight 5 months so that have bigger body weight with the average weight of 3.43 kg.

## CONCLUSIONS

There is no interaction between the cage density and sex toward quantity of the pelt (heavy, thick, and area of pelt) of New Zealand White crossbreed rabbit. Cage density affects the wide of pelt of New Zealand White crossbreed rabbit but does not affect on its heaviness and thickness. Sex affects pelt heaviness of New Zealand White crossbreed rabbit but does not affect pelt thickness and vastness.

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# TECHNOLOGIES OF THE AGRO FOOD PRODUCTS PROCESSING

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# EFFECT OF TRANSGLUTAMINASE AND NEUTRASE ON THE PROPERTIES OF PROTEIN ENRICHED RICE FLOUR

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***Abstract***

*One way to improve the functionality of the proteins within different food matrices consists on applying different enzyme treatments. The present study aimed at investigating the effect of transglutaminase catalyzed cross-linking and Neutrase catalyzed hydrolysis on the rheological properties of egg, gluten and soy protein derivatives, and on the thermo-mechanical performance of the proteins - whole rice flour mixtures. Tests performed on 15% protein suspensions indicated that the rheological behaviour varied significantly with the substrate and type of enzyme treatment. The controlled enzyme treatment improved both the consistency and strength of the egg and soy proteins based suspensions. Moreover, the values of G``, G` and flow threshold values increased significantly after the enzymes treatment. On the other hand, lower viscosity and stability were observed when investigating the effect of the enzymes on the rheological behavior of gluten suspension. Further tests were meant to investigate the effect of transglutaminase and Neutrase addition on the thermo-mechanical behavior of the protein containing doughs based on whole rice flour. Addition of both enzymes to the flour mixtures resulted in significant changes in the Mixolab curves describing in particular the behaviour of the proteins at increasing temperature, but also starch gelatinization and retrogradation.*

***Key words****: gluten, egg proteins, soy proteins, transglutaminase, Neutrase.*

## INTRODUCTION

The enzyme assisted bio-processing of flour can be successfully applied for improving dough processability and final quality of the bakery products. Applications on gluten free matrices are more challenging with respect to the wheat based ones, because of the lack of viscoelastic properties specific to the gluten network. Due to the low allergenic potential, pleasant sensory characteristics and nutritional benefits, rice flour was nominated as the most suitable cereal for making gluten free products (Gujral and Rosell, 2004; Marco and Rosell, 2008a). In order to overcome drawbacks like difficulties in retaining the $CO_2$ generated through fermentation, the addition of gums, starches and hydrocolloids to the gluten free bakery products has been proposed, resulting in very low protein contents and deficit of lysine. Therefore selection of appropriate protein sources, with balanced amino acids profile and appropriate functionality, plays a key role in obtaining bread products with desired quality. The baking performance of the gluten free batters highly depends on the particular formulation used, because different components of the mixtures can interact to different extent to each other (Hager and Arendt, 2013; Matos and Rosell, 2013). In particular, depending on the source, proteins can alter water distribution within the batter and weaken the interactions between hydrocolloids and starch matrix (Crockett et al., 2011; Renzetti and Rosell, 2016).

One way to improve the proteins behavior in the gluten free mixtures, such as to resemble the network like structure, rely on the use of different protein modifying enzymes. Starting from the main elements defining the baking functionality of different batters, Renzetti and Rosell (2016) provided a nice comparative overview focusing on the use of enzymes for enhancing the functionality of proteins from both gluten-free flours used as basis in different mixtures, and those arising from different supplements.

Transglutaminase (TG) and different oxidases such as glucose oxidase, lipoxygenase, sulphydryl oxidase, polyphenoloxidase and peroxidase, catalyzing direct or indirect cross-linking of proteins appeared to be effective

alternatives for generating effective protein networks in the gluten free matrices (Renzetti and Rosell, 2016). In case of TG, whose activity depends on the accessibility of glutamine and lysine residues in the proteins (Houben *et al*., 2012), the enzyme concentration has to be accurately adapted to the substrate, such as to avoid altering the quality of the final product. For instance, Gujral and Rosell (2004) identified the optimum bread volume and crumb softness for the medium tested enzyme concentration of 1.0 (w/w), although the viscous ($G''$) and elastic ($G'$) moduli of the rice flour based doughs displayed progressive enhancement at even higher enzyme concentration. Advanced protein cross-linking might cause excessive tightening of the dough structure, impeding the expansion during proofing.

On the other hand, proteases are responsible for peptide bonds hydrolysis in proteins, being therefore effective in standard baking application for controlling gluten related properties of doughs. Concerning the gluten free products, Renzetti and Arendt (2009) reported improvement of the rheological properties of the batters and of the specific volume and crumb softness of breads obtained from brown rice flour treated with Neutrase (N) from *Bacillus amyloliquefaciens*. Moreover, Kawamura-Konishi *et al*. (2013) indicated the possibility of improving the quality of the rice flour based bread through treatment with different commercially available protease.

The aim of the present study was to estimate the impact of enzyme catalyzed protein hydrolysis and cross-linking on the rheological properties of protein derivatives, and their thermo-mechanical performance when introduced in a gluten free matrix. In particular the effect of TG and N addition on the properties of powdered eggs, soy protein concentrate and vital gluten alone or in admixture with whole rice flour was tested.

## MATERIALS AND METHODS

### *Materials*

The wholegrain rice flour (Solaris Plant SRL, Bucharest, Romania) was purchased from a local market (Galati, Romania). The proximate composition of the commercial wholegrain rice flour was as follows: 13.46±0.11% moisture, 6.18±0.41% protein, 2.16±0.07% fat, and 0.99±0.17% ash.

The protein sources used as substrates for the enzyme treatments were: soy protein concentrate (Ubimedia S.R.L., Galati, Romania; 9.52±0.09% moisture and 74.28±0.47% protein), dried whole eggs powder (Agricola Bacau, Romania; 4.67±0.06% moisture and 49.39±1.51% protein), and vital gluten (SanoVita, Vâlcea, Romania; 6.62±0.04% moisture and 76.06±0.71% protein).

Commercial transglutaminase (Activa $^{TM}$TG, Ajinomoto Corporation Inc., Tokyo, Japan) and Neutrase 5.0 BG (Novo Nordisk, Denmark) were used in the experiment at levels recommended by the producers for bakery applications. Transglutaminase (TG) has a declared enzymatic activity of about 100 UE/g, and is active over large temperature (2–60°C) and pH (5–8) domains. Neutrase (N) has a declared activity of 5 UA/g and is active in the temperature and pH domains of 25-70$^{o}$C and 5-8, respectively. The concentration of TG and N used in the experiment was 0.1g/g protein, and 0.001g/g protein, respectively.

### *Proximate composition*

The moisture content was determined through the AACC 44-51 method, the protein content through the semimicro-Kjeldahl method (Raypa Trade, R. Espinar, S.L., Barcelona, Spain), the fat content by extraction with ether using a Soxhlet extractor (SER-148, VELP Scientifica, Usmate Velate (MB), Italy) and the ash content using SR ISO 2171: 2002 method.

### *Fundamental rheological measurements*

Rheological properties of protein based suspensions of 15% concentration, treated with TG or N for 30 min at 50$^{o}$C, were determined using an AR2000ex Rheometer (TA Instruments) equipped with Peltier control temperature jacket. The control samples for the rheological measurements consisted of protein suspensions with no enzyme addition. Due to different consistency of studied protein matrices – liquid like in case of egg suspension and solid like in case of soy protein isolate – distinct geometric systems were used. The egg based suspensions were tested in a double gap concentric cylinder (inner radius of 32 mm, outer radius of 35.03 mm, cylinder length of

54.95 mm and gap of 500 mm), while gluten and soy suspensions were tested with a single gap cylinder cup (inner radius of 28.01 mm, cylinder length of 42.01mm and gap of 5920 mm). The geometric systems where chosen such as to allow the best testing conditions, as it is known that choosing an adequate geometric system is crucial for correct rheological testing (Abu-Jdayil, 2003; Mori et al., 2006).

The rheological behavior of all studied samples was evaluated by the means of low amplitude dynamic shearing tests - strain sweep, frequency sweep and temperature ramp. The registered parameters were storage modulus (G`) and loss modulus (G``).

For strain sweep tests the controlled parameter was the oscillatory frequency of 1 Hz, while increasing strain from 0.01 to 100%. System temperature was set to 20°C. This test was used to identify the linear viscoelastic region (LVR) for all investigated samples.

The dynamic frequency sweeps were further performed in the 0.1 - 100 Hz domain, at a constant strain of 0.3% (determined to be within the LVR). The test provides information about structural assembly of the tested viscoelastic material as a function of the time scale of the applied deformation. A viscoelastic material will flow over long times and bound over short times of applied deformation. For each sample the frequency dependency was determined with power low equation: $\eta=k\times\omega^{(n-1)}$, using the TA Rheology Advantage Data Analysis Software V4.8.3., where η - viscosity (Pa·s), k - consistency index, ω - frequency (Hz), and n - frequency index.

Rheological behavior of studied suspensions as a function of system temperature was determined by ramping up the temperature from 20 to 90°C (110°C in case of SPI) with a rate of 1.5°C/min in *quasy-static* conditions (0.3% strain, 1 Hz). The temperature was then maintained for 10 min at 90/110°C. Water evaporation was avoided by covering the immersion cup with a solvent trap cover.

***Empirical rheological measurements***

The thermo-mechanical behavior of the mixtures consisting of 85% wholegrain rice flour and 15% of vital gluten, powdered eggs or soy protein concentrate supplemented with TG or N was assessed by means of Mixolab Chopin (Tripette & Renaud Chopin, Villeneuve La Garenne, France), using the Chopin+ protocol, as previously described by Patraşcu et al. (2016). In case of the samples supplemented with TG, the mixtures were kneaded in Mixolab tank with the appropriate amount of water to get doughs with maximum torque of 1.1 Nm, which were then left to rest for additional 30 minutes, after which the test was restarted.

The parameters recorded from the Mixolab curves give indications about proteins functionality (minimum C2 (Nm) torque is related to protein weakening while subjecting the sample to mechanical work and heating at 4°C/min) and starch behavior (the C3 (Nm) torque is related to starch gelatinization, C4 (Nm) gives indication on the stability of the starch gels at high temperature (90°C), and C5 (Nm) is connected to starch retrogradation when cooling the dough to 50°C) (Collar et al., 2007).

## RESULTS AND DISCUSSIONS

***Effect of enzyme addition on the rheological properties of the protein suspensions***

The strain sweep test was used to differentiate between three structure characteristics of the investigated viscoelastic materials, as described in Patrascu et al. (2016) - linear viscoelastic region characterized by constant G` and G`` values, transition phase when G` values starts to decrease while the tested material still shows a solid like behavior (G`>G``), and the yield point at G`/G`` intersection when the onset of flow occurs - material enters the viscous domain and phase angle (δ) exceeds 45°. In the case of egg and soy proteins based suspensions the enzyme treatment determined the increase of samples elasticity. The strain values corresponding to G`/G`` cross-over, marking the faze inversion and the beginning of flow, significantly increased after enzyme treatment ($p<0.05$) (Table 1). It is known that TG addition determines proteins crosslinking, improving their mechanical properties (Marco and Rosell, 2008).

Regardless of the enzyme treatment, gluten based suspensions registered decreased consistency (lower G` values) compared to the control sample. Moreover, the enzyme treatment significantly affected samples

resistance ($p<0.05$), *i.e.* lower yield point values were obtained (Table 1).

Regarding N effect on the behavior of gluten suspension during the strain sweep tests, the obtained response can be explained by advanced peptide bonds breakdown leading to higher concentration of soluble proteins. As for the poor rheological characteristics obtained for gluten suspension treated with TG, the behavior can be due to the low lysine content.

Similar observations were made by Renzetti et al. (2008) when studying the effect of transglutaminase on some gluten free flours, who declared that the weakening of corn flour structure can be due to the lysine deficiency of corn proteins.

**Table 1.** The yield point of the egg, gluten and soy protein based suspensions during strain sweep test

| | Enzyme treatment | | |
|---|---|---|---|
| | Control* | TG | N |
| Dried whole eggs | 1.88±0.18% | 4.29±0.79% | 4.44±1.03% |
| Vital Gluten | 38.2±1.24% | 19.74±2.30% | 10.98±0.82% |
| Soy protein isolate | 3.84±0.08% | 38.40±1.18% | 83.37±1.56% |

*Control – no enzyme treatment

The viscoelastic characteristics of egg, gluten and soy protein based suspensions as a function of time scale of the applied deformation are presented in Figure 1.

The egg based suspensions showed a frequency dependent viscoelastic response, with alternate prevailing of elastic (G`>G``) or viscous moduli (G``>G`) resembling plastic behavior. Enzymatic treatment of egg proteins determined an increase in consistency when compared to control sample.

Cold enzymetically set gels were obtained when treating the sample with TG.

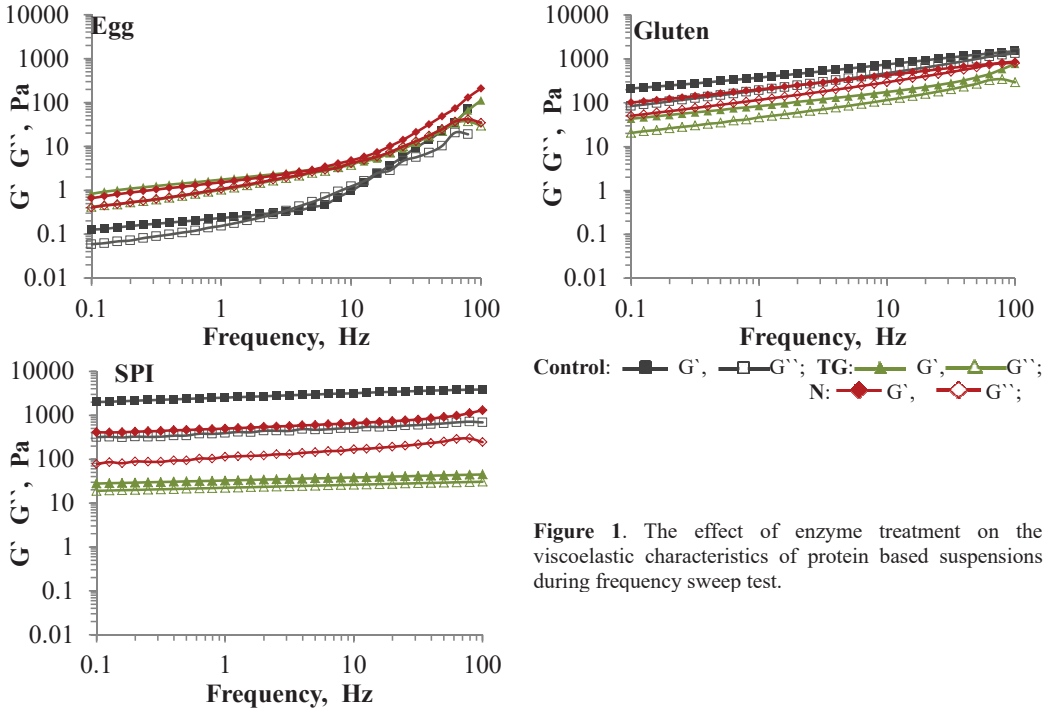


**Figure 1**. The effect of enzyme treatment on the viscoelastic characteristics of protein based suspensions during frequency sweep test.

According to observations made by Tunick (2011) and Alting et al. (2004), gels with a perfectly crosslinked structure present a minimal frequency dependency and are more solid like due to the formation of a covalent network. The TG egg proteins based suspension presented a weak gel like structure (frequency index $n = 0.664$).

Gluten and soy protein based suspensions, both controls and enzymatically treated ones, as presented in Figure 1, showed a dominant elastic response, specific to solid like materials. However, the frequency dependency behavior was more evident in case of the gluten suspensions. The lowest frequency dependency was observed in the case of soy protein based sample treated with TG. In addition, the frequency index of this sample was very low ($n = 0.06$), being characteristic to perfectly crosslinked structures.

When studying temperature dependent viscoelastic bahavior of targeted suspensions in *quasi-static* conditions, it was observed that the enzyme treatment influenced mainly the egg and soy protein based samples (Figure 2).

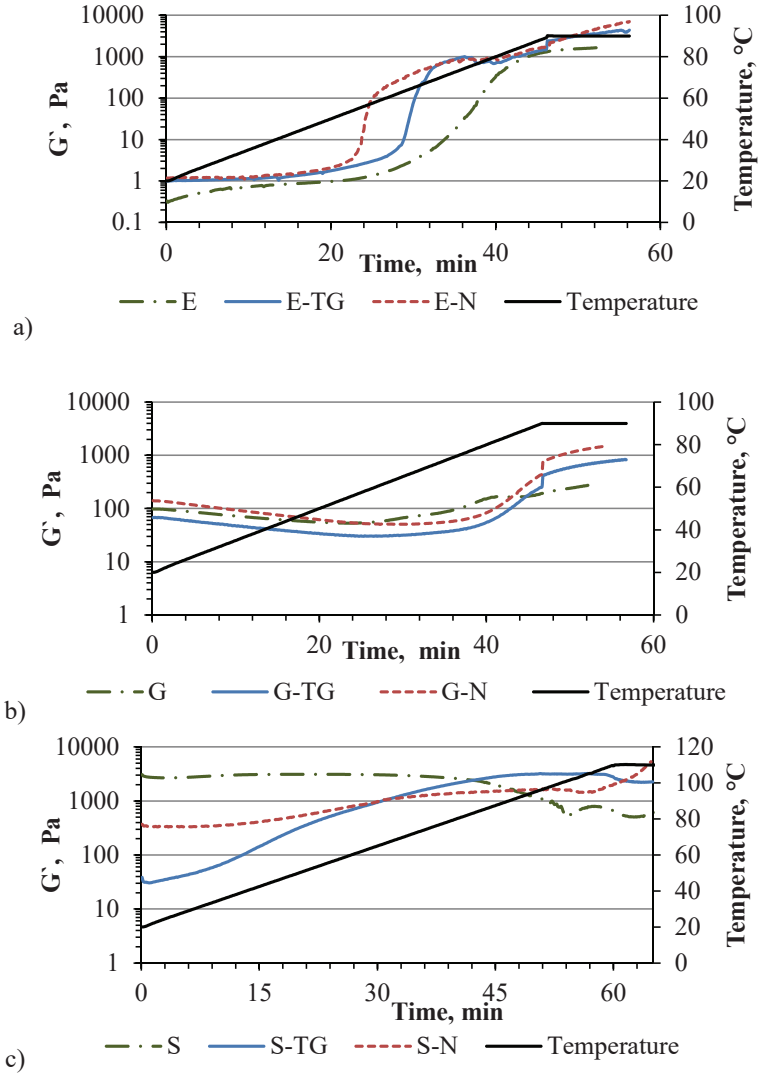


**Figure 2**. Rheological behavior of TG and N treated egg (a), gluten (b) and soy (c) protein suspensions during oscillatory temperature ramp test.

In these two cases, the G` *vs*. applied temperature curves presented a different trend with respect to the control. Thus, if control soy protein suspension presented at low temperatures rather constant values for G` which started to decrease after 50°C, the enzyme treated ones registered a constant and accentuated increase of storage modulus from the beginning of the test. Obtained results are similar to those reported by Tang et al. (2006) when studying the effect of transglutaminase on glycinin and β-coglycinin. They observed that during temperature increase from 25 to 90°C, the G` values constantly increased, most probably due to interactions between β subunits of β-conglycinin and BS subunits of glycinin. It was also observed that enzymatic treatment influenced the temperature values characteristic to proteins denaturation. The phenomenon is usually marked by the presence of inflection points in the G` curve. In this respect, it could be observed that enzymatic treatment of egg based suspensions led to a pronounced decrease of temperature domains associated with protein denaturation and gel formation. In case of the enzyme treated gluten and soy protein based samples, the temperature domains associated to sol-gel transition were rather within those of the corresponding control samples. These results indicate lower thermal stability of the gluten and soy protein aggregates and hydrolysates with respect to the native proteins.

***Effect of enzyme addition on the thermo-mechanical properties of whole rice flour enriched with proteins***

The Mixolab apparatus was further used to test the influence of TG and N on the thermo-mechanical behavior of the doughs, consisting on whole rice flour (85%) and protein derivatives (15%). Preliminary tests were carried out on whole rice flour with no protein addition, such as to get an overview on the susceptibility of the rice proteins to cross-linking and hydrolysis reactions catalyzed by TG and N, respectively. Regardless of the enzyme added, the water needed to get a maximum torque value of about 1.1 Nm (C1) at 30$^{o}$C for the whole rice flour was 62%. In case of the samples supplemented with transglutaminase, after 30 min of enzyme reaction at 30$^{o}$C the Mixolab test indicated an increase of the development time (from 1.38 to 1.92 min) required to get the maximum torque value. These results are in agreement with the observations of Marco and Rosell (2008a), suggesting higher resistance of the dough to kneading, as a consequence of the TG catalyzed cross-links formed between rice proteins. Mainly albumin-globulin and glutelin fractions, representing about 15.5% and 77.8%, respectively of total proteins in the rice flour, were reported to be involved in the cross-linking reactions catalyzed by TG (Marco et al., 2007). As a result of the increase of proteins molecular weight in the rice flour, a progressive improvement of the dynamic rheological properties of the doughs with the raise of TG concentration was observed by Gujral and Rosell (2004). Further heating and cooling of the samples over the entire Chopin+ test, which simulates the breadmaking process, resulted in rather similar values of the specific minimum and maximum torques. The C2 torque was higher in the sample treated with TG (0.789 Nm) with respect to the whole rice flour sample with no enzyme addition (0.761 Nm) (Figure 3). On the other hand, addition of the N caused the significant reduction of the C2 to 0.542 Nm, whereas all other Mixolab parameters were similar to the whole rice flour samples with no enzyme addition (Figure 3).

The influence of powdered eggs, soy protein concentrate and vital gluten addition of the thermo-mechanical properties of whole rice flour was discussed in detail elsewhere (Patraşcu et al., 2016). Therefore, only the characteristics that help understanding the effect of TG and N catalyzed reactions will be further presented. Protein addition to the whole rice flour affected the water absorption required to get the constant C1 torque of 1.1 Nm, corresponding to the optimum dough consistency (Patraşcu et al., 2016). For each type of investigated protein product no variation of the water absorption was considered when testing the effect of enzyme addition. Addition of TG to the protein enriched whole rice flour based samples caused no significant changes of the Mixolab curve parameters. Our results comply with the observation of Marco and Rosell (2008b) who studied the effect of TG on the rheological properties of a mixture consisting on rice flour

and egg proteins, and reported no significant differences in RVA parameters - peak viscosity, gel stability and starch retrogradation. The most significant changes induced by TG addition were observed in samples with soy protein concentrate, especially at temperatures over 61°C, when the highest C3, C4 and C5 values (3.01, 3.34 and 5.61 Nm, respectively) were registered in the Mixolab curves (Figure 3).

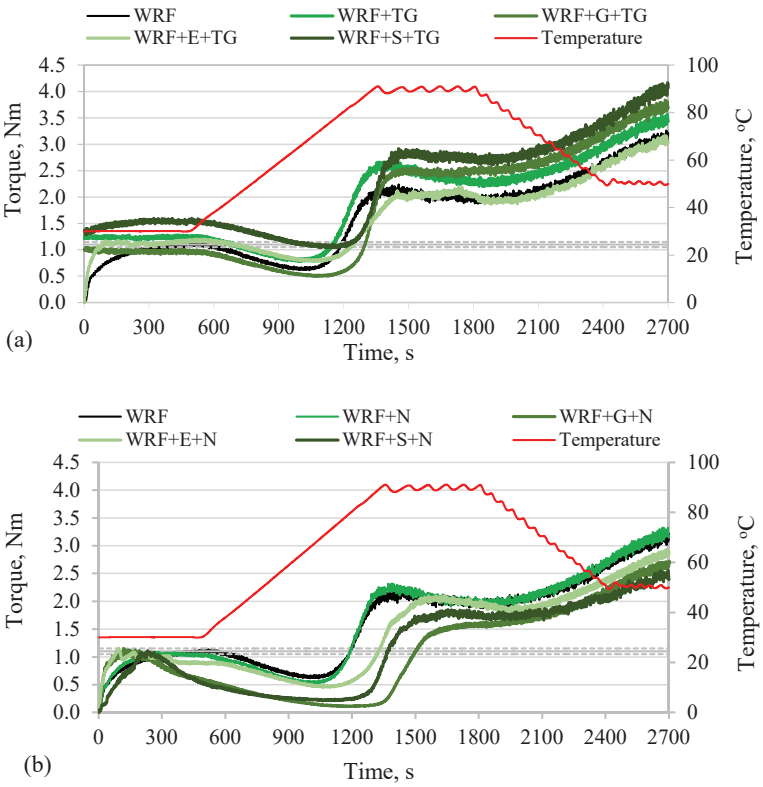


**Figure 3.** Thermo-mechanical behavior of the whole rice flour (WRF) supplemented with 15% vital gluten (G), powdered eggs (E) or soy protein concentrate (S) and treated with TG (a) or N (b)

These results might be due to the high molecular weight protein aggregates formation catalyzed by TG, resulting in a more continuous protein phase (Marco et al., 2007) in the investigated samples. Marco et al. (2008) and Tang et al. (2006) showed that TG is able to catalyze intermolecular cross-linking of proteins from soy and rice. Most of the cross-links involved β-conglycinin and glycinin from soy, and glutelin, albumin and globulin from rice (Renzetti and Rosell, 2016). In particular, the glycinin/β-conglycinin ratio appears to be decisive for the properties of the TG cross-linked soy protein based gels (Tang et al., 2006). Finally, in case of the gluten containing sample, addition of TG resulted in the increase of the C2, C4 and C5 values. A higher exposure of the lysine and glutamine residues was reported by Wang et al. (2007) when heating the gluten, therefore providing new sites recognized by TG. The TG catalyzed cross-links at thermal treatment might significantly contribute to defining the thermo-mechanical behavior of the dough in the regions of the Mixolab curve where starch is normally the main contributor. As shown by Wu and Corke (2005), gluten cross-linking might improve the water binding capacity due to the increase of overall hydrophobicity, therefore explaining the tendency observed in the torque values (Table 2).

**Table 2.** Effect of TG and N addition on the torque values of the whole rice flour (WRF) based batter samples supplemented with 15% gluten (G), powdered eggs (E) or soy protein concentrate (S). The torque values were assessed by Mixolab device during mixing and heating stages of the Chopin+ protocol.

| Sample | Enzyme treatment | C2-C2$_E$, Nm | C3-C3$_E$, Nm | C4-C4$_E$, Nm | C5-C5$_E$, Nm |
|---|---|---|---|---|---|
| WRF | TG | -0.028 | 0.017 | 0.022 | -0.035 |
| | N | 0.094 | -0.113 | -0.031 | -0.070 |
| WRF+S | TG | -0.053 | -0.179 | -0.646 | -1.539 |
| | N | 0.742 | 0.828 | 0.779 | 1.363 |
| WRF+E | TG | -0.049 | -0.030 | -0.02 | 0.039 |
| | N | 0.324 | -0.011 | 0.142 | 0.215 |
| WRF+G | TG | -0.028 | 0.034 | -0.285 | -0.267 |
| | N | 0.199 | 0.981 | 0.432 | 0.734 |

The addition of N to the whole rice flour based samples supplemented with 15% protein products caused important changes of the Mixolab curve with respect to the corresponding samples with no enzyme addition. Regardless of the type of protein used to supplement the whole rice flour, N caused the decrease of the C2 torque (Table 2). Moreover, the addition of N in the whole rice flour based samples supplemented with protein products modified the development time, the stability of the dough, and the speed of proteins softening. For instance, when N was added to the sample with powdered eggs the dough development time decrease from 8.77 min to 1.57 min, and the stability decreased from 11.6 min to 4.78 min. Similarly, a significant reduction of the development time from 9.37 min to 3.97 min, and of dough stability from 6.98 to 2.32 min was observed in case of adding N to the sample with soy protein isolate. Moreover, an increased protein softening, resulting in the decrease of the minimum C2 torque from 0.96 to 0.22 Nm, was associated to the hydrolysis of the soy proteins by N. In addition, a 4$^o$C shift of the temperature associated to the C2 values (from 64.3 to 59.3°C) was registered in the Mixolab curves, suggesting lower thermal stability of the soy peptides after the hydrolysis catalyzed by N. Unlike soy proteins, hydrolysis of the vital gluten incorporated into whole rice flour was accompanied by the increase from 55.6 to 63.6$^o$C of the temperature corresponding to the minimum C2 torque.

Regarding the toque values associated to starch behavior, except for the C3 which was higher when adding N to the powdered egg supplemented sample, all other torque values decreased in the samples where protein hydrolysis occurred (Table 2). Although acting on protein substrates, N significantly altered the Mixolab parameters related to starch gelatinization, gel stability and starch retrogradation. For instance, when adding N to the dried eggs supplemented sample, the C4 decreased from 1.986 to 1.844 Nm, and C5 from 3.095 to 2.880 Nm. Even if the most significant decrease of the torque values due to the N catalyzed hydrolysis was registered in case of the samples with soy proteins (C3 from 2.63 to 1.80 Nm, C4 from 2.49 to 1.71 Nm, and C5 from 3.83 to 2.47 Nm), the thermo-mechanical parameters indicated that the mixture is suitable to be used for gluten free bread applications. Ragaee and Abdel-Aal (2006) suggested that changes in starch behavior at thermal treatment might be a consequence of altering the starch - protein interactions, subsequent to the hydrolytic activity of proteases. Depending on the source and properties, when incorporated in certain matrices the protein products might prevent the starch granules to swell sufficiently. The proteins surrounding the starch granules confer rigidity to the starch paste, but proteins hydrolysis might induce viscosity decrease. Anyway, Renzetti and Arendt (2009) observed that lowering the viscosity of the batters based on rice flour, as a consequence of proteolytic enzymes addition can be correlated with the improvement of the volume of products.

## CONCLUSIONS

The rheological behavior of the protein based suspensions highly depended on the source of proteins and type of enzyme used for the preliminary treatment. The Mixolab curves indicated that transglutaminase and Neutrase had no significant influence on the rice proteins. The most important changes in the

thermo-mechanical behavior of the enzyme treated samples were registered in the Mixolab zones mainly showing the protein berhavior, in case of the soy protein containing samples. In addition to the protein source and composition, the cross-linking and hydrolysis reactions catalyzed by transglutaminase and Neutrase influenced the gelatinization and retrogradation of the starch from the whole rice flour.

## ACKNOWLEDGEMENTS

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# ISOLATION AND IDENTIFICATION OF YEAST IN TRADITIONAL COTTAGE CHEESE WITH STRAWBERRY AS COAGULANT

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***Abstract***

*Cottage cheese is one of the popular unripened cheese which made by acid addition for coagulate the casein. In traditional cheesemaking, fruit juice extract which has high acidity used to replace rennet as coagulants. The addition of fruit juice extract in the making of traditional cottage cheese has given specific characteristic such as flavor which also affected by the presence of microorganisms such as yeasts. The research aims to determine the presence of yeast in traditional cottage cheese that coagulated by Strawbery juice extract. Strawberry juice extract selected as cottage cheese coagulant because it has high acidity with the pH of 3.34. Cottage cheese made with pasteurized cow milk and mixed with 20%, 30%, 40% of strawberry juice extract until the pH turn to 5.85-5.93, curdled and added with 0.4% NaCl then solidified aseptically (modification of McMahon, 2005). Total yeasts counted by using total plate count method on the Malt Extract Agar with the addition of 10 ppm antibiotic (Roostita, et al., 2011) The yeasts colony identified using Remel RapID Yeast Plus to determine the species. Results showed that 40% addition of strawberry juice extract resulting the best yield of 32.07%, with the total yeasts of 5.98 x $10^7$cfu/g and Cryptococcus albidus as yeasts dominantly found in products.*

***Key words:*** *Cottage Cheese, Strawberry, Yeast, Cryptococcus albidus.*

## INTRODUCTION

It is widely recognized that yeasts can be an important component of the microflora of many cheese varieties because of the low pH, low moisture content, high salt concentration and refrigerated storage of these products. Nevertheless, yeasts play a dual role depending on the cheese. In fact, in some cheese types they make a positive contribution to the development of flavor and texture during the stage of maturation, while in other varieties, yeasts can be regarded as spoilage organisms. Yeast spoilage is recognized as a problem primarily in fermented milk and cheese (Abd. El-Gawad and Ahmed, 2011).

Yeasts in some cheese types can periodically cause both economic and public health problems. Yeasts themselves are not commonly the cause of defects in cheese unless they ferment lactose. In this case, they can grow rapidly and produce a characteristic yeasty or fruity flavor and obvious gas. There are numerous references concerning the significance of the presence of yeasts in dairy products, where they may contribute positively to the characteristic taste and flavor development during the stage of maturation or, on the contrary, may lead to product spoilage.

Cottage cheese is one of the popular unripened cheese which made by acid addition for coagulate the casein. In traditional cheesemaking, fruit juice extract which has high acidity used to replace rennet as coagulants. The addition of fruit juice extract in the making of traditional cottage cheese has given specific characteristic such as flavor which also affected by the presence of microorganisms such as yeasts.

In this study, we sought to obtain a dominant yeasts that presence on cottage cheeses with various level of strawbery juice as coagulant.

## MATERIALS AND METHODS

Cottage cheese made with pasteurized cow milk and mixed with 20%, 30%, 40% of strawberry juice extract until the pH turn to 5.85-5.93, curdled and added with 0.4% NaCl then solidified aseptically (modification of

McMahon, 2005). Total yeasts counted by using total plate count method on the Malt Extract Agar with the addition of 10 ppm antibiotic (Roostita, et al., 2011). The yeasts colony identified using Remel RapID Yeast Plus to determine the species.

## RESULTS AND DISCUSSIONS

*Cottage Cheese Yield*

Abd El-Gawad and Ahmed (2011) describes different aspects related to cheese yield: characteristics of the milk (contents of protein and fat, genetic variants of proteins, somatic cells), cheesemaking conditions (incorporation of whey proteins in the curd, homogenization of the fat, type of coagulant, use of different starters, curd firmness, type of vat, treatment of the curd). The same authors also consider different predictive formulas for determine cheese yield and strategies in order to minimize cheesemaking losses.

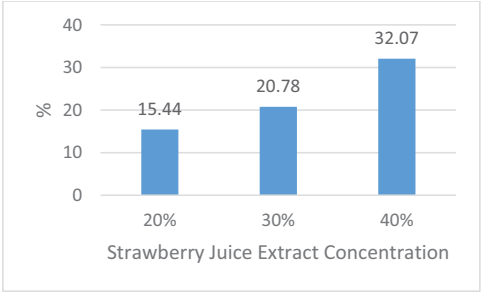


Figure 1. Cottage cheese yield with strawberry juice extract addition

The results showed that higher strawberry juice extract addition gave high cottage cheese yield and 40% strawberry juice extract addition gave the highest yield of 32.07% from the raw material.

Acidity of strawberry juice extract resulting coagulation. Yield of cottage and other cheeses is dependent upon casein content of milk.

The degrading effect of psychrotrophic proteases is much greater on casein than on whey proteins.

Any factor affecting the casein content of raw milk has a potentially great impact on yield of cottage cheese (Abd. El-Gawad and Ahmed, 2011).

Casein also sensitive to acid, the lower pH and high volume of strawberry juice extract tend to higher casein degradation that resulting the higher yield.

*Yeasts Population*

The cheese microbiota especially yeasts has long been known to be the major contributor to cheese flavor, aroma, texture, and appearance. The diversity and population of specific types of organisms present in cheese depend on the microbial quality of the raw material, handling and heat treatment of the raw material, manufacturing and curd-handling conditions, temperature and humidity during ripening, amount and manner of salting, and exposure of the cheese to exogenous microorganisms during and after manufacture (Bajara et al., 2015).

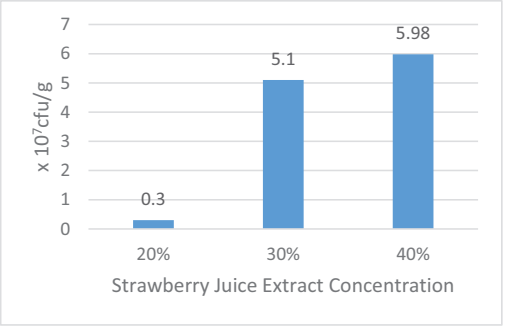


Figure 2. Yeasts population on cottage cheese with strawberry juice extract addition

Results showed that the highest strawberry juice extract addition resulting higher yeasts population. The 40% addition of strawberry juice extract resulting in $5.98 \times 10^7$ cfu/g yeasts population. Allegedly the yeasts population were came from the strawberry juice extract. Strawberry juice extract not only have a role as natural coagulant, but also as microbial contributor especially yeasts population that could gave a different characteristics on the cottage cheese product.

*Yeasts Identification*

The yeasts isolated differed between cheeses, cheese types, and samplings, as would be predicted. Yeast species that were not consistently detected between samplings or within a manufacturer's products are likely contaminants. The source(s) might be predicted

based on fungal ecology (i.e., farm environment, factory environment, or personnel), but cannot be conclusively traced. It is important to examine the production line and identify possible points in the process where the cheeses are exposed to yeast contamination (Banjara et al., 2015).

Results showed that the yeasts identified from the cottage cheese is *Cryptococcus albidus*. *Cryptococcus* spp. were one the yeasts that found on traditional cheese from Egypt (Soliman and Aly, 2011). This kind of yeasts has the ability to convert lipid from cheese whey (Seo et al., 2014).

| RapID | Yeast Plus | | | | | | |
|---|---|---|---|---|---|---|---|
| | | | | | | Run Date: | 1/11/2016 |
| | | | | | | Facility: | Universitas Padjadjaran |
| Microcode: | 736050 | | | | | Reference No: | CC1 |
| *System Tests* | +GLU 86% | +TRE 69% | -NAGA 00% | -ONPG 00% | +PHS 90% | -PRO 70% | |
| | +MAL 61% | +RAF 11% | +αGLU 90% | -αGAL 01% | -PCHO 88% | -HIST 61% | |
| | +SUC 81% | -LIP 14% | +βGLU 92% | -βFUC 18% | +URE 90% | -LGY 11% | |

**ID = Cr. albidus ..Rare Biotype**

| Choice | Probability | Bioscore | Contraindications |
|---|---|---|---|
| Cr. albidus | > 99.9% | 1/5310 | RAF [11] PCHO[88] |

Probability Level: **Adequate**
Biofrequency: **Rare**

Figure 3. Yeasts identification with RapID Yeasts Plus System

## CONCLUSIONS

Results showed that 40% addition of strawberry juice extract resulting the best yield of 32.07%, with the total yeasts of 5.98 x $10^7$cfu/g and *Cryptococcus albidus* as yeasts dominantly found in products.

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# THE OCCURRENCE OF YEASTS AND FUNCTIONAL PROPERTIES OF INDONESIAN ETHNIC FERMENTED FOODS AND BEVERAGES

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***Abstract***

*Indonesia has a great diversity of ethnic fermented foods and beverages. Besides tempe, there are many famous fermented ethnic products in Indonesia, some of them are Tape', Dadih, Dangke, Bakasam and Sie Reuboh. Tape' is ethnic fermented cassava which fermented by the consortium of lactic acid bacteria, yeast and mould contained in ragi. Dadih is buffalo milk that naturally fermented inside the bamboo, while Dangke is buffalo milk which curdled using papaya latex and then fermented in coconut shell. Bakasam is meat which anaerobic-naturally fermented with the addition of rice, salt and sugar. Meanwhile, Sie Reuboh is meat that fermented with the addition of palm vinegar.*
*Even yeasts were not the main microorganisms in the fermentation process, their functional properties were highly recognized in resulting the important foods characteristics. The yeasts isolated from Tape' such as Saccharomycopsis fibuligera shown great potential of antimicrobial and proteolytic activities (Roostita, et al., 2011). Candida curiosa, Brettanomyces custersii and Kluyveromyces lactis isolated from Dadih shown an antimicrobial activities towards B. subtilis, E. coli and S. aureus (Yurliasni, 2010). Dangke also shown antimicrobial activities towards E. coli and S. aureus. The yeasts population isolated from Bakasam and Sie Reuboh shown protelytic activities.*
*The functional properties of yeasts that isolated from Indonesian ethnic fermented foods were originally has great potential to develop as commercial products. Bioactive compound that showing antimicrobial activities towards the pathogenic bacteria can be purified and developed as food bioperservatives or even nutriceutical products. Meanwhile the proteases produced by yeasts also could developed as local enzyme that could reduced the dependency to the imported enzyme.*

***Key words:*** *Indonesia, Ethnic Fermented Foods, Beverages, Functional properties.*

## INTRODUCTION

Indonesia is a great country in South East Asia which have a great diversity of ethnic, culture and also ethnic foods and beverages. More than 200 millions population and 1.100 ethnic in Indonesia which possible to have more than 5.500 ethnic foods and beverages. Some of the foods and beverages produced by using fermentation process which resulting well-known and liked products because of their unique characteristics.

Microorganisms has important roles in specify the characteristics of ethnic foods and beverages produced. Ethnic foods and beverages usually made by natural spontaneous fermentation that involving mixed-culture with the main role of one strain of the microorganism and the other strain role as contaminants (Pawiroharsono, 2007). The contaminants in ethnic food and beverages fermentations does not always give harmful effects, many of them generate good effects for the products (Yuan, 1999).

There are many famous Indonesian ethnic fermented foods and beverages. One food that well-known worldwide was tempe which resulted from soybean fermentation using *Rhizopus sp.* especially *R. oligosporus, R. oryzae, R. Arhizus, R. Stolonifer* and *R. microsporus* (Astuti, et al., 2000). Meanwhile domestically, other fermented foods and beverages such as *Tape'*, *Dadih*, *Dangke*, *Bakasam* and *Sie Reuboh* were also liked by the peoples and the number of consumption was relatively high.

All of Indonesian ethnic fermented products mentioned above, showing the presence of microorganisms diversity that involve in the fermentation process. *Tape'* was cassava which

fermented with addition of dried mixed starter which called *ragi tape'* that naturally contains filamentous fungi, yeast and bacteria (Sujaya, et al., 2002; Sujaya, et al., 2010). Meanwhile domination of lactic acid bacteria were found in *Dadih*, *Dangke*, *Sie Reuboh* and *Bakasam* with the important role of yeasts which resulting specific characteristics of the products (Surono, 2003; Suhairi, 2007; Yurliasni, 2010; Wikandari, et al., 2012; Kesuma, et al., 2013).

The presence of yeasts in ethnic fermented foods and beverages are mostly role as contaminants that have been widely studied as giver of flavors and accelerate the maturity of the products (Wyder & Puhan, 1999; Roostita & Fleet, 1996). The population mostly range from $10^6$-$10^7$cfu/g and has an important activity in acid metabolism so as to raise the pH and has biochemical activity that produces effects on the food products (Fleet, 1990; Heard & Fleet, 1999).

Besides give good effects towards products characteristics, the presence of yeasts in ethnic fermented foods and beverages also shown functional effects. *Saccharomycopsis fibuligera* from *Tape'* shown antimicrobial and proteolytic activities (Roostita, et al., 2011). Indigenous *Dadih* yeasts such *Candida curiosa*, *Brettanomyces custersii* and *Kluyveromyces lactis* shown an antimicrobial activities towards *B. subtilis*, *E. coli* and *S. aureus* (Yurliasni, 2010). *Dangke* as a products could decrease the activities towards *E. coli* and *S. aureus* and 0.06-2.89 x $10^4$ cfu/g yeasts population isolated from *Bakasam* and *Sie Reuboh* shown protelytic activities (Roostita, et al., 2009) .

The functional properties of indigenous yeasts and Indonesian ethnic fermented foods were originally has great potential to develop as commercial products. Bioactive compound that showing antimicrobial activities towards the pathogenic bacteria can be purified and developed as food bioperservatives or even nutriceutical products. Meanwhile the proteases produced by yeasts also could developed as local enzyme that could reduced the dependency to the imported enzyme.

## Yeasts Occurrence and Functional Properties in Indonesian Ethnic Fermented Cassava

Many kind of Indonesian ethnic fermented foods and *Tape'* is one of the most popular among them. *Tape'* made from cassava that fermented with dried mixed starter which called *ragi tape'* that naturally contains filamentous fungi, yeast and bacteria (Sujaya, et al., 2002). Microorganisms especially yeasts that live on *Tape'* utilize simple and complex sugars as their carbon source (Lewis & Young, 1990).

*Tape*' is potential as yeasts habitat. Yeasts population of $2 \times 10^6$ cfu/g was found and shown antimicrobial activities in *Tape*' (Roostita, et al., 2011). Beside that, yeasts also generate proteolytic activity by producing extracellular protease (Roostita & Fleet, 1996). *Saccharomycopsis fibuligera* strain R64 were one of isolated yeast from *tape'* that produced extracellular protease with optimum pH of 5 and temperature of 25°C (Roostita, et al., 2012)

The extracellular protease produced by yeasts is well known and many people utilized it for their activities. Yeasts extracellular protease has potential in beer and wine stabilization (Ogrydziak, 1993). Proteolytic enzymes have some important role in medicine such as food digestion, protein turnover, blood coagulation, embryonic development and cell division (Reid, 2012). Therefore, the enzymes were an important group in scientific, medical research and biotechnology (Rawlings, et al., 2009).

## The Role of Yeasts and Functional Properties of Indonesian Ethnic Fermented Milk

*Dadih*, an Indonesian ethnic fermented milk of West Sumatra is made by pouring fresh raw unheated buffalo milk into a bamboo tube capped with banana leaves, and allow to ferment at room temperature for two days. The use of buffalo milk in West Sumatra aims to exploit abundant buffalo milk. Buffalo milk are less preferred when consumed in a fresh state because of the smell. Fermentation is done so the flavour will be more acceptable.

The making of *Dadih*, involves several kinds of microorganisms including lactic acid bacteria (LAB), molds and yeasts. The existence of yeasts in the fermentation of *Dadih* should be considered, because it can make a positive contribution during the fermentation process and end products such provide growth factors for other microorganisms and also as flavor enhancer. In addition secondary metabolites produced by yeasts such as acetate, succinate, propionate, fumarate and piruvat has a good influence on the taste and have the ability as antimicrobial which can inhibit the growth of pathogenic bacteria.

There are three potential yeasts isolated from *Dadih*, such as *Kluyveromyces lactis*, *Candida curiosa*, and *Brettanomyces custersii*. *Kluyveromyces lactis* has strong antimicrobial activity against *B. subtilis* with clear zones of inhibition 5mm, *C. curiosa* against *E. coli* with inhibition zone 5mm, and *C. curiosa* and *Brett. custersii* against *S. aureus* with clear zones of inhibition respectively 5.75mm and 7mm which showed that the yeast isolated from *Dadih* is able to inhibit the growth of pathogenic bacteria (Yurliasni, 2010).

*C. curiosa*, *Brett. custersii* and *Kluy. lactis* have strong activity against gram-negative bacteria compared to gram-positive bacteria. Antimicrobial activity would be seen when the interaction between yeast and bacteria occur (Golubev & Boekhout, 1992). The interaction not only indicate a positive or negative traits of fermentation process, but involves antagonistic activity against yeasts and other microorganisms with produce micocin (anti-microbial compounds).

Different with *Dadih*, *Dangke* is an Indonesian ethnic fresh soft cheese that is usually made from fresh cow milk or buffalo milk by the farmers' households in Enrekang regency, South Sulawesi province. *Dangke* made by heating with a small fire to boil, then add coagulant in the form of sap of papaya (papain) resulting in natural clotting which change the cow's or buffalo milk become solid due to the separation of protein and water (Rahman, 2013).

Cow milk *Dangke* has a high nutrient content (water content of 55%, protein of 23.8%, fat of 14.8% and ash of 2.1%) and its near normal pH value of 6.4 (Hatta, et al., 2013). Its shelf life is generally two days at room temperature, while at the refrigerator temperature, it can reach five to seven days. Preservation method is usually done by the community is the addition of salt solution.

Functional properties of *Dangke* shown by the microorganisms involves in the fermentation process. Some of microorganisms such lactic acid bacteria isolated from *Dangke*, producing bacteriocin that could inhibit pathogenic bacteria *Salmonella typhiimurium* (Razak, et al., 2009). As well as yeasts capable of producing metabolites and create condition that is not conducive for harmful microorganisms such as *E. coli* and *Salmonella spp.* which is the main contaminant of *Dangke* (Hatta, et al., 2013)

**Functional Properties and Yeasts Role in Indonesian Ethnic Fermented Meat**

*Bakasam* is ethnic fermented meat from Lampung, Indonesia. It has acid flavor. The ingredients of *Bakasam* were 20 gram of rice, 2 gram of salt, 0.2 gram of sugar, and 100 gram of top side meat. The fermentation carried out until 15 days under anaerobic condition. The microorganism grew in anaerobic situation of fermentation process were the bacteria, yeasts, or mold (Buckle, et al., 1987; Winarno & Fardiaz, 1993).

Yeasts was able to grow in many products including fresh meat and its processed products (Roostita, 2004). Meat was beneficial since it contained the nutrient needed by the body. Some yeasts found in fresh meat were *Candida, Debaryomyces, Rhodotorula,* and *Torulopsis;* while large number of yeasts found in processed meat and cured yeast meat were *Candida, Torula, Torulopsis, Trichosporon* dan *Debaryomyces* (Dwidjoseputro, 2003; Jay, 1996; Roostita, 2004). Fermentation process appeared as a result of anaerobic type of metabolism.

The growth of yeasts in *Bakasam* increased until day 3 ($23.91 \times 10^4$ cfu/g), and then it decreased until day 15 ($0.46 \times 10^4$ cfu/g). The amount of total yeasts with proteolys activity found in *Bakasam* meat ranged from

$0.06x10^4$cfu/g to $2.89x10^4$ cfu/g. The growth of yeasts increased until day 6 ($2.89 \times 10^4$ cfu/g), and then it decreased until day 15 ($0.21 \times 10^4$ cfu/g) (Roostita, et al., 2009).

At the early stage of fermentation, the number of total yeast with proteolysis activities was found to be the least. It was because the yeasts with proteolysis activities just recently broke the protein inside the meat especially proteolysis one, used protein as their energy source (Soeparno, 2005).

It happened since the yeast with proteolysis activities experienced their growth process by breaking the protein inside the meat. The activity was assumed as the activity of protease enzyme that came from high number of yeast. Then, these enzymes experienced autolysis that resulted in high number of yeasts death. Thus, high numbers of yeast colonies were found. Yeast with intracellular proteases activity was contributed in the presence of proteolysis activity (Roostita, 1993). The yeast produced the protease enzyme not only outside the cell, but also inside the cell (intracellular activity) that can only be seen and be measured only if the autolysis from its yeast cell occurred (Roostita, 2004).

Different with *Bakasam*, *Sie Reuboh* is a beef or buffalo meat products from Aceh which manufactured by using ingredients such as vinegar, fat, salt and spice inside the slice meat and then heated (Suhairi, 2007). Beside the proteolytic yeasts that grew in the beef or buffalo meat, acetic acid bacteria in vinegar were contribute in making acid condition that is not suitable for spoilage and pathogenic microorganisms. *Saccharomyces spp.* and *Acetobacter spp.* dominantly grew in vinegar that added into beef or buffalo meat which will be made *Sie Reuboh* resulting in functional effects in the end products.

## CONCLUSIONS

Indonesian ethnic fermented foods and beverages such *Tape', Dadih, Dangke, Bakasam* and *Sie Reuboh* shown presence and role of yeasts which determined from the products characteristics. Yeasts presence gave great potential of functional effects to developed i.e. proteolytic activities and antimicrobial activities towards spoilage and pathogenic bacteria. The metabolites (extracellular proteolytic enzymes, antimicrobial compound) produced by yeasts still need to be developed so that could give best functional effects if it is produced as commercial products.

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# ANTIOXIDANT AND ANTIMICROBIAL PROPERTIES OF PLANT EXTRACTS AND THEIR RECENT APPLICATIONS IN MEAT PRODUCT PROCESSING

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***Abstract***

*This review study aimed to give information about the use of plant extracts in meat product processing as antimicrobial and antioxidant agent. Microbial spoilage and lipid oxidation are the major causes of the deterioration and reduction of shelf-life in meat products. Lipid oxidation in meat products results in formation of off-flavors and undesirable chemical compounds such as aldehydes, ketones, alcohols and hydrocarbons. Growth of microorganisms in meat products causes not only microbial spoilage but also development of food borne diseases. To inhibit lipid oxidation and growth of microorganisms, especially pathogenic microorganisms in meat products, several preservation techniques, such as pasteurization, reduction of water activity (salting, drying, freezing etc.), acidification, fermentation, synthetic and natural antimicrobial and antioxidant additives have been used in meat industry. Many synthetic and natural food additives such as butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), propyl gallate, α-tocopherol, nisin and organic acids are commonly used in the meat industry to inhibit or delay the oxidation process and reduce the microbial growth. In recent years, consumer demands for natural food additives have increased because of negative and toxic effects of synthetic food additives on human health. Herbs, spices, fruits and vegetables, and their powders, oils and extracts have been reported to be a good source of various phenolic compounds, such as flavonoids, terpenoids, carotenoids, could therefore be incorporated in meat products as a source of natural antioxidants and antimicrobials to extend shelf-life and safety of meat products.*

***Key words****: Meat, antioxidant, antimicrobial, plant, extract.*

## INTRODUCTION

Meat and meat products are sensitive to quality deterioration due to their rich nutritional compound such as proteins, lipids, vitamins and minerals. Microbial growth and chemical changes are the two main causes of deterioration in meat products (Shah et al., 2014). As a result of lipid oxidation, undesirable reactions that deteriorate flavor, odor, color, sensory and textural properties of meat products can be occurred (Shah et al., 2014). Pathogenic microorganisms can also potentially cause food borne diseases (Lucera et al., 2012). Lipid oxidation and microbial growth can be reduced by applying synthetic or natural antioxidant and antimicrobial agents to the meat product processing to improve the product quality, shelf-life and safety (Kim et al., 2013).Synthetic food additives have been widely used for inhibiting lipid oxidation and microbial growth in meat products due to their strong antioxidant and antimicrobial activities, and their low production cost and easy accessibility (Falowo et al., 2014). Due to the potential toxicological effects of synthetic antioxidants, the use of alternative natural additives has become widespread due to consumer demands. Herbs, spices, fruits and vegetables, and their powders, oils and extracts were found to be a good source of natural antioxidants and antimicrobials to extend food quality and stability.

### Antioxidative Effective Plant Extracts

Antioxidants can inhibit the oxidation of lipids, proteins, carbohydrates and pigments in meat products, therefore the product quality and shelf-life can be improved by antioxidants (Karre et al., 2013). Antioxidants can delay or inhibit the oxidation process through breaking the oxidative free radical chain reaction, decomposing peroxides, deactivating singlet

oxygen, chelating metal ions, absorbing ultraviolet radiation and scavenge oxygen (Shah et al., 2014). Antioxidants, which are widely used in meat products, are divided into two groups as synthetic and natural. Natural antioxidants from plants have been obtained from different sources such as fruits, vegetables, herbs and spices (Falowo et al., 2014).

There are a number of studies on the use of natural antioxidants in meat products, and it appears that these antioxidants have been extracted from different plant parts such as leaves, roots, stems, fruits and seeds (Rather et al., 2016).The extracts of rosemary, grape seed, ginger, cinnamon, garlic, pomegranate, broccoli, onion, myrtle, mint, nettle and green tea have been widely studied for their antioxidant potential (Banerjee et al., 2012; Karre et al., 2013). The antioxidant effect of echinicea, mysore thorn, mango seed, cranberry and strawberry, citrus peel, coffee, olive leaf, oregano, adzuki bean and carob fruits extracts were also investigatedin broiler meat, beef patties, bologna type-mortodella, rabbit meat, raw chicken drumettes, pork patties, cooked beef and pork, pork sausages (Carpenter et al., 2007; Rojas and Brewer, 2008; Mirshekar et al., 2009; Jayawardana et al., 2011; Karre et al., 2013; Falowo et al., 2014; Rather et al., 2016). Furthermore, the antioxidant effects of aloe vera, fenugreek, ginseng, mustard, rosemary, sage extracts and tea catechinswere studied on pork patties (McCarthy et al., 2001).The results of some previous studies are presentedbelow.

Zhang et al. (2016) stated that cloves and rosemary extracts were highly effective against lipid oxidation and had potential to be used as a natural antioxidant in raw chicken meats. El-Zainy et al. (2016) demonstrated that grape seeds polyphenols extract was effective in terms of lowering TBARS in raw beef sausage. Qi and Zhou (2013) reported that the lotus seed epicarp extract significantly delayed the level of lipid oxidation in pork homogenates. Banerjee et al. (2012) reported that TBARS values of broccoli powder extract containing nuggets were lower than those with BHT, furthermore, 2% was the most effective concentration. Rababah et al. (2011) reported that green tea extract, commercial grape seed extract and TBHQ significantly decreased lipid oxidation of the goat meats. Wojciak et al. (2011) indicated that green tea, rosemary and red pepper extracts effectively reduced the lipid oxidation in cooked pork. Additionally, researcher reported that pepper extract showed the lowest TBARS. Kanatt et al. (2010) noted that pomegranate peel extract observed significant antioxidant activity whereas the pomegranate seed extract did not have any significant activity. Akarpat et al.(2008)reported that the lipid oxidation in beef patties was slowed down by myrtle, rosemary, nettle and lemon balm leaf extracts. Myrtle and rosemary extracts showed the higher antioxidant effects than nettle and lemon balm extracts. Ahn et al. (2007) found that grape seed extract, pine bark extract, oleoresin rosemary and synthetic antioxidants (BHA/BHT) delayed the formation of TBARS by 92%, 94%, 92% and 75%, respectively, and significantly lowered the hexanal content. Lee and Ahn (2005) reported that plum extract reduced TBARS effectively in irradiated turkey breast rolls. Rojas and Brewer (2008) found that oregano extract (0.02%) was effective at reducing lipid oxidation in vacuum-packaged cooked beef. Carpenter et al. (2007) found that grape seed and bearberry extract significantly decreased lipid oxidation in raw and cooked pork patties. Lee et al. (2006) also showed that cranberry extract exhibited 51% of TBARS formation in cooked pork. El-Alim et al. (1999) found that basil, sage, thyme and ginger extracts were effective antioxidants in meat system.

**Antimicrobial Effect of Plant Extracts**

The use of natural antimicrobials such as organic acids, essential oils, plant extracts could be a good strategy to inhibit microbial spoilage of meat products (Negi, 2012). The plant extracts and essential oils demonstrated potential antimicrobial effects according to the following mechanisms: (1) The phenolic compounds in these extracts and essential oils affect enzyme activity or cause protein denaturation, respectively. (2) It causes changes in the permeability of microbial cells.(3) It causes changes in the functions of the normal activity of cell membranes such as

electron transfer, nutrient exchange, protein synthesis, nucleic acids and enzymatic activity (Aminzare et al., 2016).There are many studies on the use of extracts and especially essential oils from different plant sources such as ginger, cinnamon, garlic, rosemary, oregano, basil, cloves, marjoram, turmeric and sage in order to determine the antimicrobial activity on meat products (Lucera et al., 2012).

Abdulla et al. (2016) indicated that the Ziziphus leaves extracts inhibited the growth of *Bacillus subtilis, Escherichia coli, Neisseria gonorrhoeae, Pseudomonas aeruginosa, Staphylococcus aureus* and *Streptococcus faecalis*, and decreased the total viable counts in sausages. Zhang et al. (2016) reported that the spice extracts (rosemary, cloves and their combination) were highly effective against microbial growth in raw chicken meats. Kramer et al. (2014) pointed out that the inhibitory activity of the hop extracts against *L. monocytogenes* was strongly reduced in a fat-containing model meat marinade system. Nejad et al. (2014) reported that the 1 mL garlic aqueous extract was effective in decreasing the growth of *S. aureus* in hamburgers. Baker et al. (2013) found that the rosemary and ginger extract or their combination with sodium lactate have an inhibitory effect against the coliform, lipolytic, proteolytic and psychrophlic bacteria. Uçak et al. (2011) reported that rosemary extract in combination with vacuum packaging was effective controlling microbial growth in fish burgers. Jałosińska and Wilczak (2009) reported that rosemary, cranberry and lovage extracts inhibited growth of the microorganisms in meatballs and rosemary extract was characterised with the strongest antimicrobial activity. Ahn et al. (2007) indicated that 1.0% grape seed and pine bark extracts were effectively reduced the numbers of *E. coli* O157:H7, *Salmonella Typhimurium, L. monocytogenes* and *Aeromonas hydrophila* in cooked beef. Kim and Fung (2004) indicated that the arrowroot tea extract slightly inhibited *S. enterica* serotype *enteritidis* and *L. monocytogenes* in ground beef. Careaga et al. (2003) reported that 1.5 mL/100 g Capsicum extract was adequate to inhibit *S. typhimurium* in raw minced beef, however, the required extract dose for a bactericidal effect against *P. aeruginosa* was 3 mL/100 g.

## CONCLUSIONS

The use of extracts for antioxidant and antimicrobial effects has been widely investigated in different types of meat and meat products. These studies demonstrate that plant extracts have antioxidant and antimicrobial effects. The results pointed out that extracts are as effective as or better than synthetic antioxidants. Antimicrobial effects of extracts have been shown to be highly effective in *in vitro* studies, however, antimicrobial effect may decrease when extracts are added into meat systems. Thus, there is need for more research to improve the antimicrobial efficiency of plant base extracts in meat systems. It can be concluded that using plant extracts in meat product processing is beneficial strategy to extend shelf-life and safety of meat products.

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# POLYUNSATURATED FATTY ACID (PUFA) CONTENTS OF MEAT AND EGG OF RAINBOW TROUT FISH (*ONCORHYNCHUS MYKISS*)

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***Abstract***

*Long-chain omega-3 polyunsaturated fatty acids (PUFA), including eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) and the ratio of n3/n6 is important factor for a healthy life. Fish and seafood are good source of PUFA. This research aimed to determine the fatty acid profile of rainbow trout meat and egg, compare the PUFA contents of these supplies and reveal their relation with health. Rainbow trout meat and egg contains high amount of PUFA, followed by MUFA and SFA. PUFA/SFA ration in trout meat is 1.64, meanwhile this ratio is 2.23 in trout egg. EPA+DHA content of the eggs are (23.23) higher than fish flesh (14.01). Rainbow trout egg meets the recommended values of PUFA/SFA, n3/n6 and EPA+DHA contents better than rainbow trout meat. Both fatty acids supplies have atherogenicity and thrombogenicity index values close to the recommended levels. Rainbow trout meat and its egg are good sources of long-chain PUFA.*

***Key words****: Rainbow trout, fish egg, Oncorhynchus mykiss, PUFA, Omega-3 fatty acids.*

## INTRODUCTION

Polyunsaturated fatty acids are fatty acids that contain more than one double bond in their carbon chain and mostly composed of 18-20 carbons. They are categorized into two main sections; omega-6 ($\omega$6 or n-6) and omega-3 ($\omega$3 or n-3) depending on the position of the first double bond from the methyl end group of the fatty acid (Venegas-Calerón et al., 2010). PUFA are reported to be in relation with prevention of cardiovascular diseases and have certain efficacy in preventing illnesses with an inflammatory component (Grosso et al., 2016). They are also reported to reduce hypertension, asthma, immune system disorders, susceptibility to mental illness, protection against heart disease, and improved brain and eye functions (Yerlikaya et al., 2013).

The essential omega-3 (n3) PUFA α-linolenic acid (ALA, C18:3n3) can be converted in humans to the long chain PUFA eicosapentaenoic acid (EPA, C20:5n3) and docosahexaenoic acid (DHA, C22:6n3) in a multistage chain elongation and desaturation process (Schuchardt et al., 2016). However, this process is slow and inefficient in human body. The conversion rate from ALA to EPA (~5%) and especially to DHA is low (~1%) (Plourde and Cunnane, 2007).

Therefore, it is essential to supply PUFA from food. Marine derived food contains a high proportion of long chain PUFA. Aquaculture is one of the fastest growing food sectors in the world.

The most common type of breeding is rainbow trout in Turkey and even in the world. Rainbow trout is a species with large eggs and these eggs are affected from dietary components as diverse as fatty acids, vitamins pigments and proteins (Fontagne-Dicharry et al., 2017).

Fish egg contains 11% albumin, 75 ovoglobulin and 13% collagen (Sikorski, 1994). Fish egg has a high content of nutritive lipids, particularly phospholipids and long chain unsaturated fatty acids (Mahmoud et al., 2008). Fish eggs are commonly consumed due to its high amount of protein, lipids, vitamins and minerals.

The objectives of this study were; (i) determine the fatty acid profile of rainbow trout meat and its egg, (ii) compare the PUFA contents of the supplies and (iii) reveal their relation with health.

## MATERIALS AND METHODS

### *Materials*

Farmed rainbow trout (*Oncorhynchus mykiss*) was purchased from the seafood market in Antalya, Turkey and fresh milked rainbow trout eggs were obtained from the fish farm located in Akçay creek (Finike-Antalya, Turkey). Fishes and fish eggs were transferred to laboratory in polystrene boxes with in an hour. The fishes were beheaded and gutted at once. The samples were packed in polyethylene pouches and stored at -80 °C prior to analysis.

### *Analytical procedures*

*FAME Analysis: Fatty Acid Esterification*

A lipid sample of 10 mg dissolved in 2-mL n-heptane was mixed with 4-mL 2-M methanolic KOH and centrifuged at 4,000 rpm for 10 min (Ozogul et al., 2007). The upper layer was injected into a gas chromatograph (GC; Clarus 500, Perkin Elmer, Waltham, MA, USA).

*FAME Analysis: Gas Chromatographic Conditions*

A GC instrument with BPX70 fused silica column (50 m × 0.22 mm, film thickness 0.25 µm; SGE Inc., Victoria, Australia) and equipped with a flame ionization detector was used. The oven temperature was held at 140°C for 5 min, and then raised to 200°C at 4°C/min and without holding, raised to 220°C at 1°C/min. T

he injection temperature was set at 220°C. Helium was the carrier with 1.0 mL/min flow rate. The detector temperature was set at 280°C.

The split used was 1:50. Fatty acids were identified by comparison with the retention times of standard fatty acid methyl esters (FAME Mix, C4-C24 Unsaturates, Supelco, Bellefonte, PA, USA). The results were expressed as a percentage of the total of the identifiable fatty acids.

*Health lipid indices*

From the data of fatty acid profile, the atherogenicity (AI, showing the inhibition of the aggregation of plaque and diminishing the levels of esterified FA, cholesterol, and phospholipids, thereby preventing the appearance of micro- and macro-coronary diseases) and thrombogenicity (TI, showing the tendency to form clots in the blood vessels) were calculated as follows (Ulbritcth and Southgate, 1991).

$$AI = [12:0 + (4x14:0) + (16:0)] / (\Sigma MUFA + \Sigma PUFA\ n\text{-}6 + \Sigma PUFA\ n\text{-}3)$$

$$TI = (14:0+16:0+18:0) / [(0.5 \times \Sigma MUFA) + (0.5 \times \Sigma PUFA\ n\text{-}6 + (3x\ \Sigma PUFA\ n\text{-}3) + (n\text{-}3/(n\text{-}6)]$$

### *Statistical analysis*

Homogenized samples were prepared as two parallels and two recurrences. Test plan was defined and analyses of variance (ANOVA) carried out. Different results are used for multiple comparison tests. Statistical analysis was performed using SAS program (Statistical Analytical Systems, Cary, NC) (Duzgunes et al., 1987).

## RESULTS AND DISCUSSIONS

The lipid contents of rainbow trout and its egg were 5.66±0.04 g and 10.34±0.84 in wet weight, respectively.

Fatty acid profile of rainbow trout meat and egg revealed that these foods are good sources of PUFA (Figure 1). Total PUFA contents in rainbow trout meat were 40.76% and 43.01% in egg. Mono-unsaturated fatty acids (MUFA) content ranged between 34.26 and 36.66%. Both sources had low saturated fatty acids (SFA) content.

It is recommended that a diet must be composed of high concentrations of PUFA. The ratio of n6/n3 should be less than 4, n3/n6 should be more than 6 and PUFA/SFA should be more than 0.4 (Wood et al., 2003). PUFA/SFA ration in trout meat is 1.64, meanwhile this ratio is 2.23 in trout egg.

The higher values are preferred in both PUFA/SFA and n3/n6 ratios which are satisfied with rainbow trout egg due to prevention of coronary hearth diseases, plasma lipid levels and cancer risks (Simat et al., 2015).

As can be seen from Figure 2, EPA+DHA content of the eggs are (23.23) higher than fish flesh (14.01).

Long-chain PUFA especially EPA and DHA are reported to have cardio-protective effects. Simopoulos (1999) reported that daily

consumption of EPA+DHA should not be less than 0.22 g.
The consumption of rainbow trout meat and egg meets this requirement.

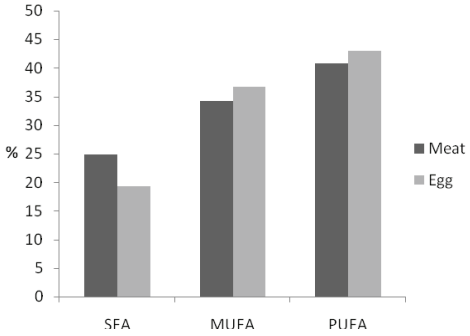


Figure 1. SFA, MUFA and PUFA contents of rainbow trout meat and egg

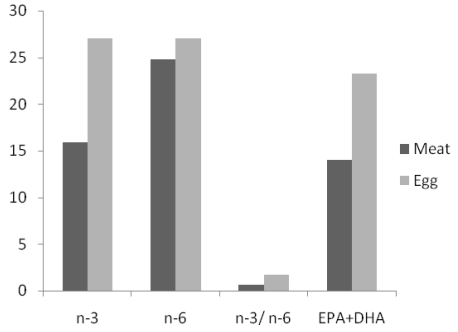


Figure 2. n-3, n-6, n3/n6 and EPA+DHA contents of rainbow trout meat and egg

Atherogenicity and thrombogenicity indexes are related with both coronary heart diseases and nutritional quality of fatty acids (Figure 3).
The recommended level is 0.4-0.5 which is considered beneficial for humans (FAO/WHO, 1994).
Both fatty acids supplies have values close to the recommended levels.
Higher levels of plant-originating C18:2n-6 presents in manufactured feeds affect the n-3/n-6 ratio in the edible part of the fish, thus reducing the nutritional quality of lipids Grigorakis, 2007).
The broodstock diets such as certain nutrients and antioxidants have direct effect on egg quality (Sawanboonchun et al., 2008).

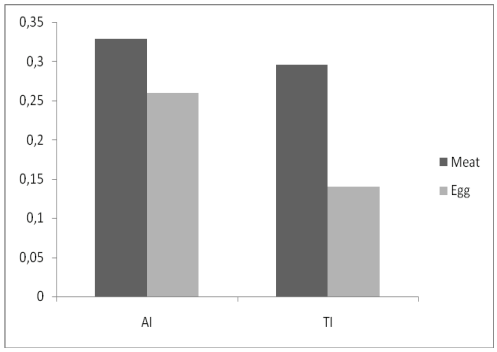


Figure 3. AI and TI values of rainbow trout meat and egg

## CONCLUSIONS

A comparison of PUFA content between fish species and its egg has not been examined before. It was revealed that rainbow trout egg has higher PUFA content than fish meat. Moreover, the eggs of rainbow trout meet the recommended values for PUFA/SFA, n3/n6 ratios and EPA+DHA content. Both rainbow trout meat and egg can be consumed as good sources of healthy fatty acids.

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# COMPARISON OF ESSENTIAL TRACE ELEMENT PROFILES OF RAINBOW TROUT FISH (*ONCORHYNCHUS MYKISS*) MEAT AND EGG

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***Abstract***

*Many mineral compounds, present in fish meat, are essential for human life at low concentrations. Humans need a steady supply of at least minerals to maintain health and optimal performance. The elements such as Fe, Cu, Mn and Zn are essential elements because of their crucial role in biological systems. In this study, essential element profiles of rainbow trout meat and its egg were investigated and compared each other. Higher concentrations of essential trace elements (Fe, Cu, Mn and Zn) were found in egg of rainbow trout compared to its meat. Highest Zn (140.28±4.24 µg/g) content was determined in trout egg samples compared to other trace elements. Fe (48.17±1.61 µg/g) was the most abundant trace elements found in trout meat, whereas Mn (1.24±0.04 µg/g) was the least.*

***Key words****: Rainbow trout egg, fish meat, Oncorhynchus mykiss, essential trace elements.*

## INTRODUCTION

Seafood is widely consumed in all over the world because of its high protein, lipid (Omega-3 fatty acids) and mineral content. Aquatic foods are very rich sources of macro and trace elements known as minerals. The total mineral content of aquatic foods is in the range of 0.6–1.5% wet weight (Erkan and Özden, 2007). Five macro (sodium, Na; potassium, K; magnesium, Mg, calcium, Ca; phosphorus, P) and four trace elements (iron, Fe; manganese, Mn; copper, Cu; zinc, Zn) are essential for regulation of healthy functions in the human body. The major minerals, Ca, P and Mg, are involved in bone health. Fe is the most abundant trace element in the human body and its insufficient daily intake results in anaemia. Mg, Mn and Zn are responsible for activity regulation of several enzymes (Taskaya et al., 2009).

Rainbow trout (*Oncorhynchus mykiss*) is a member of the Pacific trout and belongs to the Salmonidae family. They survive in cold, clear and well-oxygenated lakes, rivers and streams with the ideal temperature, ranging between 13°C and 15.5°C (Fallah et al., 2011). Rainbow trout is widely cultured, appreciated and consumed fish species. Fish flesh and egg are known nutritive seafood products because of their lipid, protein and mineral content. Fish eggs are rich source of vitamins and minerals (Fe, Mg, Mn, P, K, Cu and Zn) (Bledsoe et al., 2003).

Referring to existing scientific literature, few studies have found regarding essential elements composition of rainbow trout. In addition, no studies have been carried to determine and compare differences between trout meat and egg in terms of essential trace element contents. Thus, objective of this study was to determine and compare of essential trace elements in edible flesh and egg of farmed rainbow trout for the first time.

## MATERIALS AND METHODS

### *Materials*

The farmed rainbow trout (*Oncorhynchus mykiss*) fish was provided from the seafood market (Metro gross market, Antalya, Turkey) in January 2017. Fresh milked rainbow trout eggs were obtained from the fish farm located in Akçay creek (Finike-Antalya, Turkey). Fishes and fish eggs were transferred to laboratory in polystrene boxes with in an hour.

The fishes were beheaded and eviscerated manually to obtain fillets. The samples were packed in polyethylene pouches and stored at -80 °C prior to analysis.

***Analytical procedures***

The meat and egg samples were dried in laboratory oven at 90°C for 24 hours until a constant weight was obtained, allowed cooling. Then samples were ground in a household grinder. Digestion of the samples was performed by using microwave, pressure digestion system (Berghof Speedwave, Eningen Germany). A sample (0.20 g) was mixed with 6 ml of nitric acid in a container. The system was heated up to 190°C for 20 min. After cooling to ambient temperature, the solution was filtered through a 0.45 µm nitrocellulose membrane filter, followed by transfer to an acid-washed volumetric flask and made up to volume with double deionized water. Blank digest was also carried out in the same way. Analysis of the elements (Fe, Cu, Mn and Zn) was carried out by inductively coupled plasma-optical emission spectrophotometer (ELAN DRC-E ICP-MS, Perkin Elmer, USA) equipped with a Scott spray chamber (Norwalk, C, USA). Details of the instrumental operating conditions are depicted in Table 1.

Table 1. ICP-OES instrumental operating conditions

| Parameters* | Responses |
|---|---|
| RF generator power (W) | 1000 |
| Plasma gas flow rate (l/min) | 19 |
| Auxiliary gas flow rate (l/min) | 1.2 |
| Nebulization gas flow rate (l/min) | 0.81 |
| Sample uptake rate (ml/min) | 1 |
| Type of detector | Solid state |
| Type of spray chamber | Cyclonic |
| Injector tube diameter (mm) | 0.3 |
| Measurement replicates | 3 |
| Element (λ/nm) | Fe: 259.939; Cu: 324.754; Mn: 257.610; Zn: 213.856; |

***Statistical analysis***

All experiments were conducted in duplicate, and all analyses were done at least in duplicate. The data were recorded as mean ± standard deviation (SD) for measurements. Statistical analysis was conducted according to the statistical analysis software of SAS institute (Statistical Analysis System, Cary, NC, USA). Differences among the mean value of samples were tested by Duncan's Multiple Range Test and significance was defined at $P<0.05$.

## RESULTS AND DISCUSSION

***Essential trace element concentrations in rainbow trout meat and egg***

Table 2 shows the mean concentrations of the essential trace elements in egg and meat of rainbow trout fish.

Table 2. Essential trace element concentrations in meat and egg of rainbow trout fish*

| Element | Meat (µg/g) | Egg (µg/g) |
|---|---|---|
| Fe** | $48.17\pm1.61^{B}$ | $98.16\pm2.97^{A}$ |
| Zn** | $12.56\pm0.42^{B}$ | $140.28\pm4.24^{A}$ |
| Mn** | $1.24\pm0.04^{B}$ | $18.46\pm0.26^{A}$ |
| Cu** | $3.33\pm0.11^{B}$ | $8.15\pm0.25^{A}$ |

* In dry matter; **Essential elements

Our study showed that essential trace element concentrations of egg samples were significantly higher ($P<0.05$) than meat samples. Fe and Cu were predominant in trout egg. Fe, Cu, Mn and Zn are essential for growth, reproduction and energy metabolism in all living organisms (Fallah et al., 2011; Verep et al., 2007). Iron (Fe) deficiency is one of the most widely known nutritional disorders that affect an estimated two billion people worldwide (Pretorius et al., 2016). Pregnant women, infants, young children and adolescents have higher iron requirements and are at greater risk of developing iron deficiency (Zimmerman and Hurrell, 2007). Fe concentration (98.16 µg/g) of egg samples was twofold higher than meat (48.17 µg/g) samples. Fig. 1 shows Fe and Zn contents of rainbow trout meat and egg. Our results were significantly higher than results of Fallah et al. (2011). The researchers found that wild and farmed rainbow trout meat contains 32.46 and 15.47 µg/g Fe, respectively. Fe content (98.16 µg/g) of rainbow trout egg was found higher than those of skipjack (70.22 µg/g) and tongol (55.24 µg/g) fish eggs but lower than those of defatted bonito fish egg (122.17 µg/g) (Intarasirisawat et al., 2011). Higher Zn concentration was observed in egg samples

(140.28 µg/g) compared to meat samples (12.56 µg/g). Lower Zn concentrations have been reported for wild (46.74 µg/g) and farmed (20.97 µg/g) trout meat by (Fallah et al., 2011).
Manganese (Mn) is an essential trace metal for human and animals, since it is involved in many physiological processes. Particularly it plays an important role in the metabolism of proteins, carbohydrates, lipids and in the production of steroids sexual hormones, moreover is the cofactor of enzymes such as RNA synthetase, glutamine synthetase, pyruvate decarboxylase, Mnsuperoxido desmutase and arginase (Wedler, 1993).

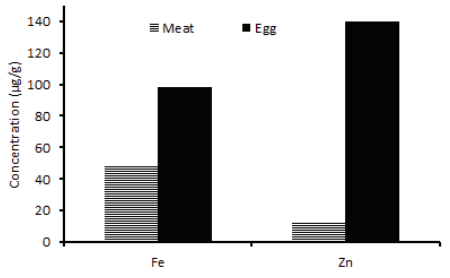


Figure 1. Iron (Fe) and zinc (Zn) concentrations in rainbow trout meat and egg

Figure 2 shows Mn and Cu contents of rainbow trout meat and egg. Mn concentration of egg samples was significantly ($P$<0.05) higher than concentration of meat samples.

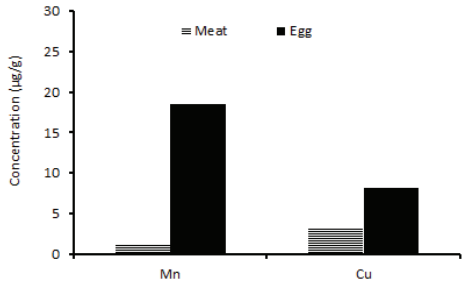


Figure 2. Manganese (Mn) and copper (Cu) concentrations in rainbow trout meat and egg

Egg samples contain 18.46 µg/g Mn, whereas Mn content of meat samples was 1.24 µg/g. Higher Mn concentrations have been reported for wild (13.93 µg/g) and farmed (6.26 µg/g) trout meat by Fallah et al. (2011). Mn content of trout egg was higher than those of skipjack (0.34 µg/g), tongol (0.80 µg/g) and defatted bonito (0.78 µg/g) fish eggs (Intarasirisawat et al., 2011).
Copper (Cu) is required for iron utilization, and as a cofactor for enzymes involved in glucose metabolism and the synthesis of hemoglobin, connective tissue and phospholipids (Celik and Oehlenschlager, 2004). Cu content of rainbow trout egg (8.15 µg/g) was significantly ($P$<0.05) higher than those of trout meat (3.33 µg/g). Higher Cu contents were found for wild (8.40 µg/g) and farmed (21.81 µg/g) rainbow trout meats (Fallah et al., 2011). Cu content of egg samples (8.40 µg/g) was lower than those of skipjack (12.72 µg/g), tongol (12.48 µg/g) and defatted bonito (34.35 µg/g) fish eggs (Intarasirisawat et al., 2011).

## CONCLUSIONS

This study gives valuable information on the essential trace element contents in meat and egg of farmed rainbow trout. Essential trace elements (Fe, Zn, Mn and Cu) content of rainbow trout egg was significantly ($P$<0.05) higher than those rainbow trout meats. It could be stemmed from owing to the fact that essential trace nutrients such as elements are necessary for growing. Thus rainbow trout eggs could be serve as a good source of essential minerals for human and animals.

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# ACCUMULATION OF LEAD IN *BARBUS BARBUS*, *ALBURNUS ALBURNUS* AND IN THEIR COMMON PARASITE *POMPHORHYNCHUS TERETICOLLIS* FROM RIVER DANUBE (VETREN AREA), BULGARIA

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***Abstract***

*During 2016, 45 specimens of barbel (Barbus barbus (Linnaeus, 1758)) and 45 specimens of bleak (Alburnus alburnus (Linnaeus, 1758)) are collected and examined from the Danube River. The aim of the study is to analyse the lead content in sediments, tissues and organs of two fishes that inhabit different water levels and in their common parasite - P. tereticollis from the Bulgarian section of River Danube. New data for the lead contents in sediments, parasites, tissues and organs of barbel and bleak from the Danube River are presented. From the tissues and organs of the studied fish specimen B. barbus, the lowest concentrations of lead were found in skin, while in A. alburnus the lowest concentrations of lead were found in muscles. The acanthocephalan Pomphorhynchus tereticollis showed significantly higher content of lead than its hosts tissues and organs. Bioconcentration factor for lead (Pb) in the tissues and organs of barbel and bleak and their common parasite Pomphorhynchus tereticollis were presented and discussed with respect to their content in sediments. Highly significant correlation ($p<0.01$) was fixed for relationship between P. tereticollis $_{Pb}$-Skin $_{Pb}$ for Barbus barbus. Highly significant correlation ($p<0.01$) was also fixed for relationship between P. tereticollis $_{Pb}$-Sediments $_{Pb}$ for Alburnus alburnus.*

***Key words****: Alburnus alburnus, Barbus barbus, Danube River, lead, Pomphorhynchus tereticollis.*

## INTRODUCTION

The content of heavy metals in different tissues and organs of fishes and in their parasites and the state of freshwater ecosystem of the Danube River has been studied from different authors (Atanasov, 2012; Gabrashanska et al., 2004; Subotić et al., 2015; Sures et al., 1994; Sures and Siddall, 1999; Schludermann et al., 2003; Thielen et al., 2004; Kirin et al., 2013; Kirin et al., 2014; Nachev and Sures, 2009; Nachev et al., 2013; Nachev, 2010; Nedeva et al., 2003; Ricking and Terytze, 1999; Woitke et al., 2003, etc.).

This paper presents the results of examinations of heavy metal contents in sediments, fish tissues and organs of two fishes that inhabit different water levels and their common parasite- *P. tereticollis* from the Bulgarian part of Danube River.

## MATERIALS AND METHODS

During 2016, sediments, fish and fish parasites are collected and examined from the Lower Danube River (village of Vetren, Bulgarian part). The village of Vetren ($44^0133$'N, $27^0033$'E) is situated on the riverside, in the northeastern part of the Danube Valley.

A total of 3 samples of sediment, 45 samples of barbel *(Barbus barbus* Linnaeus, 1758) and 45 samples of bleak *(Alburnus alburnus* Linnaeus, 1758) from the Danube River are collected and examined in 2016. The scientific and common names of fish hosts are used according to the Fish Base database (Fröse and Pauly, 2016). The barbel *(Barbus barbus* Linnaeus, 1758) and bleak *(Alburnus alburnus* Linnaeus, 1758) species chosen for examination of the heavy metal content in this study were weighed (total weigh: from 285-788 g for barbel; from 10-22 g for bleak) and measured (total length: from 29-45 cm for barbel; from 10 - 14 cm for bleak).

Helminthological examinations are carried out following recommendations and procedures described by Petrochenko (1956), Bauer et al. (1981), Bykhovskaya-Pavlovskaya (1985), Gusev (1985), etc. Identification of *P. tereticollis* was based on resurrection of the species (Špakulová et al., 2011).

Samples of sediments were collected during the spring, summer and autumn season, according

to the Guidance on sampling of rivers and watercourses – BSS ISO 5667-6:1990. Samples of muscles, skin and liver are collected from all samples of fish. The content of lead (Pb) in samples of sediment, fish tissues, organs and parasites was established by ICP Spectrometry (ISO 8288:1986; BDS EN ISO 17294-2:2016; Bíreš et al., 1995). In order to determine the relative accumulation capability of the fish tissues and parasites in comparison to the sediments, bioconcentration factor (BCF= [Chost/parasite tissues]/[Csediments]) are calculated (Sures et al., 1999). The bioconcentration factors are used for estimation of trace metal pollution in freshwater ecosystem by examined fish and their parasites. The differences in concentration factors are discussed in respect to the bioavailability of lead from sediments. A Spearman's rank correlation coefficient, $r_s$ and levels of significance were determined to test the relationships between bottom sediments, fish tissues, organs and parasites.

## RESULTS AND DISCUSSIONS

A total of 45 specimens of barbel (*Barbus barbus* Linnaeus, 1758) and 45 specimens of bleak (*Alburnus alburnus* Linnaeus, 1758) are collected and examined from the Danube River. *Barbus barbus* and *Alburnus alburnus* are estimated as least concern species (LC=Least Concern; IUCN Red List Status).

Barbel is freshwater, benthopelagic, potamodromous fish species. Inhabits from premontane to lowland reaches of clear, warm, medium sized to large rivers with fast current and gravel bottom. Occasionally found in lakes. Frequently overwinters in large group, inactive or active in slow-flowing river habitats. Adults often form shoal, hiding under overhanging trees or bridges during the day. Adults are encountered most active during dusk and dawn while larvae and juveniles are active during both day and night. Larvae and juvenile stay on the bottom in very shallow shoreline habitats and leave the shores for faster-flowing waters as they grow. Lives in the deeper, faster-flowing upper reaches of rivers with stony or gravel bottom (barbel zones). Feeds chiefly on benthic invertebrates, such as small crustaceans, insect larvae, mollusks, mayfly and midge larvae and also on small fish and sometimes algae (Fröse and Pauly, 2016).

Bleak is freshwater, brackish, benthopelagic, potamodromous fish species. It inhabits open waters of lakes and medium to large rivers. Bleak forms large aggregations in backwaters and other still waters during winter. Adults occur in shoals near the surface. Larvae live in littoral zone of rivers and lakes while juveniles leave shores and occupy a pelagic habitat, feeding on plankton, drifting insects or invertebrates fallen on the water surface. This fish species feeds mainly on plankton, including crustaceans and insects. Bleak spawns in shallow riffles or along stony shores of lakes, occasionally above submerged vegetation (Fröse and Pauly, 2016).

The result of the content of lead (Pb) in samples of sediments and samples of muscle, liver and skin of *Barbus barbus* and *Alburnus alburnus* and their common parasite *P. tereticollis* from the Danube River are presented. Based on the results of chemical analyzes, mean concentrations ($mg.kg^{-1}$) in tissues, organs of the fish, parasites and sediments, as well as the bioconcentration factor (BCF= [Chost/parasitetissues]/ [Csediments]) are defined.

From the fish tissues and organs of barbel the highest contents of lead was determined in samples from liver (2.592±2.30 $mg.kg^{-1}$), followed by those from muscles (1.727±1.317 $mg.kg^{-1}$) and skin (1.53±0.718 $mg.kg^{-1}$)(Table 1).

Table 1. Lead concentration ($mg.kg^{-1}$) in sediments, different organs of *Barbus barbus* and its parasites *P. tereticollis*

| *Barbus barbus* | Mean±SD | Range |
|---|---|---|
| Sediments | 45.256±15.958 | 33.940-67.825 |
| Liver | 2.592±2.30 | 0.52-6.715 |
| Muscles | 1.727±1.317 | 0.461-4.756 |
| Skin | 1.53±0.718 | 0.781-3.271 |
| *P. tereticollis* | 135.713±26.28 | 94.231-180.833 |

From the fish tissues and organs of bleak the highest contents of lead was determined in samples from liver (4.30±3.627 $mg.kg^{-1}$), followed by those from skin (1.642±0.8 $mg.kg^{-1}$) and muscles (1.449±0.410 $mg.kg^{-1}$) (Table

2). In general the content of lead in liver is higher in bleak than barbel.

Table 2. Lead concentration ($mg.kg^{-1}$) in sediments, different organs of *Alburnus alburnus* and its parasites *P. tereticollis*

| *Alburnus alburnus* | Mean±SD | Range |
|---|---|---|
| Sediments | 45.256±15.958 | 33.940-67.825 |
| Liver | 4.30±3.627 | 0.631-9.154 |
| Muscles | 1.449±0.410 | 0.46-1.815 |
| Skin | 1.642±0.8 | 0.943-3.779 |
| *P. tereticollis* | 172.770±18.37 | 154.40-191.139 |

The acanthocephalan *P. tereticollis* showed significantly higher content of lead (135.713 $mg.kg^{-1}$ for *Barbus barbus*; 172.770 $mg.kg^{-1}$ for *Alburnus alburnus*), than its hosts tissues and organs. This purpose remains regarding the values of BCF, set against the levels of lead in sediments of the Danube River (Biotope Vetren). Regarding *Barbus barbus* the highest BCF $_{P.\ tereticollis}$ was for skin (88.70) followed by those for muscle (78.583), liver (52.358) and sediments (2.999) (Table 3).

Table 3. Bioconcentration factor (BCF=[Chost/parasite tissues]/[C Sediments]) of *B. barbus* and *P. tereticollis*

| Sediments/ *B.barbus*/ *P. tereticollis* | BCF |
|---|---|
| $C_{P.\ tereticollis}/C_{Sediments}$ | 2.999 |
| $C_{Liver}/C_{Sediments}$<br>$C_{P.\ tereticollis}/C_{Liver}$ | 0.057<br>52.358 |
| $C_{muscle}/C_{Sediments}$<br>$C_{P.\ tereticollis}/C_{muscle}$ | 0.038<br>78.583 |
| $C_{Skin}/C_{Sediments}$<br>$C_{P.\ tereticollis}/C_{Skin}$ | 0.033<br>88.70 |

Regarding *Alburnus alburnus* the highest BCF $_{P.\ tereticollis}$ was for muscle (119.23) followed by those for skin (105.22), liver (40.18) and sediments (3.818) (Table 4).

A linear correlation coefficient (Spearman's rank correlation coefficient, $r_s$) is determined to test the association between the sediments, fish tissues, organs and sediments. Highly significant correlation ($p<0.01$) was fixed for relationship between *P. tereticollis* $_{Pb}$-Skin $_{Pb}$ for *Barbus barbus*. Highly significant correlation ($p<0.01$) was fixed also for relationship between *P. tereticollis* $_{Pb}$-Sediments $_{Pb}$ for *Alburnus alburnus*.

Table 4. Bioconcentration factor (BCF=[Chost/parasite tissues]/[C Sediments]) of *A. brama* and *P. tereticollis*

| Sediments / *A. brama* / *P. tereticollis* | BCF |
|---|---|
| $C_{P.\ tereticollis}/C_{Sediments}$ | 3.818 |
| $C_{Liver}/C_{Sediments}$<br>$C_{P.\ tereticollis}/C_{Liver}$ | 0.095<br>40.18 |
| $C_{muscle}/C_{Sediments}$<br>$C_{P.\ tereticollis}/C_{muscle}$ | 0.032<br>119.23 |
| $C_{Skin}/C_{Sediments}$<br>$C_{P.\ tereticollis}/C_{Skin}$ | 0.036<br>105.22 |

The maximum lead level permitted for fish is 0.2 $mg.kg^{-1}$ according the EU and Bulgarian food codex (Anonymus, 2004); 2.0 $mg.kg^{-1}$ for WHO and 0.5 $mg.kg^{-1}$ for FAO. Lead content in analyzed organs and tissues of *B. barbus and A. alburnus* are found to be higher than limits. These results showed human health risk with respect to the concentrations of lead in analyzed samples of freshwater bream from the Bulgarian part of the Danube River.

## CONCLUSIONS

As a result of this study is presented new data for lead content in liver, muscle and skin of *B. barbus* and *A. alburnus* and their common parasite - *P. tereticollis* from Danube River (Biotope Vetren). The acanthocephalan *P. tereticollis* showed significantly higher content of lead than its hosts tissues and organs. In general the highest content of lead was found in liver and it was higher than content of lead in muscle and skin for both studied fish species. From the tissues and organs of the studied fish specimen *B. barbus*, the lowest concentrations of lead were found in skin, while in *A. alburnus* the lowest concentrations of lead were found in muscles. These results showed human health risk with respect to the concentrations of lead in analyzed samples of barbel and bleak from the Bulgarian part of the Danube River.

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# ACCUMULATION OF LEAD IN *ABRAMIS BRAMA* AND ITS PARASITE *POMPHORHYNCHUS TERETICOLLIS* FROM DANUBE RIVER (VETREN AREA), BULGARIA

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***Abstract***

*During 2016, 45 specimens of freshwater bream (Abramis brama (Linnaeus, 1758)) are collected and examined from the Danube River. Helminth parasites are recorded in 29 freshwater bream specimens (64.44%) from the Danube River. Five species of parasites were identified: one trematode species (Asymphylodora imitans (Mühling, 1898)), three acantocephalans (Acanthocephalus lucii (Müller, 1776), Acanthocephalus anguillae (Müller, 1780), Pomphorhynchus tereticollis (Rudolphi, 1809)) and one nematode species (Raphidascaris acus, (Bloch, 1779), larvae). In the component community of Abramis brama from Danube River A. imitans and A. lucii are core species. P. tereticollis is component parasite species for the helminth communities of freshwater bream. A. lucii and R. acus are accidental parasite species for the helminth communities of A. brama. New data for the lead contents in sediments, parasites, tissues and organs of freshwater bream from the Danube River are presented. From the tissues and organs of the studied fish specimens Abramis brama, the lowest concentrations of lead were found in the muscles. The content of lead in the samples of skin and liver are higher than in the muscles. The acanthocephalan Pomphorhynchus tereticollis showed significantly higher content of lead than its host organs. Bioconcentration factor for lead (Pb) in the tissues and organs of freshwater bream were presented and discussed with respect to their content in sediments. Very significant correlation ($p<0.001$) was fixed for relationship between P. tereticollis $_{Pb}$ -Sediments $_{Pb}$.*

***Key words****: Abramis brama, bioindication, Danube River, heavy metals, Pomphorhynchus tereticollis.*

## INTRODUCTION

The Bulgarian part of river Danube has important place in the Bulgarian and European ecological network. Fish parasites are sensitive indicators for heavy metals in aquatic ecosystems, due to their ability to accumulate significantly higher concentrations of trace elements than their host (Sures et al., 1994; Sures and Siddall, 1999; Schludermann et al., 2003; Thielen et al., 2004; Nachev et al., 2013). Fish parasite communities, heavy metal content and the state of freshwater ecosystem of the Danube River are studied from different authors (Atanasov, 2012; Djikanovic et al. 2013; Gabrashanska et al., 2004; Kakacheva-Avramova, 1977, 1983; Kakacheva et al., 1978; Kirin et al., 2013; Kirin et al., 2014; Margaritov, 1959, 1966; Moravec et al., 1997; Nachev, 2010; Nachev and Sures, 2009; Nedeva et al., 2003; Ricking and Terytze, 1999; Woitke et al., 2003, etc.). This paper presents the results of examinations of heavy metal contents in sediments, fish parasites, fish tissues and organs from the Bulgarian part of the Lower Danube River (village of Vetren).

## MATERIALS AND METHODS

During 2016, sediments, fish and fish parasites are collected and examined from the Lower Danube River (village of Vetren, Bulgarian part) (Fig. 1). The village of Vetren ($44^0133$'N, $27^0033$'E) is situated on the riverside, in the north eastern part of the Danube Valley.

A total of 3 samples of sediment and 45 samples of freshwater bream (*Abramis brama* Linnaeus, 1758) from the Danube River are collected and examined in 2016. The scientific and common names of fish hosts are used according to the FishBase database (Fröse and Pauly, 2016). The freshwater bream *(Abramis brama* Linnaeus, 1758) species chosen for examination of the heavy metal content in this

study were weighed (total weigh from 24-323 g) and measured (total length from 10.5 - 31 cm). Helminthological examinations are carried out following recommendations and procedures described by Bauer et al. (1981), Bykhovskaya-Pavlovskaya (1985), Georgiev et al. (1986), Gusev (1985), Moravec (1994, 2001) etc.

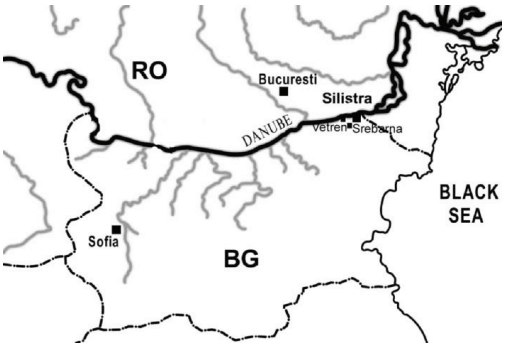


Figure 1. Danube River

The dominant structure of the component helminth communities was determined according to the criteria proposed by Kennedy (1993) on the basis of the prevalence (P%): accidental (P% < 10), component (10 < P% < 20) and core (P% >20) species. The ecological terms prevalence, mean intensity are used, based on the terminology of Bush et al. (1997). Analyses of helminth community structure were carried out during the three seasons and in both levels: infracommunity and component community. The infracommunity data are used to calculate the total number of species, mean number of helminths and Berger-Parker dominance index (d), etc. (Kennedy, 1993, 1997; Magurran, 1988).

Samples of sediments are collected according to the Guidance on sampling of rivers and watercourses – BSS ISO 5667-6:1990, introduced as a Bulgarian standard in 2002. Heavy metal concentration of the sediment samples, fish tissues, organs and parasites are carried out according to standard techniques. The samples are analyzed for content of Pb by ICP Spectrometry (ISO 8288:1986; BDS EN ISO 17294-2:2016; Bíreš et al., 1995). Samples of muscles, skin and liver are collected from all individuals. In order to determine the relative accumulation capability of the fish tissues in comparison to the sediments, bioconcentration factor (BCF= [Chost tissues]/[Csediments]) are calculated (Sures et al., 1999). The bioconcentration factors are computed to establish the accumulation order and to examine fish for use as biomonitors of trace metal pollutants in freshwater environments. The differences in concentration factors are particularly discussed in respect to the bioavailability of trace metals from sediments. A Spearman's rank correlation coefficient, $r_s$ is used to test associations between the bottom sediments, fish tissues, organs and parasites.

## RESULTS AND DISCUSSIONS

A total of 45 specimens of freshwater bream *(Abramis brama* Linnaeus, 1758) are collected and examined from the Danube River. *Abramis brama* is estimated as least concern species (LC=Least Concern; IUCN Red List Status). Freshwater bream is brackish, benthopelagic, potamodromous fish species. Adults inhabit a wide variety of lakes and large to medium sized rivers. Fish species is the most abundant in backwaters, lower parts of slow-flowing rivers, brackish estuaries and warm and shallow lakes. Adults occur usually in still and slow-running waters where they travel in large shoals. Larvae and juveniles live in still water bodies, feeding on plankton. Adults feed on insects, particularly chironomids, small crustaceans, molluscs and plants. Larger specimens may feed on small fish (Fröse and Pauly, 2016).

Helminth parasites are recorded in 29 freshwater bream specimens (64.44%) from the Danube River. Five species of parasites were identified: one trematode species *(Asymphylodora imitans* Mühling, 1898), three acantocephalans (*Acanthocephalus lucii* Müller, 1776), *Acanthocephalus anguillae* Müller, 1780), *Pomphorhynchus tereticollis* Rudolphi, 1809)) and one nematode species (*Raphidascaris acus* Bloch, 1779), larvae) (Table 1). All helminth species occurred as adults with the exception of *R. acus*. *A. imitans*, *A. lucii*, *A. anguillae* and *P. tereticollis* are autogenic species, matured in fish. *R. acus* allogenic species, matured in some species of predatory fishes as *Exos lucius*, *Lota lota*, *Salmo trutta* and others (Moravec, 1994).

Table 1. Helminth parasites of *Abramis brama* from Danube River (N – number of examined hosts, n – number of infected hosts, p – number of parasites, P – prevalence, MA – mean abundance, MI – mean intensity)

| Helminth species | N=45 | | | | | |
|---|---|---|---|---|---|---|
| | n | P | P% | MA±SD | MI±SD | Range |
| *Asymphylodora imitans* (Mühling, 1898) | 18 | 544 | 40.00 | 12.08±32.93 | 30.22±46.51 | 1-198 |
| *Acanthocephalus lucii* (Müller, 1776) | 10 | 17 | 22.22 | 0.37±0.85 | 1.7±1.004 | 1-4 |
| *Acanthocephalus anguillae* (Müller, 1780) | 2 | 2 | 3.26 | 0.044±0.206 | 1 | 1 |
| *Pomphorhynchus tereticollis* (Rudolphi, 1809) | 5 | 13 | 11.11 | 0.288±1.02 | 2.6±1.85 | 1-6 |
| *Raphidascaris acus* (Bloch, 1779), larvae | 3 | 5 | 6.67 | 0.054±0.341 | 1.67±0.94 | 1-3 |

In the component community of *Abramis brama* from Danube River *A. imitans* (P%=40.00) and *A. lucii* (P%=22.22) are core species. *P. tereticollis* (P%=11.11) is component parasite species for the helminth communities of freshwater bream *A. lucii* (P%=3.26) and *R. acus* (P%=6.67) are accidental parasite species for the helminth communities of *Abramis brama* (Table 1).

In the component community of *Abramis brama* from Danube River trematodes are presented with the highest number of specimens, with 1 species and 544 specimens. Acanthocephalans are presented with three species and 32 specimens. Nematodes are represented by one species and 5 specimens.

*Asymphylodora imitans* was found in the gut of *Blicca bjoerkna* in Bulgarian section of river Danube (Kakacheva-Avramova, 1977). *Acanthocephalus lucii* was found in *Abramis sapa*, *Leuciscus cephalus*, *Rutilus rutilus*, *Gymnocephalus schraetser*, *Benthophylus stellatus*, *Proteorhinus marmoratus*, *Silurus glanis*, *Lota lota* and *Zingel zingel* from Bulgarian section of Danube River (Margaritov, 1959; Kakacheva-Avramova 1977; Atanasov, 2012). *A. anguillae* was found in *Leuciscus idus*, *Blicca bjoerkna*, *Carassius auratus gibelio*, *A. brama* and *Barbus barbus* from Danube River (Margaritov, 1959; Kakacheva-Avramova, 1977; Nachev, 2010; Atanasov, 2012; Chunchukova et al., 2016). Intermediate host of *A. lucii* and *A. anguillae* is *Asellus aguaticus,* and definitive hosts are fish species of different families as Cyprinidae, Salmonidae, Percidae, Anguillidae and others (Kakacheva-Avramova, 1983).

*Pomphorhynchus tereticollis* was found in *Abramis brama, Ballerus sapa, Barbus barbus, Gymnocephalus schraetser* and *Neogobius fluviatilis* from Bulgarian section of river Danube (Kirin et al. 2013, Kirin et al. 2014). Intermediate hosts of *P. tereticollis* are *Gammarus* sp. (Westram et al., 2011).

Species richness in infracommunity of freshwater bream ranges from 1 to 3 species. With 1 helminth species were infected 22 fishes (75.86 %), with 2 helminth species -5 fishes (17.24%) and with three species-only 2 specimens fish (6.90%). The largest number of helminth specimens established in a single host specimen is 198. The average species richness (mean number of species for fish specimen) in infracommunity of freshwater bream is 0.84 species (Table 2).

Table 2. Infracommunities of *Abramis brama* from Danube River

| | Number of endohelminth species | | | | |
|---|---|---|---|---|---|
| | 0 | 1 | 2 | 3 | Mean±SD Range |
| *Abramis brama* | 16 | 22 | 5 | 2 | 0.84±0.787 1-3 |

Average abundance (mean number of helminths in fish) in these infracommunities is 12.64. The parasite communities of *A. brama* from the Danube River showed Berger-Parker

dominance index, d=0.706±0.256 (range 0.370-0.990).

The result of the content of lead (Pb) in 3 samples of sediments and 29 samples of muscle, liver and skin of *Abramis brama* and its parasite *P. tereticollis* from the Danube River are presented.

Based on the results of chemical analyzes, mean concentrations (mg.kg$^{-1}$) in tissues, organs of the fish, parasites and sediments, as well as the bioconcentration factor (BCF= [Chost/parasitetissues]/[Csediments]) are defined.

From the fish tissues and organs the highest contents of lead was determined in samples from skin (5.912±5.348 mg.kg$^{-1}$), followed by those from liver (5.622±4.434 mg.kg$^{-1}$) and muscles (2.495±1.709 mg.kg$^{-1}$) (Table 3).

Table 3. Lead concentration (mg.kg$^{-1}$) in sediments, different organs of *Abramis brama* and its parasites *P. tereticollis*

| | Mean±SD | Range |
|---|---|---|
| Sediments | 45.256±15.958 | 33.940-67.825 |
| Liver | 5.622±4.434 | 0.552-14.603 |
| Muscles | 2.495±1.709 | 0.652-5.923 |
| Skin | 5.912±5.348 | 1.143-19.147 |
| *P. tereticollis* | 350±104 | 246-454 |

The acanthocephalan *P. tereticollis* showed significantly higher content of lead (350 mg.kg$^{-1}$), than its hosts organs.

This purpose remains regarding the values of BCF, set against the levels of lead in sediments of the Danube River (Biotope Vetren) (Table 4). The highest BCF $_{P.\ tereticollis}$ was for muscles (140.280) followed by those for liver (62.255), skin (59.201) and sediments (7.703).

Table 4. Bioconcentration factor (BCF = [Chost/parasite tissues]/[C Sediments]) of *A. brama* and *P. tereticollis*

| Sediments / *A. brama* / *P. tereticollis* | BCF |
|---|---|
| $C_{P.\ tereticollis}/C_{Sediments}$ | 7.733 |
| $C_{Liver}/C_{Sediments}$ | 0.124 |
| $C_{P.\ tereticollis}/C_{Liver}$ | 62.255 |
| $C_{muscle}/C_{Sediments}$ | 0.055 |
| $C_{P.\ tereticollis}/C_{muscle}$ | 140.280 |
| $C_{Skin}/C_{Sediments}$ | 0.114 |
| $C_{P.\ tereticollis}/C_{Skin}$ | 59.201 |

A linear correlation coefficient (Spearman's rank correlation coefficient, $r_s$) is determined to test the association between the sediments, fish tissues, organs and sediments. Very significant correlation ($p<0.001$) was fixed for relationship between *P. tereticollis* $_{Pb}$-Sediments $_{Pb}$.

The obtained values for the content of Pb in sediments are slightly higher than those reported for the same Biotope (Kirin et al., 2013; Chunchukova et al., 2016). The obtained values for the content of lead in liver, muscles and skin of *A. brama* are slightly lower than those reported for the same Biotope (Chunchukova et al., 2016).

The maximum lead level permitted for fish is 0.2 mg.kg$^{-1}$ according the EU and Bulgarian food codex (Anonymus, 2004); 2.0 mg.kg$^{-1}$ for WHO and 0.5 mg.kg$^{-1}$ for FAO.

Lead content in analyzed fish organs and tissues of *A. brama* are found to be higher than limits. These results showed human health risk with respect to the concentrations of lead in analyzed samples of freshwater bream from the Bulgarian part of the Danube River.

## CONCLUSIONS

As a result of this study is presented new data for helminthes and helminth communities of *A. brama* from Danube River (Biotope Vetren). New data for heavy metal contents in sediments, fish parasites, fish tissues and organs from the Danube River are presented. From the tissues and organs of the studied fish specimen *A. brama*, the lowest concentrations of lead were found in muscles.

In general, the content of lead in the samples of skin and liver are higher than in the muscles.

These results showed human health risk with respect to the concentrations of lead in analyzed samples of freshwater bream from the Bulgarian part of the Danube River.

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# RESEARCH ON THE USE OF DIFFERENT HORMONAL SUBSTANCES TO STIMULATE MATURATION AND OVULATION IN PERCH (*PERCA FLUVIATILIS* L.)

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***Abstract***

*Diversification of production in fish culture by introducing valuable species for which there is demand and tradition for consumption, is one of the main directions of development of aquaculture. Perch (Perca fluviatilis L.), a valuable autochthonous species, is recognized for the quality of meat but mainly for the satisfaction offered in sport fishing. A crucial step for the introduction and expansion of perch in aquaculture, is to obtain biological material for stocking through artificial spawning. The paper presents results of experiments for artificial spawning of perch performed between March 20 and April 1, 2015. Were used a total of 120 broodstock collection in Arcesti and Ionesti reservoirs on the river Olt in the fall of 2014. For maturation and ovulation induction was used: carp pituitary extract - CPE, human chorionic gonadotropin - Chorulon HCG, GnRH or analogues, sometimes combined with dopamine antagonists - Ovopel. Technological parameters obtained from artificial spawning were within the following ranges: the percentage of maturation of females 34 -73%; embryo rate was 64 -85%, hatching rate 23 -65%. The best results were obtained when using HCG Chorulon as a stimulating agent for maturation and ovulation.*

***Key words****: artificial spawning, hatching rate, incubation, maturation, ovulation.*

## INTRODUCTION

Diversification of production in fish culture by introducing valuable species for which there is demand and tradition for consumption, is one of the main directions of development of aquaculture. Perch (*Perca fluviatilis* L.), a valuable autochthonous species, is recognized for the quality of meat but mainly for the satisfaction offered in sport fishing. Controlled reproduction is the most reliable method for obtaining a high number of perch larvae. Perch spawners from both wild and cultured stocks spawn easily in captivity. However, the spawning period is a long process that lasts for more than two weeks (Kucharczyk D., et al. 1996a). This is very inconvenient for starting incubation and rearing, and it requires more elaborate facilities. For these reasons, a method to synchronize perch spawning is still needed. Many kinds of hormonal treatments have been used to stimulate ovulation in perch females. Human chorionic gonadotropin (hCG) with common carp (*Cyprinus carpio* L.) pituitary extract (CPE) were tested by Kucharczyk et al. in 1996 (Kucharczyk et al., 1996b). Independent of temperature, spawner size and gonad maturity, or the type of hormonal stimulation applied, synchronized ovulation was observed to a lesser or greater degree in all of the above experiments. Nevertheless, the biological quality of the eggs, expressed as the percentage of egg survival to the larvae, varied widely and the improvement of spawning techniques is required.

The knowledge of physiological processes in fish has facilitated the use of substances that stimulate maturation and reproduction (Dabrowski et al., 1996). Obtaining the maturity can be stimulated in percids (perch, pikeperch) using carp pituitary extract (CPE), human chorionic gonadotropin (hCG) and luteinizing hormone-releasing hormone (LH-RH) or super-active analogs (LH-RHa), sometimes with dopamine antagonists (Ronyai, 2007).

This paper presents results of experiments of artificial spawning of perch obtained from the Fisheries Research and Development Station of Nucet, between March 20 and April 1, 2015.

## MATERIALS AND METHODS

Artificial spawning was achieved by proceeding sequentially through the following steps: broodstock collection, females selection, the stage determination of maturation of gonads, application of hormonal treatment, eggs ripening, harvesting sexual products, eggs incubation and hatching.

For maturation and ovulation induction was used: carp pituitary extrac – CPE, human chorionic gonadotropin - Chorulon HCG, GnRH or analogues, sometimes combined with dopamine antagonists – Ovopel.

**Broodstock collection**

Were used a total of 120 breeders captured in Arcesti and Ionesti reservoirs on the river Olt in the fall of 2014. Fish were parked in winter in pools of the ground the size of 3 x 25 m. There was fishing in March, at 6-7°C.

Fish were selected according to the following criteria: the belly of the females had to be fully distended, bulging and soft and resilient to the touch; the males have started the spermiation process.

Males and females selected were kept apart in the fall with volume of 2.000 litters hatchery. All breeders (Figure 1) were close in height, weighing between 180-275 g.

Figure 1. Breeders: male and female of perch

**Determining the maturation stage of oocytes**

Oocyte maturity was determined by using biopsy techniques. In this technique eggs (oocytes) are taken from the ovary, cleared with a prepared solution (e.g. Serra's solution), and viewed under a microscope.

There are many different methods of sampling oocytes from fish ovary. One of them is taking sample using a catheter. This method was tested many times in perch (Kucharczyk et al., 1996a), as well as in many other fish species, especially in cyprinids.

The main evaluation criteria of perch oocytes maturity stages, like other *Teleostei* fishes, are the location of germinal vesicle (GV) and additionally coalescence of the oil droplets.

Oocytes classified according the above-mentioned criteria are divided into four stages:

1. Oocytes in I (first) maturity stage have GV in the central position
2. Oocytes classified as II (second) maturity stage have shifted GV less than a half radius.
3. Oocytes classified as maturity stage III have positioned GV on the periphery, near the oocyte membrane.
4. Oocytes without visible GV, i.e. in which the process of GV breakdown (GVBD) has begun or GV is present near the zone, should be classified as maturity stage IV.

Only females whose oocyte maturation was between stage 2-3 and 3 were used for further investigations.

**Hormonal treatment**

For maturation and ovulation induction was used: carp pituitary extract – CPE, human chorionic gonadotropin - Chorulon HCG, GnRH or analogues, sometimes combined with dopamine antagonists – Ovopel .

The fish were divided into four groups that were injected with the following:

1. Extract from pituitary glands, i.e. carp (CPE) (Ronyai, 2007);
2. Chorionic gonadotropins human HCG;
3. Ovopel pellets. One Ovopel pellet (average weight about 25 mg) contains a mammalian GnRH analogue (D-Ala6, Pro9Net-mGnRH at dose 18-20 µg) and dopamine antagonist: metoclopramide (dose 8-10 mg) (Kucharczyk et al., 1996a);
4. 0.9% NaCl sterile solution (control group)

All spawning agents are usually prepared with 0.9% NaCl. The doses are presented in Table 1. The number of females and males in each group were 20 and 10, respectively. Injections were administered intramuscularly in the dorsal area of the body.

The range of total doses of chosen spawning agents should be as follows:

- Chorionic gonadotropins (200 – 1000 IU/kg BW) (Ronyai, 2007);
- CPE (1.0 – 3.5 mg / kg BW) (Horvath et al., 1997; Kouril et al., 1997);
- Ovopel (1.2 – 2.0 pellets / kg BW) (Ronyai, 2007).

If two injections are planned, the initial dose should be 20 – 50% in the case of chorionic gonadotropins and 10 – 20% in the case of other spawning agents.

Table 1. Hormonal treatment applied to induce artificial spawning in perch

| Specifications | Female | | Male |
|---|---|---|---|
| | Preparatory dose | Decisive dose | Single dose |
| CPE | 0.5 mg/kg BW | 2.0 mg/kg BW | 1.0 mg/kg BW |
| hCG | 200 IU/kg BW | 1000 IU/kg BW | 500 IU/kg BW |
| Ovopel | 1/10 Ovopel pellet | 1.0 Ovopel pellet | ½ Ovopel pellet |
| Control | injections from 0.9% NaCI | | |

**Manipulations with breeders**

Females of perch were screened at about 12 hours after the last injection. If the female perch is ready to give eggs usually begin to fall spontaneously genital pore.

Before stripping the genitors must be clean and dry with a soft towel. They should not be allowed to mix with water gametes because they are eliminated. The fish is held with one hand around the tail fin and the other is a slight pressure to the abdomen. If ovulation has occurred, a stream of eggs will appear. Where there is a flow of eggs, abdomen should be massaged from front to back to remove all the eggs.

The eggs are usually collected in a small plastic container. Artificial eggs stripped wait, usually for adding sperm. Sperm collection was performed using plastic syringes.

The sperm from each male was collected separately and added over eggs. After fertilization, the eggs were incubated pasted on nytal frames in Nucet incubators.

Fish from all the groups were kept for an additional ten days after the end of all the experiments in order to observe their survival.

**Statistical analysis**

Statistical differences between groups were analysed with Duncan's multiple range test ($P < 0.05$). The relationships between embryo rate to the hatching rate were calculated using regression analysis.

## RESULTS AND DISCUSSIONS

Results of different spawning agents in perch reproduction under controlled conditions are presented in Table 2.

Table. 2. Efficiency of different spawning agents for artificial spawning of perch

| Specifications | Control (0.9% NaCl) | CPE | hCG | Ovopel |
|---|---|---|---|---|
| No. of females | 20 | 20 | 20 | 20 |
| Ovulation (%) | 34 | 52 | 73 | 67 |
| Embryo rate (%) | 64.1± 3.2B | 74± 4.3C | 85± 2.1 A | 82± 3.8 A |
| Hatching rate % | 23.1± 1.2B | 50 ± 3.3C | 65± 1.1 A | 62± 3.1 A |

The percentage of females ovulate in the treated groups was between 34 and 67% highest value recorded when using Ovopel, and the smallest group of fish in the control group. Ovulation occurred after about 17 - 23 hours after the last injection.

Females stimulated agencies reproductive hCG and Ovopel yielded eggs after 17 and 19 hours at $15^0$C.

The rate of embryo was located between the limits of 64% obtained in fish in the control group and 85% for fish stimulated hormone hCG. Elevated this technological indicator was obtained and the use Ovopel.

Hatching rate was 23% in the control group and 65% in fish stimulated with hCG. The reproductive survival, before and after

spawning, was very good. The mortality in all groups was less than 10%.

All males have started the spermiation process at the moment of catch. In the present experiment, the hormonal treatment resulted in a significantly higher production of milt.

Ovulation in females from the experimental groups was synchronous. The short time between the first and last ovulation, observed in all the experiments, may be the result of the high level of synchronous oocyte maturation. The survival of perch spawners throughout the experiment was high (Dabrowski et al., 1994) and (Gillet et al., 1995) reported some problems with spawner survival, especially the females. The similar size of the perch spawners used may have also resulted in synchronous spawning.

## CONCLUSIONS

1. Controlled reproduction is the most reliable method for obtaining a high number of perch larvae.
2. Perch spawners of wild and cultured stocks spawn easily in captivity.
3. The main factors that influence the amount and quality of perch artificial spawning are: gametes; temperature, photoperiod and physiological status of the parents.
4. The use of different types of hormonal treatments can lead to getting quality material gametes and species for aquaculture development.
5. Reproduction of agents used in our experiments the best results were obtained when hCG and Ovopel.
6. The doses of spawning agents depend on many factors: time of spawning (season, out-of-season) maturation stage of fish gonads and type of hormone.

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# THE IMPORTANCE OF THE CHILIA BRANCH FOR PROTECTING AND CONSERVATION OF THE ANADROMOUS MIGRATORY STURGEONS

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***Abstract***

*The hydrotechnic works often generate aquatic ecosystem disturbances. Sometimes they are local and short-term, but sometimes they cause significant long-term impact that may be irreversible. The present paper aims to analyze the possible impact of the maintenance works performed on Bastroe channel fairway on the populations' dynamics of migratory anadromous fishes, sturgeon.*
*The methodology implied sturgeon tagging and migration monitoring using ultrasonic telemetry technique during 2011-2014. The tags were inserted into the abdominal cavity through a simple surgical intervention, being set to transmit data related on swimming depth and water temperature towards receiver stations fixed on Chilia branch, upstream and downstream of Bastroe channel, Tulcea and Old Danube branches.*
*The results showed that the distribution of the adult specimens during migration on Chilia and Tulcea branches was 53% - 47% (spring 2012/2014) and 31% - 69% in autumn 2013. A possible progressive warping of the Old Stambul and especially Musura branch, as well as an increase of the naval traffic on Bastroe channel will have major negative effects on upstream sturgeon migration for breeding.*

***Key words****: fish migration, monitoring, Old Danube, sturgeon conservation.*

## INTRODUCTION

Chilia branch and Bastroe channel are parts of the Danube Delta Biosphere Reserve, having great importance due to the many types of aquatic and terrestrial habitats existing here. The universal value of the reserve has been recognized by its inclusion in the international network of the Biosphere Reserves (1990), within the Program "Man and Biosphere" (MAB) launched by UNESCO in 1970. Bastroe channel was formed naturally and makes the connection between Chilia branch and the Black Sea. Although the area benefits of full ecological protection regime, according to which any human activity in the region is forbidden, in the area have been conducted a series of works for the riverbed arrangement through which is seeking the direct access of commercial ships in the Black Sea (Șofineți and Dobrotă, 2004). An effect of the channel arrangement will be an overall disturbance of the habitats, due to the banks transformations by digging the channel, which will cause the water flow's system change and a new way of sedimentation in the area of the Bastroe estuary. The impact on ichtyofauna may be a temporary one, generated by high mortality among fish species caused by dredging activities or their withdrawal from the area, and also a permanent impact, irreparably through the disappearance of species due to changes of habitual requirements.
One category of fish species sensitive to hydro-morphological changes of the watercourse is represented by sturgeon. It is proved the fact that human activities related to the change of the natural watercourses led to a decline of this species population over time. The best example are the dams built for the Iron Gates I and II Hydropower plants (Bacalbasa-Dobrovici, 1993, 1997; Ciolac, 2004; Reinartz, 2002). The interruption of the longitudinal connectivity of watercourses leads to the impossibility of reproduction in case these species cannot reach to specific habitats located upstream and thus,

the number of specimens decreases alarmingly from one year to another (Kerr et al., 2010; Yi et al., 2010).

Sturgeon are cartilaginous bony fish species that migrate from the Black Sea on the Danube River only for reproduction, and then return into the sea (Oţel, 2007; Antipa, 1909; Bănărescu, 1964 ). National legislation protects sturgeon species by prohibiting fishing and their commercialization (Order no. 330/2006) and by Danube programs for restocking with juvenile sturgeon specimens. Internationally, many non-governmental organizations take action for saving sturgeon species.

Sturgeons are important due to the fact that their presence on the Danube River was confirmed since ancient times and represented a source of food for coastal communities. Greek orator, Claudius Elian in his papers from the second century b.h. describes a technique for beluga capturing in the Danube River through the use of lines with hooks attached to a rope distributed transversely on the watercourse, a technique very similar to lining fishing (Palatnikov, 2010). On Chilia branch, Bacalbaşa-Dobrovici (1997) recalls the Italian monk Niccolo Barsari's visit during 1633-1639 who noted that fishermen captured daily between 1000 and 2000 sturgeon specimens.

The National Institute for Research and Development in Environmental Protection has analyzed the migration of sturgeons in the period of 2011-2014 and has noticed the possible negative effect of the arrangement works on Bastroe channel on these species.

## MATERIALS AND METHODS

The monitoring of sturgeon species migration conducted by the National Institute for Research and Development in Environmental during 2011-2014 has been performed by using ultrasonic telemetry. The method involves the use of tags attached to sturgeon specimens which transmits the information through water, with the help of ultrasounds, to a series of receiver stations fixed at certain strategic points (Badilita et al., 2013; Deák et al. 2013, 2014a, 2014b; Raischi et al., 2016a, 2016b).

Before assembling the systems, a riverbed in situ analysis has been performed. With a boat on which was installed a device for bathymetric measurements (single beam and multibeam), sections from one side of the riverbed to the other were made, on a sector of approximately 600 m (300 m downstream the location where the monitoring system was mounted and 300 m upstream). The measurements result was a 3D representation of the river morphology. For the assembly, it has been chosen only the areas with slight slope, without deep thresholds or holes, which may screen the signal transmitted by the ultrasonic mark attached to sturgeon specimens.

The ultrasonic tagging of sturgeon specimens has been conducted after a procedure with minimal stress on specimens. Thus, fish have been placed in a contention floating tube, directly into the water body provided with slots for a good oxygenation (Badilita et al., 2012). Before the tagging surgery, biometric measurements have been performed (total length, standard length, weight), the genre of the fish has been determined with an endoscope and DNA samples have been taken for each specimen. Then, the fish have been anesthetized through electro-narcosis and on the incision area has been locally injected xiline. The size of an incision was of approximately 3 cm and has been done with a sterile surgical material. The closure of the area, after ultrasonic marking, it has been done with an absorbable suture thread. The area has been swabbed with Betadine and then, a special medical adhesive that hardens in seconds and do not allow water to enter in the abdominal cavity has been applied. Finally, an "anti-poaching" spaghetti tag has been fixed, on the dorsal flipper, with a special pistol. All the identifycation data of each sturgeon specimen have been noted on the catch sheet. On every operation, a veterinarian specialist was present, who monitored the development of each phase of tagging procedure.

## RESULTS AND DISCUSSIONS

In 2011-2014 frame times, sturgeon catching, tagging and releasing was performed on the Danube River between Calarasi and Braila. During this period were studied 253 specimens of beluga, Russian sturgeon, Stellate sturgeon and Sterlet sturgeon, 186 being tagged both with ultrasonic and anti-poaching (T-bar) tags, while 67 were only tagged with anti- poaching tags.

In order to have a general overview of the sturgeon migration on Chilia branch and to perform a comparison with their behavior on Tulcea branch, the research team mounted reception systems for ultrasonic signal on Danube River at km 100, on Tulcea branch at km 70 and on Chilia at Bastroe confluence (Fig. 1).

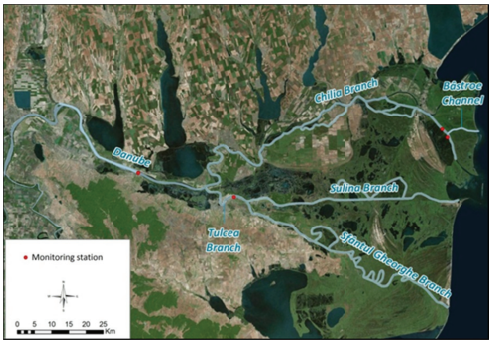


Figure 1. Location of the monitoring stations

On Chilia branch were not reported any catches of the T-bar tagged sturgeons in the studied period. Regarding the ultrasonic tagged specimens, the receivers' recordings showed that in 2012 spring 53% of the specimens that migrated towards the Black Sea used Chilia branch, while during 2013 autumn the percentage decreased at 31% and in 2014 spring reached again the value of 53% (Fig. 2).
In Table 1 is presented the distribution of sturgeon specimens detected between 2013-2014 on Chilia branch in respect with the migration direction (upstream or downstream). Therefore, for the upstream migration from the Black Sea to the spawning areas were identified also specimens tagged before 2011-2013.
The male Stellate sturgeon specimens with codes 2S5 and 3S48 came again for a new reproduction cycle after 2 years and 2 years and a half, respectively, from the tagging moment. This observation is in accordance with the previous research which showed that sturgeon do not perform reproduction actions every year (Hochleithner and Gessner, 1999). Gonads' maturation is directly influenced by the species, age, gender and hydro-climatic conditions (Reinartz, 2002). A second example is Stellate sturgeon 5S9 tagged in 2013 spring which came back after one year, in 2014 spring. An explanation for this behavior may be that the specimen did not reproduced in the previous year and intended to accomplish this action in 2014.

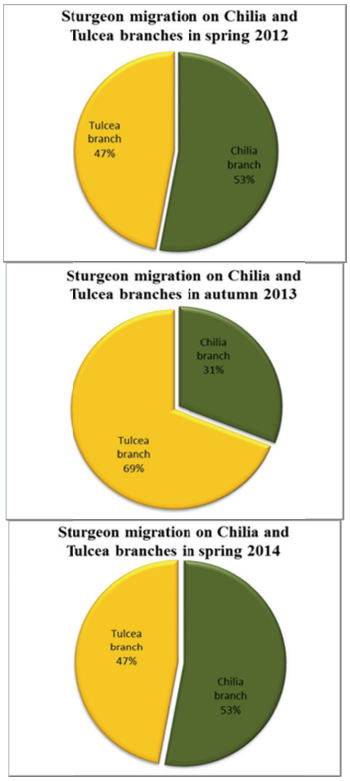


Figure 2. Distribution of sturgeon migration on Chilia and Tulcea branches between 2012 - 2014

Even that a lot of research works consider that sturgeon specimens migrate upstream during autumn and remain until the next spring migration season for reproduction purposes (Chebanov and Galich, 2011), our research revealed that some specimens have a different behaviour returning to the Black Sea in the same season (E.g.: 6S12, 6S14, 6S21, 6S22). From the 5 specimens of beluga tagged during 2013 autumn, only the 6S11 specimen was recorded on Chilia branch also in 2014 spring season.
The 7S1, 7S28, 7S32, 7S33, 7S2 specimens were tagged in 2014 spring on Calarasi-Braila sector of the Danube River and were recorded by the two receiving-recording systems mounted on the Chilia branch (upstream and downstream of the Bastroe confluence), at the end of the reproduction season when they migrated back to the Black Sea.

Table 1. Sturgeon migration routes on Chilia branch at the confluence with Bastroe channel

| Nr. Crt. | Sheet number | Tagging period | Species | Gender | Records Chilia branch | Migration Type | |
|---|---|---|---|---|---|---|---|
| **1** | 2S5 | A. 2011 | Stellate sturgeon | male | 24.03.2014 | ↗ | **Spring migration 2014 (Returning of sturgeon specimens tagged in 2011, 2012, 2013)** |
| **2** | 3S48 | S.2012 | Stellate sturgeon | male | 24.04.2014 | ↗ | |
| **3** | 5S9 | S.2013 | Stellate sturgeon | male | 04.05.2014 | ↗ | |
| **4** | 6S11 | A. 2013 | Beluga | male | 02.05.2014 | ↗ | |
| **5** | 6S12 | A. 2013 | Beluga | male | 15-16.11.2013 | ↙ | **Autumn migration 2013 (Sturgeon specimens tagged in 2013)** |
| **6** | 6S14 | A. 2013 | Beluga | male | 13-14.11.2013 | ↙ | |
| **7** | 6S21 | A. 2013 | Beluga | male | 24.11.2013 | ↙ | |
| **8** | 6S22 | A. 2013 | Beluga | male | 03.12.2013 | ↙ | |
| **9** | 7S1 | S. 2014 | Beluga | male | 07.05.2014 | ↙ | **Spring migration 2014 (Sturgeon specimens tagged in 2014)** |
| **10** | 7S28 | S. 2014 | Stellate sturgeon | male | 23.06.2014 | ↙ | |
| **11** | 7S32 | S. 2014 | Stellate sturgeon | male | 01.05.2014 | ↙ | |
| **12** | 7S33 | S. 2014 | Stellate sturgeon | male | 24.06.2014 | ↙ | |
| **13** | 7S42 | S. 2014 | Stellate sturgeon | male | 13.05.2014 | ↙ | |

| | |
|---|---|
| A. | = autumn |
| S. | = spring |
| ↗ | = upstream migration |
| ↙ | = downstream migration |

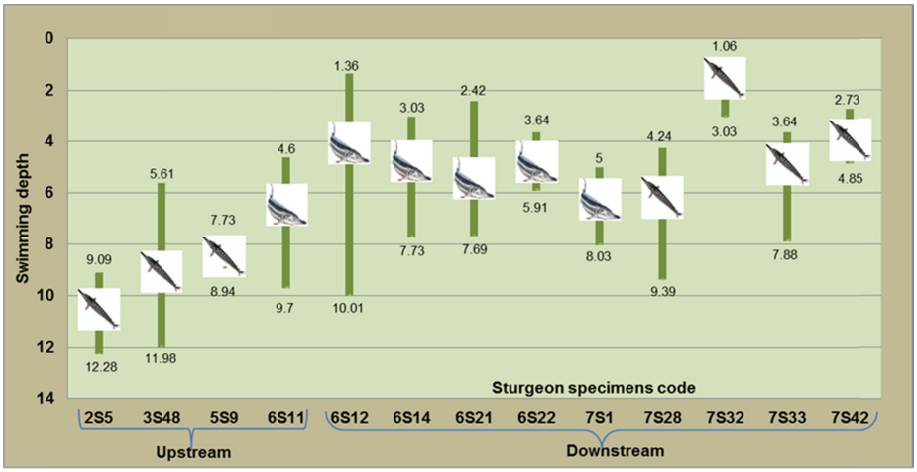


Figure 3. Swimming depths of sturgeon specimens recorded on Chilia branch

The research performed on sturgeon swimming behavior showed that they prefer deep water for upstream migration (Hochleithner and Gessner, 1999) because the water velocity is lower than in shallow water, decreasing the energy consumption. For the downstream migration they prefer the shallow water because of the high water velocity.

The graph presented below highlights the sturgeon specimens that passes through Chilia branch for both upstream and downstream migration. For downstream swimming depth it can be seen a minimum value of 1.06 m, while for downstream swimming depth the minimum value is 4.6 m (Fig. 3).

## CONCLUSIONS

The results of the research performed by the INCDPM team showed that Chilia branch has a high importance for sturgeon migration being used in both migration seasons (spring-autumn) both for upstream migration in reproduction purposes and for downstream migration to the Black Sea.

Therefore, the results revealed that in the spring season of 2012 and 2014, 53% of the monitored specimens used Chilia branch, while in the 2013 spring season only 31% used the same branch. On Chilia branch, at Bastroe confluence, the minimum sturgeon swimming depths was 1.06 m for upstream migration and the maximum value was 12.28 m.

These swimming depths are in accordance with the previously reported ones, sturgeon specimens preferring a lower water velocity at upstream migration (swimming close to the riverbed) for energy saving purposes and a higher water velocity at downstream migration.

The dredging works performed upstream on Chilia branch, including Bastroe channel, may have harmful effects such as: habitats losing or disturbing through elimination of the sediment used for reproduction, destroying macroinvertebrates fauna which constitute the main food for sturgeon species, and also increasing sturgeon larvae and juvenile death.

Knowing that at the end of 19th century was emphasized the negative effect of the dredging works on Sulina branch consisting of hampering sturgeon migration on this branch, there is a major risk for the sturgeon migration caused by the works performed on Chilia and Bastroe branches for fairway improvement.

A possible progressive clogging of the Old Stambul branch and especially of Musura branch may have a further major impact on sturgeon upstream migration.

Therefore, is necessary to undertake further research and monitoring activities for revealing the issues that may have a harmful effect on sturgeon species in order to implement, in time, the necessary measures to reduce the associated risks.

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# BROWN BEAR MAULINGS ON DOMESTIC ANIMALS IN ROMANIA – PRELIMINARY STUDY

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***Abstract***

*The study aimed to describe the morphology of injuries produced by brown bear (Ursusarctosarctos) on domestic animals such as cattle, sheep, pig and chicken.The study has been conducted during the period 2014 -2016 in Romania, ArgeşCounty.The lesions have been described in fifteen dead animals and two injured animals left alive.Indirect methods for identifying the attacking species were used by collecting samples from the site of the attack (hair, faecesand footprints). Dead animals have been examined by necropsy. Routine clinical examination has been done on alive animals. Additional information about the attacked animals has been gathered by interviewing the owners and hunting fund managers. The characteristic injuries for bear attack were represented by parallel and linear, superficial or deep wounds, bite marks, and tissue avulsions. Prey consumption appears to be characteristic in attacked animals, in humans being rarely mentioned. The specific localization of bear mauling are on head, neck, cervical and thoracic areas followed by upper hindquarters, forelimbs and hind limbs. The most serious injuries are represented by the neck and chest area features which have been observed in cattle examination.Mortality rate for the animals attacked by brown bear and found dead or alive is: 100% for chickens, 100% for pigs, 66.66% for sheep, 87.5% for cattle.*

***Key words****: brown bear, domestic animals, attack, mauling, Romania.*

## INTRODUCTION

The biggest population of brown bear, one of the largest living carnivores in Europe, is dispersed in Romanian Carpathians.

This species is also a high priority in conservation in European Union, the number of individuals being an important indicator in management of wildlife species. (Zedrosser et al. 2001; Cotovelea, 2014).

The interaction between man and bear, bear and domestic animals had been always subjected for scientific research, media and movies. Being one of the largest carnivores in Europe and occupying the top of the food chain, anthropization and habituation reflect negatively on bear behaviour.

Consequently, the bears wander closer and closer to households and livestock, resulting in attacks on human and domestic animals (Pop, 2012; Tough, 1993, Zimmermann et al., 2003). The majority of attacks reported in humans occurred in forests or at the edge of the forests. All the events can be generally characterized as "face-to-face meetings" or "close encounters" with a female bear with cubs, or with a feeding bear. All these situations are considered self-defence of the individual, as result of a surprise or unpredicted meeting.

Regarding the domestic animals, these are attacked in households situated in mountain, submontane area and at sheepfold, case in which the bear gets its food.

The importance of the study emerges from the lack of description of injuries caused by bear in animals, comparing with reported cases in humans.

The study area, Arges County, houses an average population of 700 brown bears, this population is slightly increasing in the past years. (Environmental Protection Agency Arges County – bear statistics).

## MATERIALS AND METHODS

The period analysed in this study was 2014-2016, and refers to Argeş County, Romania.

Two groups of animals were studied, as follows:

- animals killed by bear attack: 7 cows, 2 sheep, 4 chickens, 2 pigs;
- animals that survived the bear attack: 1 cattle, 1 sheep.

Indirect methods of identification have been used, such as:
- samples from the households where the attack took place (hair, faeces);
- pictures of the scratches on the walls and bear footprints on the soil;
- testimonies of ownersand hunting fund managers.

Dead animals were submitted to necropsy, using routine examination technique. Clinic examination was given to the alive animals.

## RESULTS AND DISCUSSIONS

The lesions observed during necropsy and clinical examination on dead or alive animals respectively are detailed in table 1.

Attack of the bear on domestic animals presents similar characteristics with the attack on humans, meaning that the main attacked body region are head and neck, followed by thorax and limbs. (Dhar et al., 2008)

The difference between human *versus* animals is that human is not considered as a genuine prey. Thus, bear attack on humans is considered self-defence and the bear does not eat them. Cases of consumption had been rarely reported, while the animals are attacked to be consumed.

Indirect methods of identification used in this study resulted undoubtedly in bear attack.

All gathered evidences from households within studied area pleaded for this conclusion: traces of paws, hair, scratches, faeces, type of injuries examined on animals.

The place where the attack occurred highlights traces that lead to indirect identification of the bear, such as dragging trace or dragging blood spatter, footprint on damp soil, scratches on the walls, hair, bear faeces.

The degree of specificity of these traces is very high, which makes confusion with other predators unlikely or impossible. Anatomic particularities of the limbs (plantigrade, flat-footed, five-fingered track), the type of the attack or the feeding type of this large mammalare reliable evidence for bear identification (Micu, 1998).

The injuries described both in humans and animals are also an important feature which can be used in indirect identification, being equally inflicted by teeth and claws. It is important to know the pattern of these injuries, so there can be made a differentiation from other species, such as wolves. It is known that interactions between bear and wolf involve food sources. Consequently, the same categories of criteria which support indirect identification are applied in both carnivores (Gunther and Smith, 2004).

The lesions made by bear attack on domestic animals can be summarized as follows:

-linear and parallel wounds (one to five lines corresponding to each claw) on the entire body, usually displayed on cervical, dorsal and upper hindquarters; these wounds may involve only the skin, or they can be deep, with various degree of soft tissue lacerations, reaching bone tissue.

-when claws penetrate the skin, it results in severe muscle laceration and rupture, organs rupture and perforation, leading to strong bleeding, hemopneumothorax, hemoperitoneum and hemopericardium.

-thebites, scratches and kicks lead to massive tissue loss and tissue consumption; dental marks are not so obvious on animals as they are on human skin.

-limb, ribs and spine fractures go frequently with lesions of soft tissues;

-bear may drag animals some distance, feature found out on pigs, cattle and sheep. The animals were dragged out from their shelters, dragging traces being observed on local vegetation or as dragging blood spatter.

Similarities with these injuries were reported in bear attack on human victims. Head lesions are represented by soft tissue injury; bite marks, laceration of the ear and head, avulsion of lips and eyes. Facial fractures occurred mainly in cheek, mandibula and maxillae, accompanied by similar lesions of neurocranium (mastoid bone, zygoma, occipital bones) (Ajazet al., 2010; De Giorgioet al., 2007; Prasadet al., 2013; Dharet al., 2008; Thakur et al. 2007; Roka et al., 2012; Mihailovic, 2011).

Neck and dorsal region lesions were represented by perforation of the left internal jugular vein and bite wounds. Typically, lesions were featured by deep wounds located

3–5 cm apart and six parallel excoriations between them (teeth mark and claws), scratches and bite wounds on the back. Lacerations were observed also on thigh and lumbar area (Ajaz et al., 2010; De Giorgio et al., 2007; Dhar et al., 2008).

Limbs lesions involved soft tissues and bone, such as metacarpal, radius, ulnar, humeral, clavicle and scapula fractures, lacerationswith tendon loss, biceps avulsions and gluteal laceration (Dhar et al., 2008).

The frequency of attacks may increase in the years with heavy winters such as the one of 2016-2017, and the years when bears did not receive any additional food from hunting fund managers.

Thus, more bear attacks on domestic animals are expected to occur in the next year when these carnivores need to feed for covering the losses produced during winter sleep. Romania's entry into the EU has imposed the common market milk quality standards.

Table 1.Lesions inflicted by bear on domestic animals

| 4 chickens | Injuries |
|---|---|
| 7 chickens in household, only 4 corpseswerefound<br>Figure 1. | -plucking<br>- theentire body iscoveredwithdeepwounds, musclelacerationandrupture of internalorgans<br>- multiple bone fractures (limbs, ribsand cervical spine) |
| 2 pigs | Injuries |
| pig 1<br>Figure 2. | - wounds, laceration, loss of skin and muscle tissue in the cervical region, cervical fracture;<br>- superficial chest wounds or scratches that appears rectilinear single or parallel grouped;<br>-forelimb muscle deep laceration and multiple fractures<br>-organs of the abdominal cavity were completely consumed except cecum, colon and rectum<br>- hind limb consumed 50% |
| pig 2<br>Figure 3. | - wounds and lacerations, loss of soft tissue in the cervical region<br>- superficial wounds or scratches on thoracic area that appears rectilinear single or parallel grouped;<br>- bite marks on forelimb, involving only the soft tissues<br>- internalorgans are intact<br>-hind limb consumed 5%, bitewounds are present |
| Sheep; 5 sheep in the household, one was found alive, 2 corpses, 2 were not found | Injuries |
| Sheep 1 – corpse<br>Figure 4. | - partial avulsion of head<br>- cervical fracture, muscle laceration<br>-multiplewoundson thorax, muscleruptur, multiple ribfractures<br>-total consumption of the abdominal and thoracic organs<br>-forelimbs: left forelegconsumed, multiple fractures on right foreleg multiple fractures of hindlimbs and bite wounds |
| Sheep 2 – corpse<br>Figure 4. | - total avulsion of head<br>- fracture of thoracic spine<br>- multiple ribfractures, intercostal laceration<br>-totalconsumption of the abdominal and thoracic organs<br>- total avulsion of forelimbs<br>- multiple fractures of hindlimb and bitewounds |
| Sheep 3 – alive<br>Figure 4. | -cervical and thoracic area tingedwithblood<br>- superficial skinwounds on cervical and thoracal area<br>- strayed animal in shock (traumatic shock) |
| Cattle: two corpses, onealive | Injuries |
| Cow 1 – corpse | - partial avulsion of head; absenceof tongue and masseters (bilaterally)<br>-spine: total avulsion of cervical segment (skin connects the head and thoracic limbs), partial avulsion of thoracic segment, lumbar spine fracture, pelvic fracture<br>- evisceration of thoracic organs, total heart consumption, partial lung consumption<br>- evisceration of the abdominal organs<br>-total forelimb avulsion and consumption up to acropodial level |
| Cow 2 – corpse<br>Figure 5. andFigure 6. | -thoracic spine fracture<br>- multiple wounds in parallel arrangement of the dorsal thorax, laceration and deep muscle rupture, hemorrhage, multiple ribfractures, subcutaneous emphysema, lungrupture and lung colapse associated with pneumohemotorax<br>- multiple parallel wounds on sacral andglutealarea, muscular laceration and haemorhage |
| Cow 3 – alive<br>Figure 7. andFigure 8. | - linear wounds on theside of the neck, perpendicular tothe longitudinal axis, which cross the skin, subcutaneous connective tissue and regional muscles<br>- wounds with parallel arrangement, produced by claws, on skin, subcutaneous adipose tissue and regional muscles, starting in the dorsal region of the withers, descending parallely to the shoulder, arm and forearm (left); the sides of the thorax presents superficial wounds, with parallel arrangement, cervical wounds are shorter than those of withers<br>- wounds with linear arrangement, parallel with sacral, buttock and flank that crossskin and subcutaneous connective tissue |


Figure 1. Chickens, plucking, abnormal positions that suggest multiple bone fractures

Figure 2. Pig, consumption of upper hindquarters and abdominal evisceration

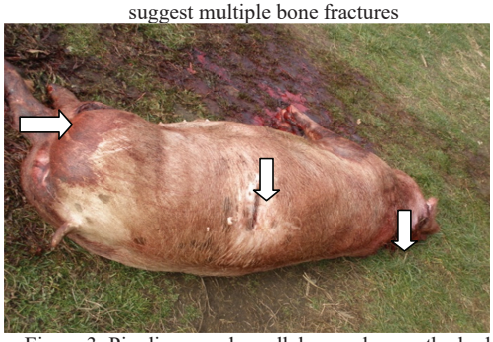

Figure 3. Pig, linear and parallel wounds over the body, deep linear wound on left hemithorax (middle arrow), loss of soft tissue in the cervical region (right arrow), left hind limb with a big bite wound and muscle rupture(left arrow)

Figure 4. Sheep, 1 alive in shock, 2 corpses with massive consumption, evisceration of abdominal and thorax organs

Figure 5. Cattle, wounds in parallelalignment on the dorsal thorax (arrow), laceration and deep muscle rupture, hemorrhage

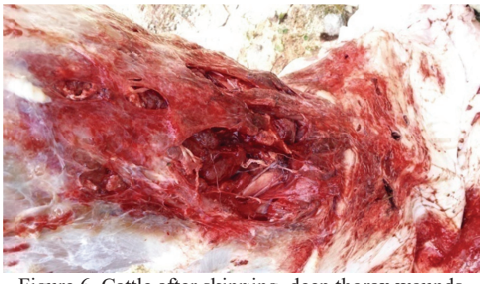

Figure 6. Cattle after skinning, deep thorax wounds made by claws, with muscle laceration, muscle rupture, haemorrhage

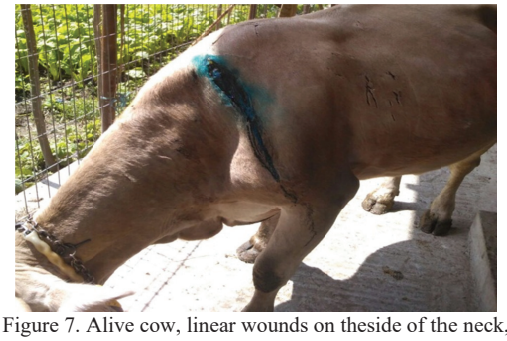

Figure 7. Alive cow, linear wounds on theside of the neck, perpendicular to the longitudinal axis, which traverses skin, subcutaneous connective tissue and muscles regional, parallel superficial wounds on thoracicarea made byclaws

Figure 8. Alive cattle, woundswith linear arrangement, parallelwith sacral, but tockand flank that traverses skin and subcutaneous connective tissue

## CONCLUSIONS

Characteristic injuries produced by bear attack are linear and parallel wounds, in number of one to five lines corresponding to each claw, usually observed on cervical region, dorsal and upper hindquarters, tissue lacerations, muscle rupture, bite marks.

Soft tissue lesions are frequently associated with bone fractures, especially of ribs, spine and limbs.

Prey consumption appears to be characteristic in attacked animals, in humans being considered self-defence.

Domestic animal consumption by bear is supported by copious missing of soft tissues, bones and organs.

Mortality rate in attacked animals reaches 88.54%.

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# FEED OIL SUPPLEMENT FROM ALTERNATIVE MATERIALS ON FEED EFFICIENCY AND PROTEIN EFFICIENCY RATIO IN RED TILAPIA FISH (*OREOCHROMIS NILOTICUS*) SEED PHASE

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***Abstract***

*Optimization of cultivation through input protein-energy efficient in the diet is very urgent. Fish need fat as an energy source and to maintain the shape and function of membrane tissue. This study aims to explore source fat from alternative materials of animal fat (caterpillar flour or larvae of Tenebrio molitor) and seed fat (hazelnut) in the form of feed oil supplement (FOS) its impact on feed efficiency and protein efficiency ratio in red tilapia fish seed phase. Methods of extraction descriptively, followed by a biological test experimentally, completely randomized design (7x3) use of feed oil supplement from caterpillar flour and hazenut (each with a dossage 1%, 2% and 2% a mixture of both) were added to the basal feed (low protein), and treatment standard feed (high protein). The extraction of hazelnut (31.72% crude protein, fat 35.32%) and the caterpillar flour (crude protein 55.11%; 15.51% fat), obtained the total yield of 30-35% material and extract oil supplements each 9.9 % and 4.05% initial weight. Biological test showed that the highest feed efficiency in the use of 2% a mixture of both caterpilar and hazelnut oil, but not significatly different with the use of standard feed and 2% hazelnut oil and caterpillar oil. Feed efficiency and Protein efficiency ratio of 2% feed oil supplement was higher compared to 1% feed oil supplement award and the basal feed. The addition of feed oil supplement will be increasing content of Energy-Protein Balance in the feed (ranging from 6.8 to 8.9 kcal.g$^{-1}$ protein). There means the protein sparring effect (effect of partial substitution of protein as an energy sources by fat) so as to conserve resources N and in turn be able to eliminate the discharge of nitrogen in the water.*

***Key words****: Feed oil supplement, the balance of energy-protein, feed efficiency, red tilapia fish seed phase.*

## INTRODUCTION

The provision of feed with a proportion of energy-protein effective quantitative (exact amount) and qualitative needs to be supported by information characteristics of energy sources and their influence on the diet (the rate of gastric emptying, the amount of feed, and interval feeding) are expected to provide benefits to improve efficiency feed on red tilapia fish (*Oreochromis niloticus*). Meat of red tilapia fish is thick and appreciated by the public abroad, especially Japan, USA, and Singapore, has an interesting color, flesh rough savory, and resembles a red snapper or red sea bream that have a high price.

Feed oil supplement is one application to add a component of fat and fatty acids. Fatty acids are a source of energy that has the ability to partially replace protein (protein sparring effect) and essential for cell permeability. Source of unsaturated fatty acids in fish feed is fish oil, linseed oil, and corn oil, but limited availability. Alternative materials locally-based dietary fat supplements showed that potentially is a caterpillar flour and hazelnut.

Caterpillar flour (insect larvae *Tenebrio molitor*) is cultured in a medium flour-based cereal. Caterpillars fresh flour contains high crude fat is equal to 32.4% and various other nutrients, but contain chitin as anti-nutrient if fed as a whole (Anguilar-Miranda et al., 2002). The nutritional value of caterpillar flour can be more helpful if the fat extracted oil (*feed oil supplement*). Hazelnut (*Alleurites mollucana*) is known as a spice native to Indonesia recommended as a source of unsaturated fatty acid linseed oil substitute for part of the fruit (seed) oil content of 55-65%, and oil content in the shell by 60%.

Fish needs for energy is expected to largely be met by non-protein nutrients such as fat and carbohydrates. If the energy derived from the non-protein sufficiently available, the majority of protein would be used to grow, but if the energy and non-protein nutrients are not met, then the protein will be used as an energy source so that the protein functions as a body builder will be reduced. The energy levels of protein in the diet also affect feed intake. If the energy level exceeds the needs of protein will decrease the consumption of so making more nutrients including protein will decline. Therefore we need the right balance between energy and protein in order to achieve efficiency and effectiveness of feed utilization.

Based on this background, it is very important to do research that aims to discover the source of the fatty acids from alternative materials as feed oil supplement in feed efficiency to achieve the balance of protein to energy.

Problems that can be identified is how far oil feed supplement extracted from alternative materials of vegetable and animal origin affect the energy balance of protein and protein utilization efficiency. The results of the study expected in the manufacture of feed supplements gained alternative sources of fatty acids and fish oil replacement strategy obtained in fish feed formulations with protein proper energy balance in order to achieve high feed efficiency.

Extraction is one of process or treatment performed on a given material as a product extracts to animals (Hartadi et al., 1986). Mechanically extracted which the extraction process from grain to heat and mechanical a way that the result is left is cake. Furthermore, the process of solvent extraction

is the term used for the extraction or removal of a material (for the purpose of fat or oil), using an organic solvent. The use a mixture of alcohols, alkanes, and water; also the use of solvents hexane and isopropanol are successfully used for the extraction of tissue. During the extraction solvent breaking of hydrogen bonds, van der Walls bonding and electrostatic interactions (Akoh and Min, 2002).

Lipid extraction procedure according Akoh and Min (2002), through the stages:

(A) Pretreatment (drying, size reduction, or hydrolysis.

Hydrolysis process can be done by using an acid (3-6 M HCl) or alkali. Acid or alkali is necessary to break the covalent and ionic binding between lipids and carbohydrates as well as fat emulsification.

(B) Homogenize network with solvent and separation / separation of the liquid component (organic and solutions) and solids.

(C) Expenditure contaminants

(D) Expenditure component solvent and drying the extract

Generally, the overall use of fish oil supplements is 5-10%, which functions other than as a source of fatty acids as well as attractant and structural improvements pellets. According Hsieh et al. (2007), the largest source of ω3 than beef tallow, linseed oil, and corn oil is fish oil with the use of the number of fish oil supplements in the diet as much as 6% that it contains 1.5% ω3. Given caterpillar flour oil and hazelnut oil fatty acids, it is expected that its use can be an alternative to fish oil.

## MATERIALS AND METHODS

Materials used in this study were: 270 fish tail test, artificial feed, and chemicals for analysis.

The fish samples used are red tilapia fish one month old 5 - 6 cm in length (5 g body weight).

Table 1. Composition of Feed Ingredients Proximate Analysis Results

| No. | Raw Feed | Crude Protein | Extract ether | Crude Fiber | Gross Energy*) |
|---|---|---|---|---|---|
| 1. | Soybean meal | 27.00 | 0.90 | 6.00 | 2240 |
| 2. | Fish meal | 46.41 | 3.22 | 1.0 | 2593 |
| 3. | Rice bran | 8.75 | 12.00 | 12.00 | 1630 |
| 4. | Pollard | 14.69 | 3.91 | 10.00 | 1300 |
| 5. | Oil/Fat | 3.00 | 100 | 0 | 9859 |
| 6. | Blood meal | 72.87 | 0.00 | 1.00 | 1208 |
| 7. | Coconut meal | 17.36 | 1.80 | 15.00 | 1597 |

*) DE (Digestible Energy) = 70% ×GE (Hepher, 1989)

**Experimental Procedure:**

1. The extraction of materials, analyzes of feed (Table 1), and Formulation (Table 2) and was measured in Energy-Protein Balance.
2. Test Feed (feeding trial), involves the collection and recording of data growth over the two-month maintenance and measurement of feed efficiency and protein efficiency ratio (Ensminger, 1997).

Table 2. Feed Formulation Treatment

| No. | Raw Feed | A | B | C | D | E | F | G |
|---|---|---|---|---|---|---|---|---|
| 1. | Soybean meal | 11 | 11 | 11 | 11 | 11 | 11 | 11 |
| 2. | Fish meal | 12 | 11 | 11 | 10 | 10 | 10 | 30 |
| 3. | Rice bran | 30 | 30 | 30 | 30 | 30 | 30 | 20 |
| 4. | Pollard | 30 | 30 | 30 | 30 | 30 | 30 | 28 |
| 5. | Oil/Fat | 5 | 5 | 5 | 5 | 5 | 5 | 5 |
| 6. | Blood meal | 12 | 12 | 12 | 12 | 12 | 12 | 6 |
| 7. | FOS seed fat (K) | 0 | 1 | 0 | 2 | 0 | 1 | 0 |
| 8. | FOS animal fat (U) | 0 | 0 | 1 | 0 | 2 | 1 | 0 |
| | | 100 | 100 | 100 | 100 | 100 | 100 | 100 |

Treatment effect was tested by F test and statistical analysis to determine the differences of each treatment used Duncan's Multiple Range Test.

## RESULTS AND DISCUSSION

### Nutrient Content and Energy Protein Ratio

Table 3. Protein and Fat Content of Materials of Alternated Fat Sources

| | Hazelnut* | Caterpillar flour* |
|---|---|---|
| Crude Protein (%) | 19 | 55.11 |
| Crude Fat (%) | 55 | 15.51 |
| Moisture (%) | 12 | 9.45 |
| Ash (%) | - | 5.11 |
| NFE (%) | 13 | 21.65 |
| Yield flour* | 60% | 70% |
| Yield of extraction** | 30% | 35% |
| Yield of oil/100 g raw material *** | 9.9% | 4.05% |
| The value of the acid number (mg KOH) | 78.5 | 10.31 |

Note:

* The results of the mechanical extraction process Steamed (steamed), Crushed (breakdown), Rolled, Blend (mixing), and Mill (flouring)

** The results of solvent extraction with hexane solvent using techniques of maceration (soaking 24 hours) the hazelnut and caterpillar flour.

*** NFA= Fat (BK100%) × yield preparation stage × yield of solvent extraction

Table 3 shows the protein content of the extracted mechanical hazelnut and caterpillar flour respectively 19% and 55.11%; whereas in the extraction process (ether extract or crude lipid) on these two materials of different hazelnut fat 55%, while the caterpillar flour by 15.51%.

Insolubility lipid in the water allow the separation of the components of carbohydrate, protein and water in a network hazelnut and caterpillar flour. Fat hazelnut was large enough that 55%, compared with fat caterpillar flour. Based on Table 3, it is also evident that the value of the acid number of hazelnut oil is high at 78.5 mg KOH while the fatty acid value of 10.31caterpillar flour.

This means triglycerides contained in pecan has an average molecular weight lower.

It allows too many parts of free fatty acids, in accordance with the opinion of Estrada (2011), as well as castor oil, generally non-food seed oils containing high free fatty acids.

Table 4. Nutrient Content (%) and Energy-Protein Balance (kcal/g protein)

| | Crude Protein | DE | Crude Fat | Crude Fiber | EPB* |
|---|---|---|---|---|---|
| Basal Feed (A) | 21.37 | 1689 | 5.47 | 9.23 | 7.9 |
| Added Fos 1% | 20.93 | 1761 | 6.44 | 9.22 | 8.4 |
| Added Fos 2% | 20.5 | 1834 | 7.41 | 9.21 | 8.9 |
| Standard Feed | 27.51 | 1871 | 4.67 | 7.11 | 6.8 |

- EPB = Energy-Protein Balance

The results of the analysis of the content of unsaturated fatty acids are dominant in caterpillar flour oils are oleic (C18: 1 ω9) amounted to 19.77% and linoleic (C18: 2 ω6) 8.51% still need to be tested further the amount of benefit to the fish. There still exists the possibility of contaminants, and the presence of residual solvents may occur. Non-lipid component separation is done by evaporation of the lipid extract by drying in a vacuum and then extraction with non-polar solvent. In this study, the solvent used is n-hexane.

**Growth Rate and Efficiency**

Growth rate of fish biomass at each sampling period showed a weight change that indicates that the fish have adapted to respond to the test feed (Figure 1).

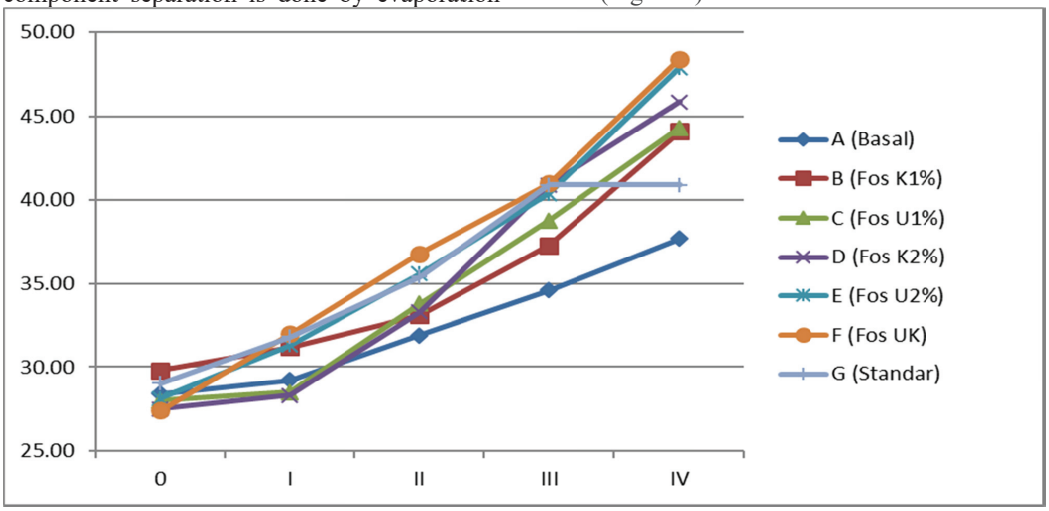


Figure 1. Growth Rate of Red Tilapia Biomass

This suggests that neither animal fat nor seed oils as feed supplements can be responded well by fish. Basal feed while increasing the weight of the lowest each week.

The growth of fish fry with standard feed at the end treatment decreased growth, which may be caused by factors of water quality due to discharge N of metabolites.

Table 4 shows that the addition of seed oil supplement will increase DE:P which means that also the mechanism of protein sparring effect (effect of partial substitution by protein sources of energy (fat) N which saves natural resources and in turn could eliminate the discharge of nitrogen in the water.

The results of the study in Japan showed that the highest growth and feed conversion in carp and eel, obtained on feed containing 1% supplement 18: 3 ω3 and 1% supplement 18: 2 ω6 (de Silva, 1989).

While tropical herbivorous fish (*Tillapia zilli*) more require 18: 2ω6 or 20: 2ω6 than ω3 series (de Silva, 1989).

Duncan Test Results on Feed Efficiency and Balance Protein Efficiency can be seen in Table 5.

Table 5. Feed Efficiency and Ratio Efficiency Protein

| Treatment | Feed Efficiency | Protein Efficiency Ratio *) |
|---|---|---|
| A (Basal feed, CP 20%) | 26.69 d | 1.25 |
| B (Added Fos Hazelnut K1%) | 38.98 b | 1.86 |
| C (Added Fos Caterpillar U1%) | 45.15 c | 2.19 |
| D (Fos K2%) | 52.73 a | 2.57 |
| E (Fos U2%) | 52.22 a | 2.55 |
| G (Standard Feed; CP 27,5%) | 54.87 a | 2.63 |
| F (Added Fos mix U1%+K1%) | 56.17 a | 2.04 |

*) Not showed significant differences in the level of 95%.

Table 5 shows that the standard feed (crude protein content 27.5%) and feed with the addition of oil feed supplement of 2% resulted in higher feed efficiency when compared with 1% feed oil supplements and basal feed (crude protein content 20%). The highest protein efficiency is in the use of 2% a mixture of both caterpilar and hazelnut oil. Protein efficiency ratio ranging ranges from 1.25-2.63, but did not show significant differences.

Energy-Protein Balance in the feed (DE/CP) ranging from 6.8 to 8.9 kcal.g$^{-1}$ protein (Table 4), which means that also the protein sparring effect (effect of partial substitution of protein as an energy by fat) so as to conserve resources N and in turn be able to eliminate the discharge of nitrogen in the water.

## CONCLUSIONS

In the manufacture of feed oil supplements gained as much as 30-35% oil yield, while purified extract obtained only by 9.9% and 4.05% of the raw material origin.

Feed efficiency and Protein efficiency ratio of 2% feed oil supplement was higher compared to 1% feed oil supplement award and the basal feed.

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# TRACEABILITY SYSTEM STRUCTURE DESIGN FOR FISH AND FISH PRODUCTS BASED ON SUPPLY CHAIN ACTORS NEEDS

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***Abstract***

*This paper presents the structure design of a traceability system in fishery supply chain based on artificial intelligence and information technology for data acquisition and processing. The design activity takes into consideration the need of the Romanian fisheries to get an effective and practical quality safety monitoring tool for fish and fishery products. The traceability system development is based on the European and national legal framework, which was reviewed and on the all stakeholder's informational needs, which were identified by interviews with the stakeholders within the fish and fish products supply chain.*

***Key words****: design, fish and fishery products, legal framework, stakeholders' needs, traceability system.*

## INTRODUCTION

The fishery supply chain is long and sinuous so the combinations of upstream and downstream components are difficult to be managed and traced. This is mainly due the fish industry includes different types of production and distribution chains, which make the tracing of the information to be very difficult.

In order to reduce the perceived risk associated with food consumption and to increase the consumers trust, countries from all over the world have introduced the Food Traceability System, which provides relevant information about the food supply chain, by introducing the concept from the farm to the table for fish and fish products as well (Choe et al., 2009). As a result, the inefficiency of the existing food safety systems together with the international developments linking food safety with trade, have determined the development of a new food legislation focused on assuring high levels of food safety (Van der Meulen, 2004).

Regarding the situation in Romania, there wasn't implemented an integrated traceability system for the fishery supply chain able to provide the complete and continuous monitoring of food safety and quality and the traceability at the national level.

On the other hand, there is a concern for complying with the EU legislation and also for development of a national legislation accordingly to EU legislation.

A traceability system is considered an effective tool to guarantee safety in fish products and improve the supply chain transparency (Nicolae et al., 2014).

Its purpose is appropriate when its development is based on the need of the fish supply stakeholders and, in the same time, in developed in the respect of the UE and national legal framework for food security and safety.

Rabade and Alfaro (2006) analyzed the influence of relationship between supplier and consumer on vegetable safety traceability, and then built an evaluation model on this basis. Resende-Filho (2007) analyzed the excitation mechanism of traceability system by building a supplier-consumer model.

An accurate achievement and implementation of a traceability system significantly reduces the risk exposure of the economic agents from the food chain by helping them to identify, isolate and correct any problem in an efficient and fast way. In this way food safety is guaranteed and the negative economic impact of this kind of incidents is minimised (Popa et al., 2010).

## MATERIALS AND METHODS

The Informatic traceability systems are involved in all important data record keeping in the product evolution, in this case fish and fish products along the production and supplying chain.

The main components of the traceability system are the path identifying and information. The identification represents a process of recognition of the evolving elements in the production and distribution chain; information represents the process of sending information through different stages of the production and supplying chain. Each stage is described by different demands that need to be completely satisfied in order to build a strong and efficient traceability system.

The structure design of the information traceability system for fish and fish products is part of a research project that intends to realize this kind of a system. First of all, enforced national and EU food safety legislation has been revised and the demands that a traceability system must comply with have been identified.

A review of the literature (printed and/or available online) referring to identification of the main stakeholders of the supplying fish and fish product chain has been also researched. On the field, we have identified the elements and requests of the interested parties on traceability as well as their involving into the traceability chain for fish and fish products by interviews and questionnaires. All these elements are reflected in the design of the structure of the fish and fish products traceability system, so the interested one to be willing to implement and use it in order to improve food safety and economic performance.

## RESULTS AND DISCUSSION

### Fish and fish products traceability legal framework in the design of the traceability system structure

Drafting of the functional specifications of the traceability system as a stage of the establishment of an Informatic system for fish and fishery products has a starting point in the need to improve the selling activities and the degree of consumers need to be informed. This is in line with the measure proposed by the "Fishing and aquaculture Law" (2014) issued as a method to develop the aquaculture in Romania (Moga and Neculita, 2016b). Monitoring the information from fish and fish products distribution chain has been also considered when realising a specific adapted production system in Romania, as well as the complying to the EU and national legislative framework. In order to ensure the safety of the fishery supply chain, many countries, including Romania, issued food traceability policy for the adoption of the traceability system, which is becoming a helping tool for fishery stakeholders in order to manage the inputs and products and to improve food safety. Governments, particularly in developed countries, argue that existing food safety requirements have been ineffective in reducing the growing burden of food borne illnesses (Kelepouris et al., 2007).

Info storage eases the sending of the product history to control institutions as well as facilitate the access of consumers to these data via different access tools. This is in respect of the request issued by the National Sanitary Veterinary and Food Safety Authority (NSVFSA) in /order to assure food traceability based on the principle "one step back, one step forward" regulated by Regulation (EC) No 178/2002.

The projected system is offering the tools to help food operators to comply with suppliers and clients identification to find all the products from each supplier and also to have special info on the products sold to clients. By using the implemented processes, the system can fulfil the NSVFSA request to assure external and internal traceability.

The system helps the food operators to prove external traceability by using initial documents for raw materials, batches identification, delivery documents of finished products and batch marking in official documents. Regarding internal traceability, food operators need to have their own batching system, an internal batching chart and identification methods for precooked products and ingredients during processing, product batching and sampling. Registering of these traceability related information into the system can be available on request, on the spot (Moga and Neculita, 2016b).

The traceability system assures fish and fish products following by analysing the batch production numbers given to each move on the harvested products or processing at fisheries, production, processing units, warehouses and selling points. Thus, it is assured the implementing of the requests of the Regulation (EC) No 178/2002 which states the pathway of foods - known as distribution chain, that include the production steps to the final consumer (Moga and Cretu, 2016). Regulation (EC) No 178/2002 establishes the correct naming of production, processing and distribution stages as "any stage, primary production, storage, transportation, selling to the final consumer or to animal feeding". Furthermore, the system is following the Regulation (EC) No 1224/2009 which states that all fish products to be "batched before the first selling. In Romania, according to the Fishery and Aquaculture Law (2014), "products from commercial and aquaculture fishing placed on the market must be labelled consequently for each batch. All labelled data and the labelling procedure are regulated by the central public authority responsible for the fishery sector".

**The stakeholders needs and the traceability system design**

Different stakeholders have different requests and approaches towards the traceability based on their different role in the distribution chain for fish and fish products. Implementing and using the traceability system depends on the tight coordination between the parties involved in the distribution chain. When the traceability system meets all stakeholders needs (consumers and producers), the expected benefits are shown. For information data gathering, identifying of the main stakeholders of the fish product delivery chain, need to be done properly. These include: fish biologic material farms, fish rearing farms, production units, sales units and also consumers. Processing factories are in the centre of the supply chain, where the main capital, technology and human recourses are concentrated, compared to supplying and selling of fish products. At the processors stage, were also the main changes applied to raw materials.

The main Romanian stakeholders involved in fish and fish products traceability are:

- Producers - based on fish provenance - raw material:
  - for aquaculture products:
    - fish rearing farms;
    - recirculating aquaculture system.
  - commercial fishing:
    - wild fish: industrial fishing (natural interior ponds and Black Sea);
    - traditional fishing (natural interior ponds and Danube Delta).
- First selling points represent the link stage between the producer and processor/distributors;
- From producers or first selling points, fish and fish products are delivered to others (processor and/or distributors);
- The last representative in the commercial chain is the final consumer who needs to identify the information found in database of the product.

Regarding the Romanian business transaction system, these include engross markets, distributors, supermarkets, chains of supermarkets, direct buyers form local producers, small and independent shops and less known and used - electronic commerce. The consumers are the final stage in the supply chain. When the traceability system meets all participants - consumers and producers' requirements, then the anticipated benefits will be obtained. Therefore, a system of traceability intended for fishery products should harmonize the requirements of each of the categories presented (Moga and Neculita, 2016a).

The IT system for fish and fish products is made on a portal (platform) that allows inserting access to information on the entire distribution chain. The portal is structured in 3 access zones, based on the role in the system and intelligence needs of each involved stakeholder. The portal sections are presented in Figure 1: public area for consumers; private area 1 - for first selling points and processors; private area 2 - for portal administrator. Private areas 1 and 2 are available only to users that have allowance for access. For private access on the portal, users must have digital certificate or SSL, but also the authentication can be made using an ID and a password based on the email used for registration.

The structure of the traceability system is related to the starting point of the distribution chain of fish and fish products, represented based on provenance by the first selling points of the raw material, fish farmers or importers followed by processors, distributors e.a.

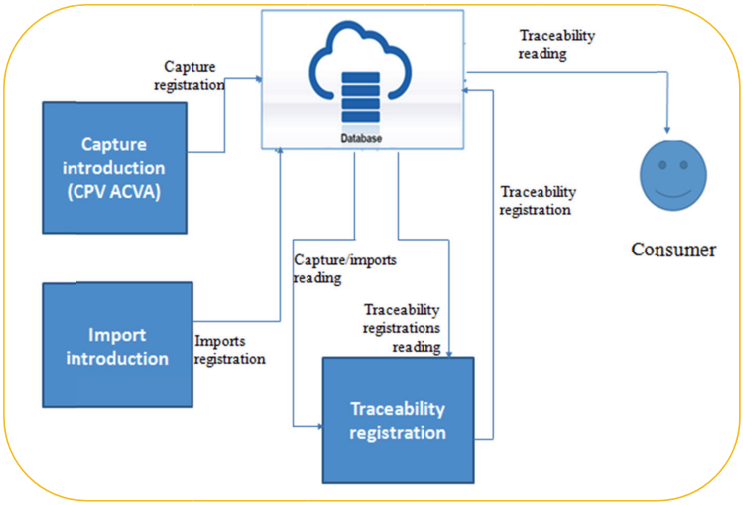


Figure 1. General scheme of the traceability system (original)

**The information framework design in correlation with the stakeholders needs**

In the traceability system, information is registered on the specific moments of the technological flow named as Reception, Harvesting, Acquisition, Processing and are from the informational point of view perceived as Batches or/and Re-batches.

When harvest the fish in their own ponds, the **fish farmers** have to batch the harvested fish and to mark the moment in the system (for every batch). They will introduce information related to harvesting date and hour, capture procedure, type of the pool form where the fish was harvested, its way of filling with water, treatments and selling procedures.

When receive the harvested fish from the fish farmers, **the first selling point** batches the products and mark the moment with the registration of harvesting date and hour, way of capture, hydrographical pool and the area of fishing and selling procedure.

**Importers** need to also batch the fish and fish products when receive it and mark the moment with register the information: import date, fishing date, FAO area, authorisation number of the producer, and ways of selling.

**Distributors** will identify the purchased fish batches based on the suppliers type and then re-batches and mention for each new batch the selling procedure. Processors are assimilated to distributors with the slight difference that when receiving the fish and fish products they can re-batch these products based on transforming after processing.

**Consumers** represent the essential component of this flow because the information received on different stages (by all the partners involved) is available and can be used to follow the traceability of the fish and fishery products.

In this regard, the last distributor/processor (the one who is selling the fish) will have to give access to information on traceability by marking (when at weighing and labelling) with 2D bar codes. This procedure allows the reading of the primary information on the product and also analysing of the product path (by accessing the application TRASIPESC where all partners have been loaded information).

The traceability chain of fish and fish products can differs based on the status of actors involved. So, it can be short or long and it can change in time. If the fish and fish products are

intended for export or intra-community (EU countries) commerce, there is a need for labelling and certification.
For achieving competitive advantages there is the need to assume voluntary certification for fish and fish products of the importing countries (Nicolae et al., 2015).

## CONCLUSIONS

1. Fish traceability can be perceived as a tool to provide a higher value of the product, by managing and controlling the processes, the stocks, and the products quality which conducts to an increasing of the consumers trust in food safety, growing of the operational efficiency for all the partners in the supply chain and ease a potential increasing of the profit for the companies from food industry.
2. Compliance to the legal framework for traceability in the European Union which is focused on the big importance of labeling of fish products and of the supporting documents serving as certificates, permits the achivement of all of the advantages provided by a traceability system.
3. As regarding Romania, although consumers are more aware, focused and interested in fish products safety, the implementation of a national integrated system that answers to the needs of safety and traceability is becoming more obvious and useful.
4. The traceability system designed for fish and fish products takes into consideration the requests imposed by the National Sanitary Veterinary and Food Safety Authority (NSVFSA) that state the importance of information sharing, organizing the traceability register of recorded database in order to be easily accessed "on request" with no delay. Although there is no obligation for economic agents for fish industry to adhere to an integrated system, considering the fact that such a system is in line with the NSVFSA requests, this could be a good reason for implementing / using the system by the fish industry.
5. For the food safety increasing, the responsible actors of the field must adopt and apply a responsible marketing policy that involves knowing of the path of foods from “from farm to fork”.
6. The availability of the information regarding fish and fish products quality by marking the selling products (at weighing or labelling) represents an advantage for the economic agents but also for the consumers which are interested the traceability system for products.

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# SOME BIOLOGICAL ASPECTS OF LESSEPSIAN *SARGOCENTRON RUBRUM* (FORSSKÅL, 1775) IN THE NORTH CYPRUS, MEDITERRANEAN SEA

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***Abstract***

*This study was carried out by trammel nets in the waters of North Cyprus, 0-50 m, between January - November 2016. The size frequency distribution and length/weight relationships of Sargocentron rubrum were determined. A total of 148 individuals of lessepsian fish species were sampled during the study. Length and weight of the samples varied between 11.1 - 20.1 (15.1 ± 2.6) cm total length and 23.73 - 153.33 (66.19 ± 33.66) g respectively. The relation between total length (L) and weight (W) was determined as* $W = 0{,}0138L^{3{,}0915}$ $R^2 = 0{,}9773$. *It was determined that Sargocentron rubrum showed positive allometric growth.*

***Key words:*** *Lessepsian fish, Sargocentron rubrum, biological aspects, North Cyprus, Mediterranean Sea.*

## INTRODUCTION

Since the opening Suez canal about 130 red sea species have become successful colonizers of the Mediterranean Sea (Safriel and Ritte, 1986). Some lessepsian fish species in the eastern Mediterranean were very well colonized, such as Indo-Pacific species *Sargocentron rubrum, Siganus rivulatus*, *Etrumeus teres Fistularia commersonii*, *Lagocephalus sceleratus* in the eastern Mediterranean.

*Sargocentron rubrum* is one of lessepsian fish species can be found between the depths of 1-84 m, in the Mediterranean Sea (Randall, 1998). It is inhabits in caves and cracks (Kuiter and Tonozuka, 2001); coastal reefs (Lieske and Myers, 1994), silty reefs, wreck in lagoons, bays, and harbours (Randall, 1998). This species feed on the small fishes, shrimps, and crabs (Randall et al., 1990; Göthel, 1992). This species is distributed in Red Sea to the western Pacific (from southern Japan to New Caledonia, Vanuatu and New South Wales, Australia) (Randall et al., 2003). It can reach maximum 32 cm in length (Williams and Greenfield, 2016).

In this present study, some less known properties (population dynamics and growth performance) of lessepsian fish species *S. rubrum* in the Cyprus, eastern Mediterranean Sea were reported.

## MATERIALS AND METHODS

The study was carried out from 4 different stations (Table 1) in the north Cyprus (Figure 1), eastern Mediterranean Sea, between January and November 2016. Samples of *S. rubrum* (Figure 2) were caught by the trammel nets (22 mm mesh) from the depths 0 - 50 m. 4 - 6 m fishing boats (20 - 30 HP) were used to catch the fish species. The bottom structure of the four fishing ground were rocky.

Table 1. Coordinates of stations in the Mediterranean Sea

| Stations | Coordinates |
|---|---|
| 1 | 35°14'44.5"N, 33°57'04.1"E |
| 2 | 35°24'12.8"N, 32°55'12.6"E |
| 3 | 35°21'10.5"N, 33°09'44.9"E |
| 4 | 35°33'26.2"N, 34°13'04.9"E |

A total of 10 fishing operation were performed. Trammel net used in the study had 22 mm bar length in the inner panels and consisted of PA multifilament webbing made of 210 d/2 and 60 meshes depth with a hanging ratio of 0.59. The outer panels had a mesh size of 100 mm with 8.5 meshes depth those used by local commercial fishers were used in the north Cyprus. Float lines of the nets were equipped

with PP Ø4 no floats and 30 g lead sinkers. The experimental trammel net with a total length of 210 m was obtained using one sheet of each mesh size in 70 m long.

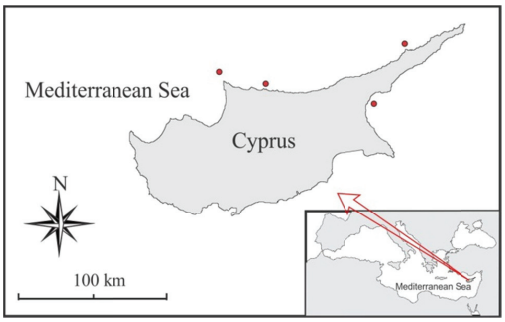


Figure 1. Sampling areas

Figure 2. Research material *Sargocentron rubrum*

Samples were caught and total length (TL) was taken from tip of snout to caudal fin end (TL) measured to the nearest centimeter and then weighed to the nearest grams in the laboratory. In the laboratory, fishes were identified to species level, based on following Smith and Heemstra (1986).

In the study, the relationship between length and weight were calculated by using the formula $W = a\ TL^b$, in which W is the total weight (g) and TL is the total length (cm). The parameters *a* and *b* were estimated by functional regression. In the equal *b* value for each species was tested by t-test at the 0.05 significance level to verify that it was significantly different from isometric growth (Froese, 2006).

## RESULTS AND DISCUSSIONS

A total of 148 specimens of *S. rubrum* were caught and analyzed during the research period. The mean length was estimated as 15.06 ± 2,56 cm, ranging from 11.1 cm to 20.1 cm TL; and weight was 66.19 ± 33.65 g , varying from 23.7 g to 153.3 g. The length and weight frequency distribution diagrams were given in Figure 3, 4.

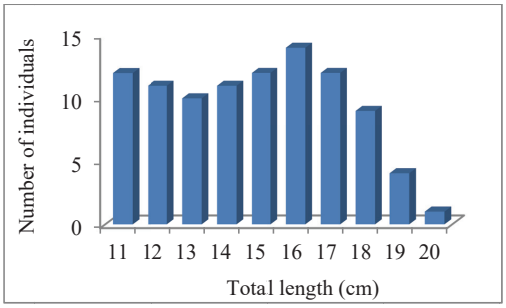


Figure 3. Length frequency distribution of *Sargocentron rubrum*

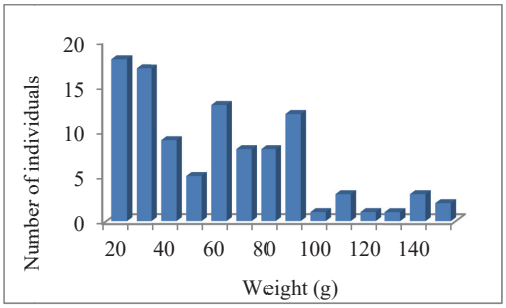


Figure 4. Weight frequency distribution of *Sargocentron rubrum*

The length/weight relationships were calculated and showed in Figure 5. According to table the length-weight relationship curves, allometry in growth is observed positive.

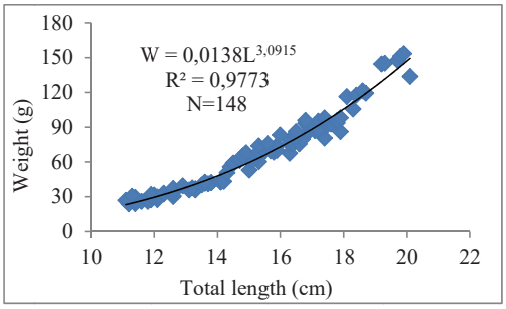


Figure 5. Length-weight relationship of *Sargocentron rubrum* in the North Cyprus

The largest individual of *S. rubrum* caught in the present study was recorded as 20.1 cm TL (153.33 g).

In the previous study, Krishna et al. (2015) were estimated the minimum and maximum lengths, the length/weight parameters a and b, coefficient of determination ($r^2$) in Table 2.

Table 2. Length-weight relationship for sargocentron rubrum in the north Cyprus, Mediterranean Sea

| Specifi-cation | n | Length range (cm) | a | b | $R^2$ | Growth type |
|---|---|---|---|---|---|---|
| Present Study | 148 | 11.1-20.1 | 0,0013 | 3,0915 | 0,9773 | + |
| Krishna et al., 2015 | 44 | 11.1-21.2 | 0.0089 | 3.102 | 0.849 | + |

Our results for *S. rubrum* were found similar with the findings of Krishna et al. (2015), from Visakhapatnam coastal waters, India.

Generally, parameters of length/weight relationships can be affected by several factors such as season, sample size, habitat, gonad maturity, sex, diet and stomach fullness, health, fish activities, seasonal growth rates and preservation techniques (Benegal and Tesch, 1978).

Even if both studied fishing ground were far from each other, similar results were recorded for *S. rubrum*.

## CONCLUSIONS

Even if, *S. rubrum* has a minor commercial value in the Mediterranean Sea, present study results are provided the basic information on the length-weight relationships of *S. rubrum* from the north Cyprus rocky substrate can be useful for the management of fishery resources.

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# SOME BIOLOGICAL ASPECTS OF LESSEPSIAN PENAEID SHRIMP *PENAEUS JAPONICUS* (BATE, 1888) IN THE GULF OF ANTALYA, MEDITERRANEAN SEA

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***Abstract***

*This study was carried out through monthly deep trawls applications in the waters of 20-100 m of depths in the Gulf of Antalya, between June 2013 and November 2014. The species composition, size frequency distribution and length/weight relationships for each sex of the commercially important shrimp, Penaeus japonicus in the Gulf of Antalya, in Mediterranean Sea were studied. A total of 108 individuals of lessepsian penaeid shrimp were sampled during the study. Length and weight of the samples varied between 10.6-20.3 (12.3±2.2) mm total length and 11.33-80.17 (29.17 ± 13.05) g respectively. The smallest individual was sampled in February and the biggest was in April. A total of 63 (% 58) samples were female and 45 (% 42) were male. The relation between total length (CL) and weight (W) was determined as* $W = 0.0038CL^{3,1916}$ $R^2 = 0.9437$. *This was calculated for females as* $W = 0.0036CL^{3,2158}$ $R^2 = 0.953$ *and for males* $W = 0.0121CL^{2,7561}$ $R^2 = 0.8582$. *It was determined that total and females showed positive allometric growth and males were showed negative allometric growth.*

***Key words****: Lessepsian shrimp, Penaeus japonicus, biological aspects, Gulf of Antalya, Mediterranean Sea.*

## INTRODUCTION

The Mediterranean Sea is one of the seas of the world most affected by biological invasions (Streftaris et al., 2005). A total of 955 alien species are known in the Mediterranean and the vast majority of them were reported from the eastern Mediterranean (718 species), less from the western Mediterranean (328), central Mediterranean (267) and Adriatic Seas (171). Of these, 535 species (56%) have become established in at least one area. It is worth noting that aliens have increased the total species richness of the Mediterranean Sea by 5.9% (Zenetos et al., 2010). A total of 119 alien crustaceans have been reported in the eastern Mediterranean and 58 species belong to decapod crustaceans, presenting an accelerating entrance rate (Koukouras et al., 2010). According to Galil et al. (2015), 14 identified alien penaeids in Mediterranean Sea, eight of which probably were introduced through the Suez Canal (Scannella et al., 2017).

Turkey is surrounded by four seas (Levantine Sea, Aegean Sea, Sea of Marmara and Black Sea) with different hydrographical characteristics. A total of 400 alien species belonging to 14 taxonomic groups occur along the Turkish coasts up to 2010, with the crustacean being the third group (64 species) after Mollusca (105 species) and Polychaeta (75 species). The majority of these species (306 species, 76% of the total number of species) have become established in the area (Çinar et al., 2011). The proximity of Turkey to the Suez Canal has resulted in dense settlements of Indo-Pacific migrants (66% of the total alien species in Turkish waters), especially in habitats along the Levantine coast of Turkey. In the last years, some of them have expanded their distributional ranges to other areas of the Aegean Sea, i.e. Gökova Bay (Ateş et al., 2007; Yokes et al., 2007).

*Penaeus japonicus* is a commercially important species in the Mediterranean region around Egypt, Israel, and Turkey. It lives on sandy mud and sandy bottoms waters up to 90 m depth. This shrimp has a maximum length of 25-30 cm, and can survive and grow at low water temperatures (10°C). It is a native species in the Indian Ocean and the southwestern Pacific Ocean. It is distributed along the east coast of South Africa, Red Sea, Indian Ocean, Korea, Japan, Taiwan, Malaysia, Philippines, Indonesia, New Guinea, Fiji Island and north Australia (Hayashi, 1996).

In this study, some biological aspects (sex ratio, substrates features, maximum - minimum lengths and weights, lengths frequency distribution etc.) of lessepsian *P. japonicus*, a shrimp of high commercial value, were determined in the Mediterranean Sea.

## MATERIALS AND METHODS

The data were from monthly catches in the Gulf of Antalya, June 2013-November 2014. Trawling's were carried out in the Gulf of Antalya on 36° 50' N, 30° 34' E - 36° 45' N, 30° 55' E Mediterranean Sea (Figure 1).
Samplings of shrimps were made by means of a bottom trawl net (22 mm mesh size) every month during a period of 9 months. Trawl shots of about 180 minutes were undertaken at each sampling station at depths of between 20 and 100 m. The samples were used to determine species composition, size frequency, length/weight relationship of the shrimp. For this reason, the species was identified; females and males were sorted by visible thelycum or petesma. All individuals were weighed to the nearest 0.1 g and measured with vernier calipers for their carapax length (CL) from tip of the rostrum to end of the carapax.

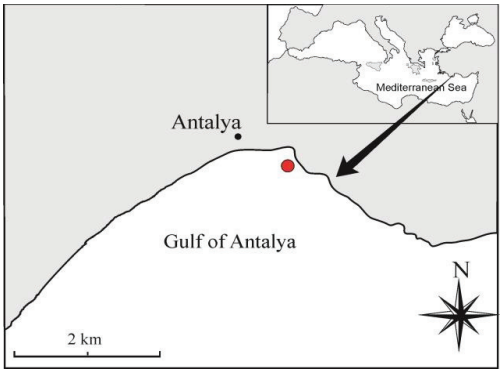


Figure 1. Sampling area in the Gulf of Antalya

The relationship between length and weight were calculated using the formula $W = a\ CL^b$, in which W is the total weight (g) and CL is the carapax length (mm). The parameters *a* and *b* were estimated by functional regression. In the equal *b* value for each species was tested by t-test at the 0.05 significance level to verify that it was significantly different from isometric growth (Froese, 2006).

## RESULTS AND DISCUSSIONS

### *Length frequency distribution*

During this study, a total of 108 specimens of *P. japonicus* were analyzed throughout the research period, 63 (58 %) being females, 45 (42 %) males. The mean size for females was 34,22341 ± 2,160858 mm CL, ranging from 12.2 mm to 22.0 mm; for males 15,16 ± 1.21mm TL, varying from 12.1 mm to 17.4 mm TL. The mean size of females was significantly larger than the mean size of males ($P < 0.05$). The largest female and male were 22.0 mm and 17.4 mm, respectively. The length-frequency distribution diagrams for female, male are given in figures 2, 3.

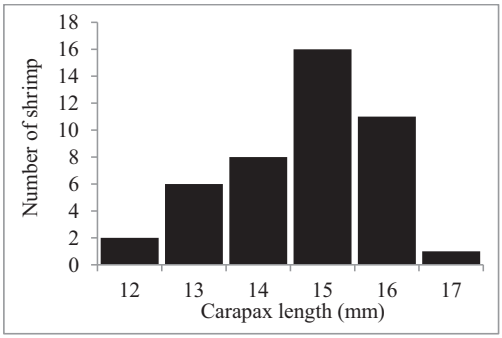


Figure 2. Size frequency distribution of males *Penaeus japonicus* in the Gulf of Antalya

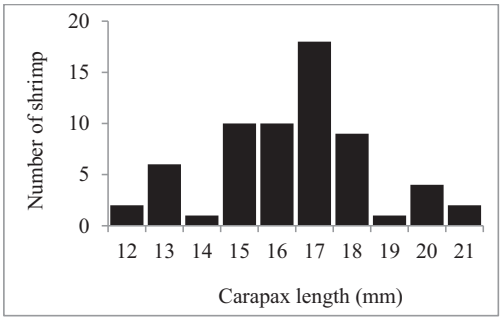


Figure 3. Size frequency distribution of females *Penaeus japonicus* in the Gulf of Antalya

### *Length/weight relationship*

The length/weight relationships were calculated for pooled data, males and females separately and were showed in Figures 4, 5, 6. These figures show that males of *P. japonicus* have

fusiform body shape. From visual inspection of the length-weight relationship curves, allometry in growth is observed positive in both pooled data and females. Only males were showed negative allometry.

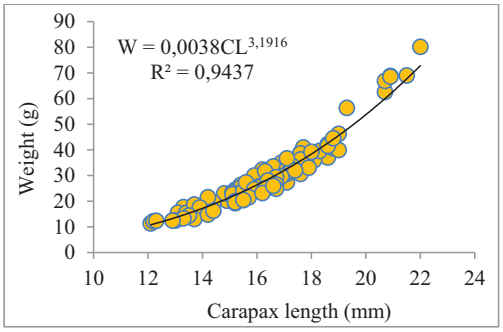


Figure 4. Length-weight relationship of *Penaeus japonicus* in the Gulf of Antalya

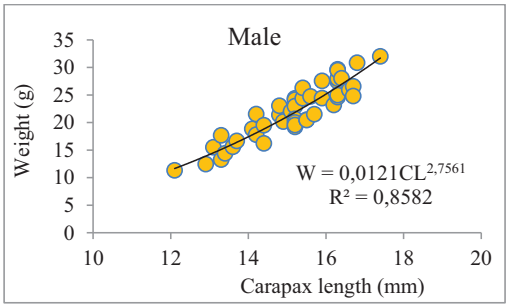


Figure 5. Length-weight relationship for males of *Penaeus japonicus* in the Gulf of Antalya

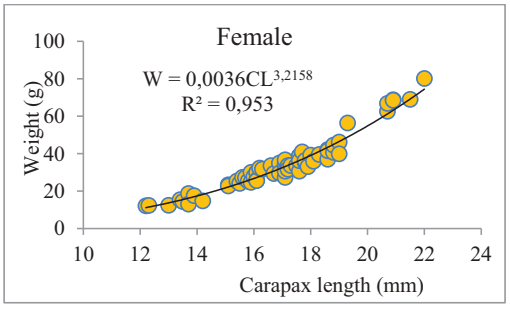


Figure 6. Length-weight relationship for females of *Penaeus japonicus* in the Gulf of Antalya

*Catch composition and habitat features of the research area*

In this study, while *Penaeus semisulcatus* (de-Hann, 1844), *F. aztecus* and *Metapenaeus monocerus* (Fabricus, 1798), species were living in muddy bottom, *P. japonicus* and *M. hathor* were found in sandy substrates. *P. japonicus* was caught with *M hathor* and *P. kerathurus* by trammel nets intensively especially in shallow water, in research areas. *P. semisulcatus, M. monoceros, Merlicertus (Penaeus) kerathurus* (Forskal, 1775), *M. hathor*, *Metapenaeopsis aegyptia* (Galil & Golani, 1990), *Parapenaeus longirostris* (Lucas, 1846), *Trachypenaeus curvirostris* (Stimpson, 1860), *F.* Aztecus were observed in the catch composition by bottom trawl.

*Salinity and temperature*

The salinity of the gulf did not change much during the course of this study. Salinity was 37.0-39.5 ppt. The water temperature was 17-24°C during study period.

A total of 108 individuals of lessepsian penaeid shrimp were sampled during the study between June 2013 and November 2014.
In this species, it is clear that females grow to a larger size than males. The length/weight relationships showed in Figures 2, 3 also indicated this suggestion. The largest female and male of *P. japonicus* caught in the present study were recorded as 22.0 mm CL (80.17 g) and 17.40 mm CL (30.02 g), respectively.
Our results for *P. Japonicus* were found similar with the findings of Kumlu et al. (1999) who studied same species on the Yumurtalık Bight (North eastern Mediterranean).

## CONCLUSIONS

*P. japonicus* is an indo-Pacific shrimp species and migrated to the Mediterranean Sea. According to study results, this species found similar environmental features in the gulf of Antalya or it could adapt itself to this new habitat. In the study area, it is found maximum 22 mm CL in length and 80.17 g in weight in the gulf of Antalya.
This shrimp species has become one of the commercial shrimp species caught in the Antalya bay.

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# HELMINTH FAUNA OF WHITE BREAM (*BLICCA BJOERKNA*) (LINNAEUS, 1758), FROM THE SREBARNA BIOSPHERE RESERVE, BULGARIA

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***Abstract***

*The aim of the study was to reveal the helminth diversity and the parameters of infection of white bream (Blicca bjoerkna) from Srebarna Biosphere Reserve, North-East Bulgaria. This is the first study of helminth fauna of white bream from Srebarna lake. The hosts were examined by standard techniques. Five species of helminths were found: trematodes (metacercariae of Diplostomum paraspathaceum, Diplostomum pseudospathaceum, Posthodiplostomum cuticola, Tylodelphys clavata) and monogenean (Paradiplozoon homoion). All helminth species identified in the present study are new host-records for the white bream in the Srebarna Lake. Blicca bjoerkna was reported as a new host record for digeneans, namely Diplostomum paraspathaceum, D. pseudospathaceum, T. clavata and T. monogenean, P. homoion from the territory of Bulgaria. Blicca bjoerkna was reported as a new host record for D. paraspathaceum, D. pseudospathaceum and monogenean P. homoion from Balkan Peninsula. In the present study, was reported for the first time the trematodes for D. paraspathaceum, D. pseudospathaceum as parasite of white bream from Basin of Danube River.*

***Key words****: parasite, helminths, Blicca bjoerkna, Srebarna Lake, Bulgaria.*

## INTRODUCTION

Srebarna Lake is a hyper-eutrophic lake, located on the Bulgarian right bank of the Danube River between r.km 391 and r.km 393, near the village Srebarna, 18 km west of town Silistra. Srebarna Lake is connected via an artificial canal with the Danube. Srebarna Reserve is included in the List of Wetlands of International Importance (Ramsar Convention) and among Important Bird Areas (BirdLife International) and being listed as a site of the Natural Heritage and a Biosphere reserve under the Programme on Man and the Biosphere (UNESCO). This reserve is characterised by a significant diversity of highly protected species, including fish-eating birds; it is one of the major European nesting sites of the Dalmatian pelican (*Pelecanus crispus*) (Michev et al., 1998; Uzunov et al., 2012). The fish populations are the main participant in the circulation of helminths in lake ecosystem.

This is the first study of helminth fauna of *B. bjoerkna* (Linnaeus, 1758) from Srebarna Lake, although there are several studies of helminths of fish from Srebarna Lake (Chunchukova et al., 2016; Kirin et al, 2013; Kirin et al., 2014; Margaritov,1959; Shukerova, 2005; Shukerova, 2006; Shukerova, 2010; Shukerova and Kirin, 2008; Shukerova et al., 2010, Shukerova and Kirin, 2012).

## MATERIALS AND METHODS

During period May-September 2013, sixteen (total length of body 85 -150 mm) specimens of white bream *Blicca bjoerkna* were collected from Srebarna Lake (Figure1).


Figure 1. Srebarna Lake

The hosts were examined for helminth parasites using standard techniques. Fish were captured by local fishermen or technical staff members using various methods (netting, angling or electrofishing). The fish were weighed and measured. The parasites were counted and identified by Bauer, 1987; Gusev, 1985; Moravec, 1994, 2001; Niewiadomska, 1986, 1996; Scholz, 1999; Scholz Hanzelová, 1998; Shigin, 1986. The parasites were fixed and preserved in 70% ethanol (Bauer et al., 1981; Moravec, 1994). Trematodes and monogeneans of the genus Paradiplozoon were stained in iron acetocarmine, dehydrated in ethanol series with increasing concentrations, cleared in eugenol (metacercariae of Diplostomum spp. were cleared in dimethylphthalate) and mounted in Canada balsam (Bykhovskaya-Pavlovskaya, 1985; Georgiev et al., 1986; Shigin, 1986).
The ecological terms prevalence (P%), mean abundance (MA) and mean intensity (MI) are used here based on the terminology of Bush et al. (1997) and Marcogliese (1999). Mean abundance (MA) and mean intensity of infection (MI) were calculated using Microsoft Excel and STATISTICA 6.0 program.

## RESULTS AND DISCUSSIONS

### Fish communities

The white bream *B. bjoerkna* is an European freshwater fish of the Cyprinid family. The white bream occurs in a wide variety of shallow, warm lowland lakes and slow-flowing lower reaches of large rivers and canals. *B. bjoerkna* is freshwater, brackish, demersal and potamodromous fish. Frequently very abundant on bottom of large sandy rivers. The juvenile fish live in still water bodies. *B. bjoerkna* feeds on benthic invertebrates (Kottelat and Freyhof, 2007).
The white bream is estimated as least concern species (LC=Least Concern; IUCN Red List Status).

#### Helminth diversity and parameters of infection

The present study revealed the presence of five helminth species: *Diplostomum paraspathaceum* (Shigin, 1965), larvae, *Diplostomum pseudospathaceum* (Newiadomska, 1984), larvae, *Tylodelphys clavata* (von Nordmann, 1832), larvae, *Posthodiplostomum cuticola* (von Nordmann, 1832), larvae, *Paradiplozoon homoion* (Bychowsky et Nagibina, 1959) (Table 1).
The white breams from Srebarna Lake were infected from one to three helminth species, 20.08 % of hosts were infected with only one helminth species, 69.23 % with two species and 7.69% with three helminth species. The total number of helminths varies from 1 to 17 specimens per host (4.63 on average).

Table 1. Species diversity of helminth parasites in the white bream *B. bjoerkna* from Srebarna Lake

| Helminth species | P% | MA±SD | MI±SD range | Site |
|---|---|---|---|---|
| *Diplostomum paraspathaceum* | 62.5 | 2.06±3.43 | 3.3±3.89 1-14 | lens |
| *Diplostomum pseudospathaceum* | 6.25 | 0.38±1.5 | 6±0 6 | lens |
| *Tylodelphys clavata* | 12.5 | 0.38±1.02 | 3±0 3 | vitreous humour |
| *Posthodiplostomum cuticola* | 43.75 | 1.50±3.22 | 3.43±4.28 1-13 | skin, fins muscu-lature |
| *Paradiplozoon homoion* | 25.00 | 0.31±0.60 | 1.25±0.5 1-2 | gills |

The first intermediate hosts of trematoda *P. cuticola* are freshwater snails (*Planorbis planorbis*, *P. carinatum*), second intermediate hosts are fish and the definite host are birds of genus *Ardea* and *Nycticorax*. The first intermediate hosts of trematoda *T. clavata* are freshwater snails *Radix ovata*, second intermediate hosts are fish and the definite host are grebes - *Podicipes cristatus, P. griseigena* etc. The first intermediate hosts of for *D. paraspathaceum*, *D. pseudospathaceum* are freshwater snails from genus *Lymnea* (*Lymnea ovata, L. fortinalis*, *L. bactriana*) and *Radix (Radix auricularia, R. ovata*), second intermediate hosts are fish and the definite host are different fish-eating birds (*Larus munutus*, *L. canus*, *L. ridibundus*, *L argentatus*, *Chlidonias hybrida*, *Sterna albifrons*, *Pelecanus crispus*, etc.) (Bauer, 1987; Bykhovskaya-Pavlovskaya, 1985; Shigin, 1986).
The monogenean *P. homoion* is with a direct life cycle without intermediate hosts (Gusev, 1985). All trematoda species are endoparasites and monogenean is ectoparasite. Four helminth species were determined as allogenic parasites

the trematode species, *D. paraspathaceum*, *D. pseudospathaceum, P. cuticola* and *T. clavata*. Their life-cycle includes fish as intermediate host and fish-eating birds as final hosts. The monogenean *P. homoion* was determined as autogenic parasite for the examined lake ecosystem, it uses fish as definite host in its life-cycle (Esch et al., 1988). All allogenic helminth of white bream were at larval stage and an autogenic was in an adult form.

The species *Diplostomum paraspathaceum* is showed the highest prevalence and mean abundance (P% = 62.5, MA = 2.06 ± 3.43), followed by *P. cuticola* (P% = 43.75, MA=1.50±3.22). However, both species were showed low mean intensity (3.3±3.89 and 3.43±4.28, respectively). Other species form a descending order of prevalence, mean intensity and mean abundance: *P. homoion* (P%=25, MI=1.25±0.5, MA=0.31±0.60) and *T. clavata* (P = 12.5%, MI = 3 ± 0, MA=0.38±1.02). The species *D. pseudospathaceum* is showed the lowest prevalence (P%=6.25) and the highest mean intensity (MI=6±0).

The species *D. pseudospathaceum* was reported of *Perca fluviatilis* (Linnaeus, 1758) from dam Jrebchevo (reported as *D. volvens*) (Nedeva and Grupcheva, 1996), of *Scardinius erythrophthalmus* (L., 1758) from Black Sea Lakes (Kostadinova, 1993), of *Abramis brama* (L., 1758), *Blicca sapa* (Pallas, 1811), *Leuciscus aspius* (Lineus, 1758), *Barbus barbus* (L., 1758), *Carassius gibelio* (Bloch, 1782), *Chondrostoma nasus* (L., 1758), *Cyprinus carpio* (L., 1758), *Rutilus rutilus* (L., 1758), *S. erythrophthalmus*, *Pelecus cultratus* (L., 1758), *Vimba vimba* (L. 1758), *Esox lucius* (L., 1758), *P. fluviatilis*, *Gimnocephalus schraetser* (L., 1758), *Sander lucioperca* (L., 1758), *Silurus glanis* (L., 1758) from Bulgarian part of Danube river (Atanasov, 2012).

*D. pseudospathaceum* was found of *Alburnus alburnus* (L., 1758), *L. aspius, S. erythrophthalmus* (reported as *Diplostomum chromatophorum*), *P. fluviatilis*, *Lepomis gibbosus* (L., 1758), from Srebarna Lake (Shukerova, 2010; Shukerova and Kirin, 2008; Shukerova et al., 2010; Shukerova and Kirin, 2012).

Metacercariae of *P. cuticola* were recorded of *B. bjoerkna, Pelecus cultratus, Leucaspius delineatus, S. erythrophthalmus, C. chalcoides, C. carpio, S. cephalus* and *R. rutilus* from Danube River, Provadiiska River, Mandra Lake and Durankulak Lake (Margaritov, 1959; Margaritov, 1992; Kakacheva–Avramova et al., 1978; Kostadinova, 1993) of *Pelecus cultratus* (L., 1758), *L. cephalus*, *C. nasus* from Danube River Bulgarian part (Atanasov, 2012). The species *P. cuticola* was established of *P. fluviatilis, C. gibelio, Cyprinus carpio, S. erythrophthalmus, A. alburnus* and *L. aspius* from Srebarna Lake (Margaritov, 1959; Shukerova, 2005; Shukerova, 2006; Shukerova, 2010; Shukerova and Kirin, 2008; Shukerova et al., 2010).

The species *T. clavata* was found in *Misgurnus fossilis* from Danube river (Kakacheva – Avramova, 1977), of *B. petenyi* from Palakariya and Shipolnica River (Kakacheva and Menkova, 1978; Menkova, 1977); of *S. cephalus* from Shipolnica River (Menkova, 1977); of *P. fluviatilis* from dam Jrebchevo (Nedeva and Grupcheva, 1996), in *P. fluviatilis*, *S. erythrophthalmus* and *R. rutilus* from Durankulak Lake (Kostadinova, 1993). The species *T. clavata* was found in *P. fluviatilis, A. alburnus, L. aspius* and *L. gibbosus* (Shukerova**,** 2010; Shukerova et al., 2010; Shukerova and Kirin, 2012).

The acantocephalan *P. homoion* was recorded on gills of *R. rutilus* from Palakaria River and Danube River (Kakacheva–Avramova, 1977; Kakacheva and Nedeva, 1978), of *C. carpio*, *S. cephalus*, *C. nasus* and *B. barbus* from dam Pchelina and rivers Maritsa, Danube Tundza, Struma and Gradevska (Nedeva, 1991), of *C. gibelio* from dam Jrebchevo (Grupcheva and Nedeva, 1999) and of *Abramis brama* from Danube River (Chunchukova et al., 2016).

In Bulgaria as parasite of *Blicca bjoerkna* are established the following species *Nicolla skrjabini* (Iwanitzky, 1928), *Asymphylodora imitans* (Muhling, 1898), *Cotylurus pileatus* (Rudolphi 1802), *P. cuticola, Rhipidocotyle campanula* (Dujardin, 1845), *Dactylogyrus cornu* (Linstow, 1878), *D. distinguendus* Nybelin 1937, *D. similis* (Wagener, 1909), *D. sphyrna* Linstow, 1878, *Gyrodactylus prostae* (Ergens, 1963), *Diplozoon gussevi* Glaser and Glaser, 1964, *Caryophyllaeides fennica* (Schneider 1902), *Pomphorhynchus laevis* (Müller, 1776), *Acanthocephalus anguillae* (Müller, 1780)

(Margaritov, 1959, 1964, 1966; Kakacheva-Avramova, 1973, 1977, 1983). All parasites were reported of white bream from Danube River, Bulgaria part, with exception of *D. similes* (from Kamchia River).

In the countries of the catchment area of Danube River under *B. bjoerkna* were also established the following parasites: Trematoda – *Aspidogaster limacoides, Tylodelphys clavata*, *Phyllodistomum folium, Apophallus muehlingi*, *Palaeorchis unicus*, *Sphaerostomum bramae, Opisthorchis felineus* (Djikanović et. al., 2012; Gelnar et al., 1994; Hering-Hagenbeck and Schuster, 1996; Ozcelik and Deufel, 1989, Reimer, 2002). Monogenea – *Dactylogyrus cornoides, D. crucifer*, *D. difformis*, *D. nanus*, *Diplozoon paradoxum*, *Paradiplozoon bliccae, Gyrodactylus elegans*, *G. vimbi* (Gelnar et al., 1994; Kritscher, 1988; Matejusova et al., 2001; Matskasi and Sey, 1993; Ozcelik and Deufel, 1989; Reimer, 2002). Cestoda – *Archigetes sieboldin, Caryophyllaeus laticeps*, *Neogryporhynchus cheilancristrotus*, *Ligula intestinalis*, *Proteocephalus torulosus* (Barus and Prokes, 1994, 1995; Hanzelova and Rysavy, 1999; Kritscher, 1988; Macko et al., 1993; Scholz, 1989). Nematoda – *Anguillicola crassus*, *Philometra ovata*, *Philometra rischta, Rhabdochona denudata*, *Schulmanela petruschewskii* (Djikanović et. al., 2012; Moravec, 2001; Moravec et al., 1997; Szekely 1994). Acanthocephala - *Neoechinorhynchus rutili*, *Acanthocephalus lucii*, *Acanthocephalus tenuirostris*, *P. laevis*, *Pomphorhynchus bosniacus* (Djikanović et. al., 2012; Kiskarolj and Cankovic, 1969).

Common helmitnh species for helminth fauna of white bream from Lake Srebarna and previous studies from Bulgaria is *P. cuticola* from Danube River. Mean intensity of *P. cuticola* is with lower in white bream from Srebarna Lake than from this host from Danube River.

## CONCLUSIONS

This is the first study of helminth fauna of *B. bjoerkna* (Linnaeus, 1758) from Srebarna Lake. All helminth species identified in the present study are new host-records for the white bream in the Srebarna Lake. *Blicca bjoerkna* was reported as a new host record for digeneans, namely *Diplostomum paraspathaceum*, D. *pseudospathaceum, T. clavata* and monogenean *P. homoion* from the territory of Bulgaria. *Blicca bjoerkna* was reported as a new host record for *D. paraspathaceum*, *D. pseudospathaceum* and *D. monogenean, P. homoion* from Balkan Peninsula. In the present study, was reported for the first time the trematodes for *D. paraspathaceum*, *D. pseudospathaceum* as parasite of white bream from Basin of Danube River.

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# A CASE REPORT ON FISH EUSTRONGYLIDOSIS (PH: NEMATODA) IN ZANDER (*SANDER LUCIOPERCA*)

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***Abstract***

*This is a case report out of a series of epidemiological studies within fish populations inhabiting the Danube Delta area, of which aim is to gather data on Eustrongylides spp epidemiology, with emphasis on the relationship between host, agent and environment-associated factors, as well as on food safety potential hazards. This investigation has been carried out on zander (Sander lucioperca) between August-November 2016, into a fish processing plant which currently produces fillets for internal and external markets. The fish had been gathered by industrial fishing from the Razelm-Sinoe area. Following the investigation it resulted that the disease prevalence was over 90 %, with an average of 5-7 larvae/fish fillet (min. 3, max. 10 larvae per fish). Main sites of location are: muscle, gonads, intestine and peritoneum. Most of the larvae were encysted (parasitic nodules of 0.5-1.0 cm in diameter). The study results indicate that there is an increasing consumer's risk associated with the existence of Eustrongylides* spp. *larvae in fish destined to human consumption, as well as there is a potential of releasing the nematode into new areas, including on fishery farms. Hence, communication of the risk associated to the existence of Eustrongylides* spp. *within the Danube Delta area, and recommendation of specific biosecurity measures to help prevent the nematode from releasing/establishing into fishery farms in Europe should be called upon.*

***Key words****: case report, Eustrongylides spp, zander, pike perch.*

## INTRODUCTION

Larval infestations with *Eustrongylides* spp. (Ph: Nematoda, Cl: Adenophorea) have been reported in marine, brackish and freshwater fish species, worldwide. Fish eustrongylidosis is caused by the larvae of a nematode of the Order Dioctophymatida. Although a total of 19 Eustrongylides species have been described based on the morphology of the adult and larval stages (Moravec, 1994; Molnar, 2006) three species are commonly being referred to into literature: *E. tubifex*, *E. ignotus* and *E. excisus*. It is generally accepted however, that these nematodes have a complex life cycle, requiring a definitive host (wading birds) and intermediate hosts, *i.e.* oligochaetes or annelid worms (for earlier larval stages (L) – to ensure development from L1 to L2, or only for L2) and finfish (either for both L3 and L4, or just for L4), (Anderson, 2000; Arthur et al., 2002). *E. ignotus* may be able to complete its life cycle without a tubifex worm, whereas some fish species may act as definitive hosts of the nematode (Ibiwoye et al., 2005). Amphibians (frogs), reptiles (alligators, caimans, grass and dice snakes) and humans are occasional hosts of the nematode.

Infestations with *Eustrongylides* in fish are alleged to generate economic loss through impairment of reproduction, alteration of flesh coupled with sensorial devaluation of the meat, commercially displeasing appearance and faster deterioration of the fish or fish products, which all lead to marketer/consumer rejections. Although *Eustrongylides* is pathogenic also to humans, fish eustrongylidosis is currently among the least studied fish-borne parasitic zoonoses in Europe. Humans become infested by consumption of raw or undercooked infested and/or contaminated fish or fish products. An increasing number of reports of eustrongylidosis in humans have been recorded in Africa, Asia, U.S. and Europe, with more frequent reportings in fish meal-based, less developed countries (Ibiwoye et al., 2005). To our knowledge, in Europe, with few exceptions (*i.e.* the case report on *Eustrongylide*s in fish caught in Trasimeno lake, Italy) (Branciari et

al., 2016), case reports communicated in Romania, Serbia, Bulgaria (Kirin et al., 2002; Novakov et al., 2013), and Moldavia showed *Eustrongylides* as sourcing from the Danube River and the Danube Delta. Fish with eustrongylidosis have been occasionally found by local fishermen - who named the nematode "*the wire worm*", but also on public markets. The parasite has been subjected to public warnings by mass media, on the health-associated risks should the fish is consumed.
Also, there are reports of additional fish species and environments affected by *Eustrongylides*, worldwide. The parasite is commonly found in areas of denser, polluted human habitats.
In the absence of adequate measures to mitigate the spread of this parasite, it is expected that the number of infections in both humans and animals would become more prevalent in the future.

## MATERIALS AND METHODS

To assess the disease occurrence in zander, we used point prevalence (the proportion of animals in a population that are diseased, classifying each animal as either diseased or not). Zanders found infested with the Eustrongylides larvae during the study were classified as *diseased*.
The study was conducted during August-November 2016, into a plant processing fish fillet for the national and UE markets.
There were two harvests per month, with batches of about 500 kg/month, collected from the Razelm-Sinoie area, through industrial fishing. The observations were conducted on each of the fish caught, as a routine action into the processing plant. The total sample size used in the study was of cca 3600 zanders. Each zander weighted between 400 and 700 g, with body lengths of less than 40 cm.
All fish were cut open for filleting, those found infested/diseased being retained for study (fish necropsy and parasitological exams).

## RESULTS AND DISCUSSIONS

The fish under investigation displayed generally a good body condition. Discrete haemorrhages in musculature, peritoneum and viscera (gonads, intestines and liver) as well as red or brownish parasitic nodules in the organs and tissues were common findings in most cases (Figures 1 - 4). On each zander fillet there were between 3 and 10 *Eustrongylides* encysted and free larvae. Most of the larvae were encysted, forming brownish parasitic nodules with a diameter of 0.5-1.0 cm (Figures 1 - 3) supposedly accounting for the discreteness of the lesions found in the investigated zander.

Figure 1. Encysted larva in muscle (zander fillet)

Figure 2. Encysted larvae in fillets
(lighten background to distinguish the cysts in fillets)

During the last 20 years the number of case reportings referring to fish eustrongylidosis has increased, especially within industrial fisheries and among fishermen.
There is a progressive risk that the parasite to escape from natural environments to fishery farms, by introducing infested fish from natural waters into farms, using contaminated water/feed with eggs/larvae, or by infested wading birds that may have access to fish farms.

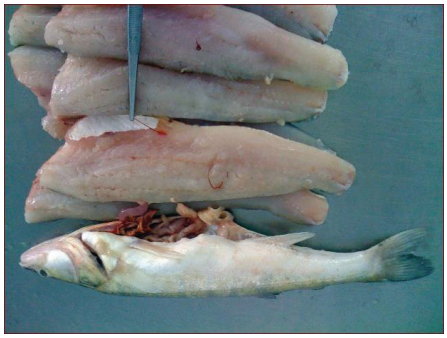

Figure 3. Free and encysted *E.* spp larvae in muscle

Figure 4. *E.* spp larvae in intestines of zander

The technological importance of the problem lays in the fact that Eustrongylides larvae establish into viscera (including gonads) and muscle of common fish species destined to human consumption, such as perch, pike, catfish, rapacious carp, perch, rudd, roach, carp, bleak and eel, thus affecting the spawn production and causing losses through meat impairment. It has been argued that the incidence of eustrongylidosis in fish (by population) and the intensity of infestations (by individual) vary according to the species and the individual resistance to infestation, but also to climatic conditions.

To diagnose infested fish, a prior necropsy is necessary. The sole reliable control measure of the fish destined to the public consumer is a randomized control of fish batches at the reception within the processing and storage units.

In fish processing facilities, the larvae are mechanically extracted, usually manually, with tweezers. The measure - possible only were evisceration and filleting take place as part of the processing technique, is appropriate providing the frequency and intensity of the parasites are low; the method proves unfeasible, demanding and time consuming at high infestation rates.

## CONCLUSIONS

Given the scarcity of scientific data required to apply efficient measures of prevention and control within fish and human populations, as expected, the parasitic disease is becoming a considerable problem by its zoonotic potential (by transmission to human consumers, under certain conditions) and epizootologically (including crossing from one species of animals to another). Therefore, there is a clear requirement for provisions of evidence-based data followed by dissemination of practical information, mainly towards the public health and industrial sectors.

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